Removal of estrogens and mitigation of estrogenic activity in natural water matrix using GAC-biofiltration system combined with photolysis as pretreatment

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by

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I, Fan Huang confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

This thesis is aimed at investigating the removal of estrogens in a natural water matrix using a granular activated carbon (GAC) sandwich biofiltration (GSBF) system, combined with the photolysis pretreatment process. To elucidate the main mechanisms of the decontamination process, the adsorption of estrogens onto GAC particles and photolysis of them under the irradiation of ultraviolet light (UV) in the batch experiment was studied firstly, in which the impacts induced by the presence of humic acid and matrix in natural water on the removal of estrogens was investigated, and corresponding kinetic models were adopted to explain the adsorption and photodegradation mechanisms. Then, a bench-scale GSBF system composed of fine sand and a GAC layer joining a photolysis reactor was installed for investigating elimination of free estrogens—estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethinyl estradiol (EE2) and estrogenic activity based on the adsorption and degradation models recorded earlier. Furthermore, the capacity of photolysis pretreatment for facilitating removal of estrogens was evaluated. The result showed that good removal for estrogens (96.2 % to 99.5 %) was constantly achieved by the GSBF over the eight weeks of the operation stage, while limited removal (20.95 % to 60.88 %) for both estrogens was observed in the slow sand filtration (SSF). Additionally, a significant reduction of estrogenic activity was achieved in GSBF, while a modest reduction was obtained for SSF. The involvement of photolysis was found to be effective to control the increase of head loss as a result of inhibiting the overgrowth of microorganisms. Therefore, photolysis pretreatment, coupling with the GSBF, is a promising treatment system for removing estrogens and estrogenic activity sustainably.
Impact Statement

Inside academia:

1) A novel detection method based on the derivatization reaction was established; this method remarkably enhanced the sensitivity of estrogens and allowed quantification of estrogens at trace level, which could only be achieved with high-budget instruments such as ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) before. This method can be employed by other researchers for estrogen analysis.

2) The adsorption of free estrogens onto GAC was investigated and this study provided a systematic insight into the adsorption mechanisms regarding the properties of GAC and influences of natural organic matter (NOM), being helpful in further understanding the adsorption capacity of GAC.

3) The photodegradation of E1, E2, E3, and EE2 varied under the irradiation of UV-C light due to the different molar absorption coefficients and quantum yields at a given wavelength. This finding can help further investigation into the photodegradation of estrogens.

4) A great removal of estrogens was achieved in the GSBF and photolysis-SSF, but the reduction of estrogenic activity in photolysis-SSF was observed. In addition, the conventional SSF was also found to be ineffective in mitigating the estrogenic activity. This finding can be a reference if other researchers want to measure estrogenic activity.

Outside academia:

1) The measurement of estrogen concentration in blood is of importance for clinical application, thus the developed detection method can be used after modifying the extraction method for the blood matrix instead.
2) The adsorption capacity of GAC was found to be vulnerable to NOM in water. This discovery is informative for water treatment plants and the pretreatment of NOM can guarantee the removal of contaminants.

3) The applicability of GSBF was evidenced via the high removal of estrogens and estrogenic activity, its lower cost than GAC contactors, and better removal performance than SSF, makes it a promising technique. This technique can be scaled up in water treatment plants for practical use.
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Abbreviation

OH = hydroxyl radicals

AS = activated sludge

AFG = acidic functional groups

AOPs = advanced oxidation process

APPI = atmospheric pressure photoionization

BAC = biological activated carbon

BF = Biofiltration

BSTFA+TMCS = N, O-bis(trimethylsilyl)trifluoroacetamide +1% trimethylchlorosilane

BSTFA = bis(trimethylsilyl) trifluoroacetamide

C18 = octadecyl

CEGE = Civil, Environmental and Geomatic Engineering Department

DFT = density functional theory

DMIS = 1,2-dimethyl-1H-imidazole-5-sulphonyl chloride

DO = dissolved oxygen

DOC = dissolved organic carbon

DWTPs = drinking water treatment plants

E. coli = Escherichia coli

E1 = estrone

E1-3G = estrone-3-glucuronide sodium salt
E1-3S= estrone-3-sulfate sodium salt

E2= 17β-estradiol

E2-17G= 17 β-estradiol-17-(β-D-glucuronide) sodium salt (E2-17G)

E2-3G= 17β-estradiol-3-(β-D-glucuronide) sodium salt (E2-3G)

E2-3S= 17 β-estradiol-3-sulfate sodium salt

E3= estriol

E3-3S= estriol-3-sulfate sodium salt

EA/EA₀= relative estrogenic activity

EDCs= endocrine disrupting chemicals

EE2= 17α-ethinyl estradiol

ELISA= enzyme linked immunosorbent

ER-CALUX= estrogen responsive chemical activated luciferase genre expression assay

ESI= electrospray ionisation

FA= formic acid

FDA= the Food and Drug Administration

G= growth factor

GAC= granular activated carbon

GC-MS= gas chromatography tandem mass spectrometry

GSBF= GAC sandwich filtration
HA= humic acid
hERα= human estrogen receptor
HPLC-MS-MS= high performance liquid chromatography tandem MS
HRT= hormone replacement therapy
IC= ion chromatography
ID= inner diameter
IR= induction ratio
LC-MS= liquid chromatography tandem mass spectrometry
LLE= liquid-liquid extraction
LODs= limit of detection
Log P= partition coefficient
LTQ= linear ion trap
MSTFA= N-methyl-N-(trimethylsilyl) trifluoroacetamide
MTBSTFA= N-methyl-N-(tertbutyldimethylsilyl) trifluoroacetamide
NOM= natural organic matter
NPOC= non-purgeable organic carbon
PAC= powder activated carbon
PBS=Phosphate buffer saline
PFBBBr= pentafluorobenzyl bromide
pHPZC = point of zero charge
pKa = acid dissociation constant

PSm = mean pore size

PVC = polyvinyl chloride

RIA = radioimmunoassay

RIC = reconstructed ion chromatogram

ROS = reactive oxygen species

RTD = residence time distribution

SBET = surface area

SEM = scanning electron microscopy

SPE = solid phase extraction

SRT = solid retention time

SSF = slow sand filter

STPs = sewage treatment plants

SWCNTs = single wall carbon nanotubes

TBFG = total basic functional groups

TOC = total organic carbon

TPV = total pore volume

UPLC-MS-MS = ultra performance liquid chromatography tandem MS

UV254 = absorbance at wavelength of 254 nm

UVT = transmittance of UV-light
VTG = vitellogenin

Yeast strain = Saccharomyces cerevisiae

YES = yeast estrogen screen
Chapter 1 Introduction

1.1 Research problems

The occurrence of endocrine-disrupting compounds (EDCs) in the aquatic environment has drawn scientific and public attention over the past decades, owing to their direct or indirect interventions in the endocrine system of wildlife, and even humans via interfering synthesis, migration, recognition, and binding of hormones in bodies (Combalbert et al., 2012, Sarkar et al., 2014, Younes, 1999). Specifically, EDCs exert disrupting actions by altering the enzymatic pathways, changing the signalling of nuclear steroid receptors, non-nuclear steroid receptors and nonsteroid receptors, and hindering the transcriptional processes (Diamanti-Kandarakis et al., 2009, Schug et al., 2011). Apart from the reproductive system, organs such as the pancreas, thyroid and central nervous system, are subject to EDCs (Schug et al., 2011). Over the past decades, there has been an increasing body of evidence addressing the association of many diseases with EDCs, like chronic diseases, metabolic disorders, genetic mutations and cancers (Hampl et al., 2016). Also, it was indicated that exposure to EDCs not only induced a decrease in the count and quality of sperm, but also potentially cause an alteration in children’s physical and mental development according to the previous study (Younes, 1999). According to the evaluation on the economic burden for the health outcomes due to EDCs exposure, the annual cost of €163 billion in the EU and $340 billion in the US was respectively estimated (Kassotis et al., 2020). Nevertheless, this informative data was probably underestimated as merely a subset of EDCs was chosen as examined targets.

EDCs are comprised of natural substances discharged from living organisms and a range of artificially synthetic products. The former is mainly from metabolites of plants,
microbes and mammals. The latter originated for serving human society and agricultural demands, consisting of pharmaceuticals, personal care products, plastic materials, insecticides, herbicides, heavy metals, etc., which are mostly equipped with phthalates, polycyclic aromatic hydrocarbons and alkylphenol structure, furthermore, most of them are persistent after entering the environment (Hampl et al., 2016, Vosges et al., 2010, Combalert et al., 2012, Salgueiro-Gonzalez et al., 2015). Upwards of millions of tons of EDCs are produced per year, and the accumulation of them occurring in the food chain system frequently exposes wild species, and even humans, to potential health risks (Kawa et al., 2021).

Global concerns over the threat of EDCs contamination were quickly deepened in the 21st century, especially in the EU and the US, which propose the most far-reaching regulations pertaining to EDCs (Kassotis et al., 2020). These regulations highlight the exposure limit of EDCs via the pathways such as pesticides, cosmetics, medical devices, drinking water, and other sectors that may involve the environment or human life. Additionally, countries including Brazil, Australia, South Korea, Canada, Japan, China, and Israel also established corresponding approaches for restricting the risk induced by EDCs (Kassotis et al., 2020). However, it is also known that the categories of EDCs are complex and there has not been an exhaustive list for EDCs up to now (Karthikeyan et al., 2021, Rahman et al., 2009). Furthermore, it is also challenging to define the adverse effects of EDCs regarding exposure and what are the indication endpoints (Karthikeyan et al., 2021). Therefore, to date no explicit concentration criteria are provided by governments or environmental agencies for most of EDCs, even in drinking water.
To attenuate the threat of EDCs, some studies about traditional water treatment technologies and advanced or tertiary treatment technologies have been conducted to minimize their discharge into receiving water (Ribeiro et al., 2015).

As a substantial subset of EDCs, estrogens are reported to occur at trace content in the aquatic environment and their occurrence is a primary cause of ecosystem issues like developmental abnormality of wild species, especially for fish (Armstrong et al., 2015). At present, the development of removal methods for estrogen is challenging on account of the limit of analytic methods and their high biological activity exerted at a low level (Yang et al., 2008, Lei et al., 2009).

The preliminary treatment processes, such as coagulation, flocculation and precipitation, exhibited limited removal efficiency for EDCs, including estrogens, according to early studies (Kim et al., 2007). A similar conclusion was also reported by (BÁRÁNY, 2013) and Haig et al. (2016).

However, some treatment technologies are also found to be promising to reduce the concentration of EDCs in influents. Biological processes such as activated sludge and biofiltration (BF), have been proved to attenuate some EDCs which are readily biodegradable (Zhang et al., 2017, Haig et al., 2016), and the removal performance was ascribed to the properties of EDCs and technology parameters. In addition to biological degradation, adsorbent materials such as granular activated carbon (GAC), powder activated carbon (PAC) and novel sorbents (e.g. carbon nanotubes, graphene) are also applied to achieve rapid removal of EDCs via physical process (Zhang and Zhou, 2005, Snyder et al., 2007). In recent years, the concept of advanced oxidation processes (AOPs) has also been put forward. They are technologies that mineralise organic contaminants through reacting with intermediate products of hydroxyl and
other oxidation species (Mohapatra et al., 2014). In research about a breach of EDCs like 2-chloropyridine and benzene under AOPs reaction, (Skoutelis et al., 2017, Lhotsky et al., 2017), it is implied that AOPs are attractive alternatives to the removal of estrogens. Furthermore, these processes were put into use at drinking water treatment plants (DWTPs) across the US, and played a key role in eliminating pharmaceuticals (Benotti et al., 2008).

At present, economically and sustainably feasible techniques are getting increasing attention in the water treatment field (Singh and Hankins, 2016). Thus, it is of importance to develop an effective technique with the aforementioned advantages to alleviate the contamination of estrogens.

1.2 Research aim and objectives

This study aims to develop an economic and efficient treatment system combining photolysis and biofiltration processes for eliminating recalcitrant estrogens and their estrogenic activity. For achieving this aim, the specific objectives are:

1. Establish a sensitive and accurate method to render simultaneous measurement of free and conjugated estrogens using solid phase extraction (SPE) pretreatment followed by the LC-MS analysis;
2. Investigate the removal mechanisms of free estrogens by GAC adsorption, photolysis under the ultraviolet light, and evaluating the positive or negative influences caused by the presence of natural organic matters (NOM);
3. Evaluate the extent to which the free estrogens and their biological potency would be removed in a lab-scale BF system coupling with photolysis pretreatment.
1.3 Thesis outline

This thesis comprises of eight chapters in total (Figure 1.1). Following the general introduction of Chapter 1, Chapter 2 provides a specific description of the overview on estrogen contamination, current technologies for profiling them, and treatment methods to mitigate their risks. Chapter 3 gives a general methodology employed in all studies of the whole thesis. Chapter 4 establishes a novel and sensitive derivatization based-method to quantify estrogens using LC-MS coupling with SPE preconcentration procedures, which was further used for measuring the concentration of estrogens in experiments described in Chapter 5, 6 and 7.

Chapter 5 investigates the adsorption mechanisms of free estrogens on GAC absorbents and evaluates the impacts of NOM on the adsorption capacity. Chapter 6 assesses the photolysis removal of free estrogens under the irradiation of UV-C light; the influence of NOM is also investigated in this chapter. Chapter 7 investigates the performance of the combined photolysis-BF system that was used for the removal of free estrogens and their total estrogenic activities were examined with different system configurations. Chapter 8 lists the main conclusions obtained from each experimental chapter and gives recommendations on future work.
Figure 1. 1 Thesis outline: simple introduction of each chapter and corresponding specific objectives.
Chapter 2 Literature review

In order to develop a promising treatment method to solve the environmental issues posed by estrogens, a comprehensive understanding of their related knowledge was highly required.

This chapter firstly introduced an overview of estrogen contamination in the aqueous environment and described their physiochemical properties; then their fate in the natural environment, the current technologies for detecting and quantitating them in the different matrix (e.g. concentration method and available analysis instruments) and the method developed for mitigating their pollution (e.g. adsorption, membrane filtration, biodegradation and advanced oxidation process) were respectively addressed in detail in terms of their mechanisms and advantages/disadvantages.

In light of the literature review, the likelihood of removing estrogens using a hybrid application of photolysis and bio-filtration was proposed, considering their low cost, and their potential of reducing by-products (Moussavi and Mohseni, 2007).

2.1 Overview of estrogens contamination

2.1.1 Definition of estrogens

The term ‘estrogens’ is known as female hormones, which can be divided into natural and synthetic origins, two categories in reference to their sources. Natural estrogens such as estrone (E1), 17β-estradiol (E2) and estriol (E3), are produced by humans and animals via the enzyme transformation from androgens in bodies, at which they are also involved in inter-cell communication. Thus estrogens are crucial to maintain the health of reproductive tissues and development of the brain and secondary sexual characteristics of females (Nie et al., 2009), but they are discharged into domestic wastewater through excretive pathways (Chen et al., 2014, Salvador et al., 2007b, Lai
et al., 2000). In addition, synthetic estrogens are another group of this estrogen family, among them exogenous estrogen 17α-Ethynyl estradiol (EE2) is the most common one, since it is the main component of contraceptive tablets (Tyler et al., 2009, Kuch and Ballschmiter, 2001b).

Compared to other EDCs such as bisphenol A or alkylphenols, the former is one of the most abundant EDCs worldwide and is usually applied as a plasticizer for the production of resins and plastics (Kawa et al., 2021). The latter is the main ingredient for producing detergents, emulsifiers and solubilizers, also being found to cause potential dysfunction of the reproductive system (Toor and Sikka, 2017, Soares et al., 2008), and estrogens exhibited stronger biologically active potency at the same concentration level; a range from ten to one million times was observed depending on their diverse chemical structures and properties (Grover et al., 2009, Schäfer et al., 2003, Desbrow et al., 1998a).

2.1.2 Biologically active effects of estrogens

It was widely reported that the presence of estrogens in an aqueous environment-induced many cases associated with deleterious reproductive impacts and development disorders, which took place in several kinds of species at a ng L⁻¹ exposure concentration, especially notable for fish (Fernandez et al., 2008, ˘Cedat et al., 2016, Tyler et al., 2009, Chen et al., 2014). These severe outcomes have also been specifically described in previous studies demonstrating the occurrence of estrogens in natural water (at trace level) gave rise to the production of vitellogenin (a female-specific egg protein, VTG) in male organisms (Lai et al., 2000, Nie et al., 2009, Shappell et al., 2008) and increased incidences of intersex (Wang et al., 2016, Chen et al., 2017). Consequently, it resulted in the feminisation and reduction of fertility in direct or indirect ways (Nash et al., 2004) and then threatens the sustainability of
aquatic species population (Kidd et al., 2007, Bisesi et al., 2017). Besides, some
research showed that over the period of pregnancy, the fetal and neonatal
development was sensitive and susceptible to exogenous hormone substances and
resulted in irreversibly negative impacts both in short and long term exposures
(Langston, 2010, Delbes et al., 2006).

Xenogenous estrogens, are estrogenic compounds produced from outside the body
(Kramer and Giesy, 1995). A growing number of scientific research illustrated that
xenogenous estrogens exhibited estrogenic potency directly or indirectly via: (i)
imitating the function of endogenous estrogens; (ii) inhabiting or antagonizing the
effect of endogenous estrogens; (iii) disrupting the synthesis of endogenous estrogens
and their metabolites by affecting the synthesis of enzymes or, (iv) altering the binding
behaviour with the specific hormone receptors which are transcription factors in charge
of gene expression under exposure of hormones (Silva et al., 2012, Roy et al., 2009,

In addition to the interference phenomena, the estrogens were also found to be
associated with the development of breast cancer (Qin et al., 2008, Xu et al., 2004,
Poschner et al., 2017). After the hydroxylation of position C-2 or C-4 next to the C-3
phenolic hydroxyl (specific chemical structure with numbered carbon positions are
described in Figure 2.1) took place, free estrogens (e.g. E1, E2, E3 and EE2) were
transformed to catechol estrogens, which consisted of two neighbouring phenolic
hydroxyl groups. As a result, catechol estrogens would mostly form quinones that
could react with DNA via nucleophilic addition reaction and produce relative DNA
adducts (e.g. 4-OHE-1-N3Ade and 4-OHE-1-N7Gu), which easily induce the
depurination of DNA, leaving behind the apurinic sites and thus resulting in the
mutations (Figure 2A.1) (Cavalieri and Rogan, 2011, Cavalieri and Rogan, 2004).
Therefore, carcinogenicity of estrogens acted on the basis of two mechanisms: 1) estrogen-induced modification induced the damage of DNA and further mutation via erroneous repair, thus initiating a tumour in the model system; 2) because of the mitogenic and antiapoptotic function, estrogens could promote the development of a tumour (Xu et al., 2007).

2.1.3 Application and regulation of estrogens

The presence of estrogens in the water environment has been the subject of concern due to the over-concentrated feeding operation (Shappell et al., 2008, Yang et al., 2012) and anthropogenic activities from domestic life and industries (Chiu et al., 2009, Cedat et al., 2016) with the explosion of the population.

![Figure 2.1](image)

Figure 2.1 Specific chemical positions of free estrogens. 1) for E1. R1: =O, R2: —H; 2) for E2. R1: —OH, R2: —H; 3) for E3. R1: —OH, R2: —OH; and 4) for EE2. R1: —OH; R2: —C≡C—.

In the aspect of agriculture, animals such as cattle received estrogen supplements as growth promoters, correspondingly their excreted waste contained the residue of estrogens which resulted in the contamination of steroid estrogens, via either the runoff from the farm into closed water bodies or infiltration into groundwater through the soil when the animal waste was employed as fertilizer for agricultural activity (Tomšíková et al., 2012).
In many cases, however, raw and treated sewage discharges are regarded as the chief sources of steroid estrogens to natural waters (Griffith et al., 2014). After the disposal of pharmaceutical factories waste and the excretion of synthetic estrogens and metabolites of endogenous estrogens from organism bodies, the incomplete removal in domestic wastewater treatment plants causes the occurrence of estrogens in treated effluent, receiving rivers, lakes, the ocean, ground water and even drinking water to some extent (Salvador et al., 2007b).

In the 1970s, the observation that the usage of contraceptive pills might lead to deleterious ecological issues regarding their persistence and estrogenic activity in environmental media was reported by Norpoth et al. (1973). Apart from this application for birth control, estrogens have also been the most important ingredients of medicines for hormone replacement therapy (HRT) since the mid 19th century, and are mainly prescribed for women both during and after the cessation of the menstrual cycle, which typically happens in their late 40s to 60s (Tyler et al., 2009). Considering the great increase of population all over the world and the extension of human longevity, the total dose of estrogens prescribed for HRT in the future is expected to keep rising. To achieve alleviation of estrogen contamination in the environment, the United States Environmental Protection Agency (USEPA) has added E1, E2, E3 and EE2 to Contaminant Candidate List 3 (Bermudez et al., 2012). Despite this, estrogens were not included in the European Union Water Framework Directive of 2000/60/EC due to their unclear impairment to organisms (Grover et al., 2009), while along with the establishment of pertinent research, E2 and EE2 have been added to the “surface water watch list” under the Water Framework Directive in the European Decision EU 2013/39 (Haig et al., 2016, Sanches et al., 2016, Ribeiro et al., 2015). Then, an extra ten kinds of organic chemicals, including E1, were recently put on the “watch list” of
Decision 2015/495 (Rubirola et al., 2017, Richardson and Kimura, 2016). At the same time, very low method detection limits of 0.035 ng L\(^{-1}\) for EE2 and 0.4 ng L\(^{-1}\) for E1 and E2 were also proposed by the EU (Glineur et al., 2020). In other words, authorities recognised the necessity to monitor the environmental content and occurrence of estrogens.

2.2 Occurrence, fate and properties of estrogens

2.2.1 Occurrence of estrogens

The existence of steroid estrogens was detected in influents, effluents of domestic sewage treatment plants (STPs), sludge, rivers, river sediments and drinking water in Canada, the UK, the US, Italy, the Netherlands, the Czech Republic, Poland, Germany, Japan and China (Table 2.1). Their concentration levels ranged from ng L\(^{-1}\) to µg L\(^{-1}\) (Belfroid et al., 1999, Baronti et al., 2000, Kuster et al., 2004, Kuch and Ballschmiter, 2001a, Ternes et al., 1999, Dudziak and Luks-Betlej, 2004, Desbrow et al., 1998b, Vega-Morales et al., 2011, Nieto et al., 2008, Nie et al., 2009, Kuch and Ballschmiter, 2001b, Fan et al., 2013).

Table 2.1 Estrogen concentration occurring (ng L\(^{-1}\) or ng g\(^{-1}\)) in influents and effluents of STPs, rivers, river sediments and drinking water.

<table>
<thead>
<tr>
<th>Matrix (location)</th>
<th>Compounds</th>
<th>Concentration</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Influent of STPs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>E1, E2, E3, EE2</td>
<td>0.40–188 ng L(^{-1})</td>
<td>Baronti et al. (2000)</td>
</tr>
<tr>
<td><strong>Effluent of STPs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>E1,E2, E3, EE2</td>
<td>0.44-82.1 ng L(^{-1})</td>
<td>Baronti et al. (2000)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>E1, E2, EE2</td>
<td>0.4-47 ng L(^{-1})</td>
<td>Belfroid et al. (1999)</td>
</tr>
<tr>
<td>Germany</td>
<td>E1, E2, EE2</td>
<td>LOD(^{a})-70 ng L(^{-1})</td>
<td>Ternes et al. (1999)</td>
</tr>
<tr>
<td>Canada</td>
<td>E1, E2, EE2</td>
<td>3-64 ng L(^{-1})</td>
<td>Ternes et al. (1999)</td>
</tr>
<tr>
<td>Country</td>
<td>Location</td>
<td>Estrogens</td>
<td>Concentration</td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
<td>-----------</td>
<td>---------------</td>
</tr>
<tr>
<td>UK</td>
<td></td>
<td>E1, E2</td>
<td>1.4-76 ng L⁻¹</td>
</tr>
<tr>
<td>Activated sludge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>E1, E2, EE2</td>
<td>&lt;4-37 ng g⁻¹</td>
<td>Kuster et al. (2004)</td>
</tr>
<tr>
<td>Spain</td>
<td>E1, E3, E1-3S, E2-3S</td>
<td>0.64-406 ng g⁻¹</td>
<td>Nieto et al. (2008)</td>
</tr>
<tr>
<td>Spain</td>
<td>E2, E3, EE2</td>
<td>LOD-100.4 ng g⁻¹</td>
<td>Vega-Morales et al. (2011)</td>
</tr>
<tr>
<td>Rivers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td>E1, E2, EE2</td>
<td>LOD-1.3 ng L⁻¹</td>
<td>Dudziak and Luks-Betlej (2004)</td>
</tr>
<tr>
<td>Germany</td>
<td>E1, E2, EE2</td>
<td>0.1-5.1 ng L⁻¹</td>
<td>Kuch and Ballschmiter (2001a)</td>
</tr>
<tr>
<td>Italy</td>
<td>E1, E2, EE2</td>
<td>0.04-1.5 ng L⁻¹</td>
<td>Baronti et al. (2000)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>E1, E2, EE2</td>
<td>0.1-5.5 ng L⁻¹</td>
<td>Belfroid et al. (1999)</td>
</tr>
<tr>
<td>China</td>
<td>E1, E2, E3, EE2</td>
<td>LOD-55.3 ng L⁻¹</td>
<td>Lei et al. (2009)</td>
</tr>
<tr>
<td>Japan</td>
<td>E1, E2, EE2</td>
<td>LOD-19.7 ng L⁻¹</td>
<td>Kawaguchi et al. (2004)</td>
</tr>
<tr>
<td>River sediments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>E1, E2, EE2</td>
<td>&lt;0.2-2 ng g⁻¹</td>
<td>Kuster et al. (2004)</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>E1, EE2, E1-3S</td>
<td>LOD-2.37 ng g⁻¹</td>
<td>Matejicek et al. (2007)</td>
</tr>
<tr>
<td>Japan</td>
<td>E2, E1-3S</td>
<td>0.03-3.6 ng g⁻¹</td>
<td>Isobe et al. (2006)</td>
</tr>
<tr>
<td>China</td>
<td>E1, E2, E3, EE2</td>
<td>LOD-21.6 ng g⁻¹</td>
<td>Lei et al. (2009)</td>
</tr>
<tr>
<td>Drinking water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>E1, E2, EE2</td>
<td>0.15-2.1 ng L⁻¹</td>
<td>Kuch and Ballschmiter (2001b)</td>
</tr>
<tr>
<td>China</td>
<td>E1, E2</td>
<td>LOD-1.7 ng L⁻¹</td>
<td>Fan et al. (2013)</td>
</tr>
</tbody>
</table>

*Limit of detection.*

It is noticed that the concentration of estrogens in influent of STPs is higher than that measured in other sources, and that the accumulation of estrogens may occur in activated sludge due to the sorption process, leading to high concentrations of estrogens as well (Silva et al., 2012). By comparison, the concentration of estrogens in the river, river sediments and drinking water, are generally much lower. However, it is known that the estrogenic potency of estrogens could be aroused in bodies of
organisms or humans at a concentration of merely 0.5 ng L\(^{-1}\) (Schäfer et al., 2003). Therefore, since most of the current treatment plants are not specifically designed for removing estrogens, their incomplete removal in wastewater treatment plants makes it a significant point source of estrogen discharge in the aquatic environment (Nie et al., 2009). So, more suitable and specific technologies are required for preventing further deterioration from estrogen contamination.

Among the natural steroid estrogens, E1 and E3 are the metabolites of E2, and both exert a lower biological activity than E2 (Nie et al., 2009, Goeppert et al., 2015). It was found that the heterotrophic bacteria was able to realise the transformation of E2 to E1 (Huang et al., 2016), and this transformation is reversible. It has also been found that under anaerobic conditions E1 would undergo reduction reaction to form E2 (Sarkar, Ali et al. 2014). In addition, man-made EE2 exhibited the greatest genotoxic strength in comparison to the other three natural estrogens. The VTG induction caused by EE2 was reported to be 20 times stronger than E2 when they were assayed at the same concentration (Anderson et al., 2012, Caldwell et al., 2012). Therefore, because of their high estrogenic activity and common presence in an aquatic environment (Haig et al., 2016, Silva et al., 2012), the natural hormones E1, E2, E3 and synthetic EE2 were selected as the representatives of female hormone steroids in this research.

2.2.2 Fate of estrogens

Under the participation of aromatase enzyme, E1 and E2 are produced in the body via transforming androgens (e.g. androstenedione and testosterone) in the aromatization process, then the reversible metabolization between E1 and E2 takes place after reduction and oxidation, subsequently as a result of hydroxylation reaction, E1 and E2 are metabolized to E3 in irreversible orientation (Rakholia et al., 2019, Samson et al., 2009, Xu et al., 2017, Zhang, 2006); finally the estrogens enter the circulation system
of the body and act on the relevant tissues and organs. Estrogens are mostly in
sulphate, glucuronide and sulfoglucuronide conjugate forms when they are excreted
in urine by living organisms and human beings, and then they are deconjugated by
enzymes such as arylsulfatase and beta-glucuronidase, which are derived from
microorganisms present in sewer biofilm or waste water treatment plants, activating
estrogens before or during entering the treatment process (Dai et al., 2016, Liu et al.,
2015, Goeppert et al., 2015, Sandor and Mehdi, 1979, Johnson and Williams, 2004).
The metabolism pathways of estrogens in the body and environment are depicted in
Figure 2.2.
Figure 2. 2 The fate and transformation of estrogens in human bodies and environment. Abbreviation— A: aromatisation; G/S: glucuronic acids/ sulphuric acids; R/O: reduction/oxidation; H: hydroxylation.
It was noticed that the composition of conjugated estrogens varied and sulphate conjugates became dominant on reaching the sewage works with very few glucuronide conjugates left (Johnson and Williams, 2004). Furthermore, partial E2 was also oxidized into E1 in the same course (Schäfer et al., 2003). In Heffley et al. (2014)’s study, 28.75% of E2 in sewers was observed to convert to E1, and the increased influent load of E1 was expected to contribute to its stability in sewers. Additionally, no removal of EE2 in sewers was observed (Heffley et al., 2014). From a few publications, the estrogen conjugates were detected in effluents of wastewater treatment plants, which indicated that the migration in the sewer and treatment process could not achieve complete cleavage of estrogen conjugates (Ben et al., 2017). A proportion of sulphate conjugates like E2-3S survived after undergoing the activated sludge process, and merely 50% of it was deconjugated to its free parent form E2 (Kumar et al., 2012). In summary, the rise of free estrogens would be expected, owing to the presence of their conjugates and the involvement of the deconjugation course.

As addressed by Johnson and Williams (2004) and Anderson et al. (2012), the secretion content of estrogens including free and conjugated forms (E, f&c) varied with the gender, age and pregnancy stage. Overall, data from different studies presented in Table 2.2 suggested that women typically excreted 7–27.3 µg day\(^{-1}\) of E1, f&c, 2.6–9.8 µg day\(^{-1}\) of E2, f&c, and 4.6–15.5 µg day\(^{-1}\) of E3, f&c in urine, in comparison, the content of estrogen in urinary excretion was far greater for pregnant women, increasing to 1200–1480 µg day\(^{-1}\) for E1, f&c, 330–360 µg day\(^{-1}\) for E2, f&c and 23600–52100 µg day\(^{-1}\) for E3, f&c. Here, it is worth noting that the women taking contraceptive pills excreted less E1, f&c, E2, f&c and E3, f&c than those who did not, in the meantime; it was predictable that more EE2 might be produced in their urinary excretion than in the other two groups of women, as it was mentioned by Xiao and
McCalley (2000) that EE2 was not detected in samples owned by the group not taking contraceptive pills. According to the calculation in Johnson and Williams (2004)'s work, the ingesting concentration of EE2 could be calculated as 26 µg day\(^{-1}\) when the contraceptive pill was used by 17% of the total female population; after the metabolism process, there was still about 10.5 µg day\(^{-1}\) of free EE2 being excreted. From other studies, approximately 30,000 kg year\(^{-1}\) of natural estrogens E1, E2 and E3 and another 700 kg year\(^{-1}\) of synthetic estrogens EE2 were predicted to be released from people all over the world (Shrestha et al., 2012). Apart from the urinary excretion, estrogens were also excreted in feces, while the concentration was much less (Johnson and Williams, 2004). Moreover, it was noticeable that a small amount of them were excreted in unconjugated form (Gruber et al., 2002).

For males, the average excretion of E1,f&c in urine was ranging from 2.8 to 3.9 µg day\(^{-1}\) (Key et al., 1996, Xiao and McCalley, 2000) and another 0.4 µg day\(^{-1}\) of E1,f&c was excreted in fecal excretion (Pusateri et al., 1990, Hämaläinen et al., 1987).

Table 2. 2 Urinary excretion amount of endogenous estrogens at different stages (µg day\(^{-1}\)).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of Samples</th>
<th>E1(_{f&amp;c})</th>
<th>E2(_{f&amp;c})</th>
<th>E3(_{f&amp;c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Non-pregnant Premenopausal Women not on Contraceptives – Urinary Excretion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D'Ascenzo et al. (2003)</td>
<td>50</td>
<td>14.0</td>
<td>7.5</td>
<td>14</td>
</tr>
<tr>
<td>Xiao and McCalley (2000)</td>
<td>1</td>
<td>15</td>
<td>5.4</td>
<td>9.4</td>
</tr>
<tr>
<td>Adlercreutz et al. (1986)</td>
<td>23</td>
<td>7.1</td>
<td>2.6</td>
<td>4.6</td>
</tr>
</tbody>
</table>
2.2.3 Properties of estrogens

The physiochemical properties of E1, E2, E3 and EE2 remarkably contribute to their variance in dissociation pathways, removal performance, and their estrogenic potency. Except that free estrogens would be studied in the following chapters, their glucuronide and sulphate conjugates were also chosen for conducting the experiments described in Chapter 4 and Chapter 8; so, the detailed information for all of them was thoroughly listed in Table 2.3.

Among these properties, the water solubility and partition coefficient (Log $P$) are critical for studying the fate and movement of estrogens in an aquatic environment. In addition, these two parameters are also helpful to study the mechanisms of treatment techniques like the adsorption process (Chen and Hu, 2009). For free estrogens, their different solubility was noted, although E1, E2, E3 and EE2 have very similar structure; the solubility of E1 and E2 are nearly the same with values 1.3 and 1.51 mg L$^{-1}$, while the solubility of E3 and EE2 in water is around ten times and seven times higher than them, respectively. These discrepancies were deduced mainly owing to the different
type and steric arrangement of functional groups located at the C-16 and C-17 positions (See Figure 2.1). Compared to free estrogens, their conjugated forms have much greater water solubility and tend to be mobile in water environments (Hanselman et al., 2003).

With respect to Log $P$, it is considered as a reasonable factor accounting for the sorption potential (Gomes et al., 2011). The values listed for free estrogens are between 2.45 and 4.01, which means that they are hydrophobic. It was introduced in previous studies that chemicals possessing log $P$ values greater than 4 were regarded as strongly hydrophobic, which tended to show low solubility in water and high adsorption coefficient in soil (or sediment), and at the same time, they were also bio-concentrated in territorial organisms. On the contrary, the chemicals with log $P$ less than 1 were found to easily dissolve in water and had weak sorption capacity (Lei et al., 2009, Ren et al., 2017b, Jones-Lepp and Stevens, 2007). So, conjugated estrogens are more hydrophilic due to the substitution of sulphate or glucuronide group at the position of C-3 or C-17. Therefore, the higher endocrine-disrupting potency of free estrogens could be explained because of their structures and properties, which allowed strong binding to the estrogen receptors (Griffith et al., 2014). Moreover, Griffith et al. (2014) and Goeppert et al. (2015) also mentioned that conjugated estrogens with sulphate and glucuronide groups were more polar and had higher solubility in water, they further exhibited weak binding affinity to the estrogen receptors owing to their low sorption potential, rendering them more biologically inactive.

The weak acid property of free estrogens was indicated by their high pKa values within a range of 10.24-10.27, in a comparison, the introduction of glucuronide and sulfate acids makes conjugates have a remarkable reduction in pKa values (-1.8-3.63), in other words, the stronger acidity was displayed by the conjugate form. The
deprotonation and pronation of a compound were closely related to their pKa values and pH of solution matrix, consequently the sorption mechanism of chemicals was also found to be associated with the pKa value (Lorphensri et al., 2006). For example, the outstanding absorption was observed when the solution pH was below the pKa of the compound, at which the compound molecule was in the protonated form and there was a tendency to interact with the solid phase (Zhang et al., 2016). As the pH of environmental water usually ranges from 6 to 8, the conjugated estrogens are mainly in deprotonated form, while the unconjugated estrogens are mostly protonated.

The Henry’s law constant could be calculated via dividing the fraction of compound dissolved in water by another fraction dissolved in air, which is usually indicative of the volatility of compounds. The compounds having Henry’s law constant < $10^{-10}$ atm m$^3$ mol$^{-1}$ were regarded to have low volatility under the ambient pressure and temperature (Hamid and Eskicioglu, 2012). Similarly, the low vapour pressure listed in Table 2.3 also indicated that both free and conjugated estrogens were weakly volatile (Friedgood et al., 1948, Speight, 2020). This characteristic demonstrated that the direct detection of estrogen using the gas chromatography tandem mass spectrometry (GC-MS) was challenging (Abdel-Khalik et al., 2013).
Table 2. 3 Physiochemical properties of free and conjugated estrogens.

<table>
<thead>
<tr>
<th>Estrogens</th>
<th>CAS</th>
<th>Molecular weight (g/mol)</th>
<th>Solubility (mg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Vapour pressure (mmHg)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Henry’s Law constant (atm m&lt;sup&gt;3&lt;/sup&gt; mol&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Log P&lt;sup&gt;a&lt;/sup&gt; (at 25°C)</th>
<th>pKa&lt;sup&gt;c/d/e&lt;/sup&gt;</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>53-16-7</td>
<td>270.37</td>
<td>1.3</td>
<td>2.49×10&lt;sup&gt;-10&lt;/sup&gt;</td>
<td>3.80×10&lt;sup&gt;-10&lt;/sup&gt;</td>
<td>3.13</td>
<td>10.25</td>
<td><img src="image" alt="Chemical structure of E1" /></td>
</tr>
<tr>
<td>E2</td>
<td>50-28-2</td>
<td>272.38</td>
<td>1.51</td>
<td>6.38×10&lt;sup&gt;-9&lt;/sup&gt;</td>
<td>3.64×10&lt;sup&gt;-11&lt;/sup&gt;</td>
<td>4.01</td>
<td>10.27</td>
<td><img src="image" alt="Chemical structure of E2" /></td>
</tr>
<tr>
<td>E3</td>
<td>50-27-1</td>
<td>288.4</td>
<td>13.25</td>
<td>9.93×10&lt;sup&gt;-12&lt;/sup&gt;</td>
<td>1.33×10&lt;sup&gt;-12&lt;/sup&gt;</td>
<td>2.45</td>
<td>10.25</td>
<td><img src="image" alt="Chemical structure of E3" /></td>
</tr>
<tr>
<td>EE2</td>
<td>57-63-6</td>
<td>296.40</td>
<td>9.2</td>
<td>1.95×10&lt;sup&gt;-9&lt;/sup&gt;</td>
<td>7.94×10&lt;sup&gt;-12&lt;/sup&gt;</td>
<td>3.67</td>
<td>10.24</td>
<td><img src="image" alt="Chemical structure of EE2" /></td>
</tr>
<tr>
<td>E1·3S</td>
<td>438-67-5</td>
<td>372.41</td>
<td>6328.6</td>
<td>1.44×10&lt;sup&gt;-12&lt;/sup&gt;</td>
<td>2.04×10&lt;sup&gt;-12&lt;/sup&gt;</td>
<td>2.5</td>
<td>-1.75</td>
<td><img src="image" alt="Chemical structure of E1·3S" /></td>
</tr>
<tr>
<td>E1-3G</td>
<td>15087-01-1</td>
<td>468.47</td>
<td>8471.7</td>
<td>9.48×10^{-19}</td>
<td>8.45×10^{-22}</td>
<td>1.6</td>
<td>3.3</td>
<td></td>
</tr>
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<td>-----</td>
<td></td>
</tr>
<tr>
<td>E2-3S</td>
<td>4999-79-5</td>
<td>374.4</td>
<td>15893</td>
<td>3.36×10^{-13}</td>
<td>1.95×10^{-13}</td>
<td>2.1</td>
<td>-1.8</td>
<td></td>
</tr>
<tr>
<td>E2-3G</td>
<td>14982-12-8</td>
<td>470.49</td>
<td>8829.8</td>
<td>6.79×10^{-19}</td>
<td>8.09×10^{-20}</td>
<td>1.1</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>E2-17G</td>
<td>15087-02-2</td>
<td>470.49</td>
<td>1.12×10^{5}</td>
<td>1.06×10^{-18}</td>
<td>2.28×10^{-22}</td>
<td>1.1</td>
<td>3.63</td>
<td></td>
</tr>
<tr>
<td>E3-3S</td>
<td>481-95-8</td>
<td>390.43</td>
<td>1.75×10^{5}</td>
<td>4.13×10^{-15}</td>
<td>7.13×10^{-15}</td>
<td>1.9</td>
<td>-1.75</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) PubChem and Sigma Adrich; \(^{b}\) Shareef et al. (2006); \(^{c}\) Tomšíková et al. (2012); \(^{d}\) He and Aga (2019); \(^{e}\) Yu et al. (2019).

CAS, chemical abstract service; Log *P*, water-octanol partition coefficient; pKa, acid dissociation constant.
2.3 Analysis methods of estrogens

The presence of estrogens is usually at a trace level in an aqueous environment, therefore the analytic methods capable of achieving the measurement at ng L\(^{-1}\) level concentrations were required for precisely quantitating them (Lin and Reinhard, 2005). As a whole, the biological analysis and chemical analysis including GC-MS and liquid chromatography tandem mass spectrometry (LC-MS) are currently the most common analytic methods employed to profile the concentration of estrogens.

2.3.1 Biological analysis

Biologically based techniques have been used to analyse estrogens in many studies (Armstrong et al., 2015, Hinteman et al., 2006). These analysis methods work by quantitating the biological effects exhibited by the whole organisms, cells or estrogen receptors chosen in studies under the exposure to estrogens. The main mechanisms specifically consist of cell proliferation, ligand bonding, VTG induction, luciferase effects and antibodies production (Campbell et al., 2006).

The availability of aqueous species for measuring the potency of estrogens was proved via a series of experiments (Yan et al., 2012, Vosges et al., 2010, Nash et al., 2004). Here, the rainbow trout, fathead minnow, sheepshead minnow and zebrafish are the most commonly used fish (Caldwell et al., 2012). In these toxicity tests, various indication endpoints for showing qualitative and quantitative analysis of estrogens were set, such as deformities, reproductive deficiencies, egg and offspring development and VGT in assayed fish groups (Campbell et al., 2006).

Apart from the whole organisms, cellular and immuno-based analyses are also widely used in biological detection techniques. Yeast and human cells like the breast cancer cell, are usually applied to monitor the toxic potency of estrogens after the genetic
modification (Heub et al., 2015, Campbell et al., 2006). The specific cellular bioassays include the yeast estrogen screen (YES) and estrogen responsive chemical activated luciferase genre expression assay (ER-CALUX) which were carried out via making the genetically engineered cells be in contact with estrogenic compounds. In the former, the exposure to estrogenic compounds resulted in the generation of galactosidase and then the concentration could be measured via the colorimetric determination using UV-Vis spectrophotometer (Murk et al., 2002). For the latter, the concentration results could be obtained via measuring the light output derived from the formation of luciferase with the presence of estrogens using the scintillation counter (Legler et al., 2002).

In the past decades, immunoassays such as enzyme linked immunosorbent (ELISA) and radioimmunoassay (RIA) have also been developed for estrogens quantification based on the competition between labelled and unlabelled antigens (estrogenic compounds) for the binding sites (antibody molecules) (Zhang, 2006, Sharma et al., 2014a). The colour enzyme or radio label was introduced for indicating the concentration results through measuring the colour using UV-vis spectrophotometer or the radioactivity using the scintillation counter (Hanselman et al., 2004, Sharma et al., 2014b).

In summary, biologically based assays are convenient because of the facile instrument requirement and low cost (Campbell, Borglin et al. 2006). Moreover, they are able to demonstrate the estrogenic activity of the input estrogens and the intermediates after treatment processes, showing the synergistic or antiestrogenic influences when the solution matrix has complicated the constitution of compounds (Barreiros et al., 2016, Heub et al., 2015). However, though the limit of detection (LODs) of ER-Calus or YES method was at the same order of magnitude (ng L⁻¹) as what was obtained by the
chemical analysis method (GC-MS or LC-MS) (Murk et al., 2002), the biological assays are time-consuming and susceptible to cross reactivity, so the results derived from these methods suffer from poor accuracy and reproducibility (Nelson et al., 2004, Pacakova et al., 2009). In addition, it was also noted that biological analysis merely allows quantitating the total biological effects of compounds rather than the exact content of each estrogen (Barreiros, Queiroz et al. 2016).

2.3.2 GC-MS analysis of estrogens
As one of the most widely used instruments for detecting EDCs, GC-MS allows the simultaneous analysis of both synthetic and natural estrogenic steroids considering its better capabilities for the separation and identification of these compounds. However, given the properties of estrogens, they are not volatile, even under high temperatures, which means that derivatization with some volatile moiety is necessary to improve sensitivity and peak separation (Azzouz and Ballesteros, 2014). And derivatization is able to increase the thermal stability of the steroid hormones (Abdel-Khalik et al., 2013).

For example, several silylation reagents such as N-methyl-N-(tertbutyldimethylsilyl) trifluoroacetamide (MTBSTFA), bis(trimethylsilyl) trifluoroacetamide (BSTFA), N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and N, O-bis(trimethylsilyl)trifluoroacetamide +1% trimethylchlorosilane (BSTFA+TMCS) were used to react with free estrogen (Quintana et al., 2004, Gonzalez et al., 2015). The specific derivatization procedures occurred at both the aromatic hydroxyl of C-3 and the aliphatic hydroxyl positions at C-17, and the competition between them showed that derivatization reaction acted on the C3 position firstly (namely aromatic hydroxyl
position, in Figure 2.1) (Zhang et al., 2006), the level and yields of reaction depended on the dose of silylation reagents, reaction time and temperature controlled in the reaction.

Nonetheless, it was found that the LODs with GC-MS (at ng L⁻¹ level) was not as low as what was detected using the liquid chromatography tandem mass spectrometry (at pg L⁻¹ level). Furthermore, in a real case, detection of estrogen metabolites with GC-MS is achieved by cleavage of their conjugated groups using glucuronidase and sulfatase firstly, then followed by the derivatization process (Tso and Aga, 2010), so simultaneous quantification for free estrogens and estrogen conjugates is complex and time-consuming.

2.3.3 LC-MS analysis of estrogens
Compared with GC-MS, LC-MS exhibits better performance in previous scenarios regarding the sensitivity and throughput, so it is usually considered as a reference methodology (Nelson et al., 2004, Goh et al., 2016, Dai et al., 2012).

With the application of advanced instruments such as ultra performance liquid chromatography tandem MS and MS (UPLC-MS-MS), high performance liquid chromatography tandem MS and MS (HPLC-MS-MS) and LC-MS-MS, it would mostly achieve direct analysis of estrogens with non-derivatization protocols though the estrogens inherently have weak ionisation (Griffith et al., 2014, Gentili et al., 2002, Goh et al., 2016). Table 2.4 listed the LC-MS method developed for determining estrogens in water samples; the low LODs obtained suggested the applicability of the LC-MS technique.

However, if the available LC-MS (e.g. LC-ion trap MS) was not advanced enough, derivatization would be a feasible alternative to increase the response of analytes via
altering the moieties of targeted compounds and enabling their ionisation to intensify. Furthermore, due to the similar structures of free and conjugated estrogens, the separation of them in the analysis part is also challenging, but the increased sensitivity allowed the remarkable recognition of compounds of interest by their specific m/z values. The principle of strengthening response in LC-MS is that derivatization reactions introduce a permanent charged group to estrogen molecules, which makes estrogens easier to ionise (Abdel-Khalik et al., 2013). The existence of ketone, hydroxyl and phenolic groups in a molecular structure facilitated the reaction between estrogens and derivatization reagents (Faqehi et al., 2016). In the past, derivatization reagents including dansyl chloride (Salvador et al., 2007b, Fan et al., 2013), pyridine-3-sulfonyl chloride (Xu and Spink, 2008), 4-(1H-pyrazole-1-yl), pentafluorobenzyl bromide (PFBBr) and isomers of 1,2-dimethylimidazole-sulfonyl chloride (Xu and Spink, 2007, Keski-Rahkonen et al., 2015) were employed to react with estrogens for the improvement of determination in different matrix (e.g. plasma, urine, and water).

After the derivatization processes, the LODs of estrogens using LC-MS could get to 0.02-2.9 ng L\(^{-1}\) depending on different matrix, instruments and derivatization reagents (Table 2.4). It was reported that the LODs of derivatised estrogens were one or two orders of magnitude lower than that of non-derivatised estrogens (Faqehi et al., 2016, Lin et al., 2007, Fan et al., 2013).

Over the course of analysis using the LC-MS, the selection of ion source was also influential to the sensitivity of detection. In matrix with little interferences (e.g. Milli-Q water and drinking water), the much greater signal response was observed under the electrospray ionisation (ESI) mode (Lin et al., 2007). For environmental samples with complicated matrix, the co-present substances might inhibit the ionisation of targeted compounds when ESI sources were used (Lin et al., 2007). Correspondingly, a
atmospheric pressure photoionization (APPI) source was recommended for its higher tolerance to complicated matrix than ESI (Kuehnbaum and Britz-Mckibbin, 2011). Nevertheless, according to the literature listed in Table 2.4, the more general usage of ESI source was noticed.

The analysis fulfilled with LC-MS is expensive due to the high-budget equipment, additionally the pre-treatment methods such as the solid phase extraction and liquid-liquid extraction, are inevitable for samples having complicated matrix. But not surprisingly, analysis work achieved with LC-MS show great accuracy, high sensitivity and prominent repeatability. Moreover, LC-MS could not only achieve the simultaneous detection of multiple compounds in one port sample, but also get the LODs of estrogen to pg L\(^{-1}\)--ng L\(^{-1}\) (Azzouz and Ballesteros, 2014). Therefore, LC-MS was selected as the analytic method for measuring the content of estrogens in the following studies.

2.4 Solid phase extraction

After the development of the solid phase extraction (SPE) method after the 1940s (Liška, 2000), the incessant liquid-liquid extraction (LLE) method originally applied to concentrate the pollutants in water was gradually substituted due its tedious procedures (Locatelli et al., 2016). Except for the enrichment function, the SPE process also helped remove the background interferences in samples, mitigating the impairment of instrument and ionisation suppression induced by impurities (He and Blaney, 2015).

To date, online or offline SPE coupled with GC-MS or LC-MS has been employed for measuring organic micropollutants such as antibiotics (Xu et al., 2021, He and Blaney, 2015), endocrine-disrupting chemicals (Nie et al., 2009), pesticides (He and Aga, 2019)
and pharmaceuticals (Li et al., 2017). In regard to estrogens, the frequent application of SPE for environmental samples could also be demonstrated by previous studies listed in Table 2.4.

The significant factors to affect the enrichment behaviour of SPE were the selection of adsorbent (to retain targeted compounds), washing solution (to wash away interferences) and elution (to release the targeted compound) (Dean, 2003).

The extraction process taking place around the adsorbents is associated with various interactions such as the adsorption, size exclusion, hydrogen bonding, ion exchange and polar and non-polar interactions (Su et al., 2017). Lots of cartridges based on these principles including Oasis MCX, Oasis MAX, Sep-Pak octadecyl (C18) and HLB are commercially available (Bertol and Vaiano, 2016). Among these options, HLB is versatile for concentrating a wide range of compounds due to its dual functional (e.g. hydrophilic and hydrophobic) filling sorbents, though the property also lessened the selectivity of sorbent and retained more matrix from sample (Zhu et al., 2015). In addition, the interaction types between cartridge and compounds and the solution matrix should also be taken into account for the optimisation of washing solution and elution.
Table 2. Measurement of estrogens in environmental water using SPE and LC-MS techniques.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Preconcentration</th>
<th>Instrument</th>
<th>Derivatization reagent</th>
<th>Water matrix</th>
<th>LODs (ng L(^{-1}))</th>
<th>Recovery (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1, E2, E3, EE2, E1-3S, E1-3G, E2-3G, E3-3G, EE2-3G</td>
<td>Off-line SPE, Empore SDB-XC disk</td>
<td>UHPLC-MS/MS, UPLC-ESI-MS/MS</td>
<td>N.U, N.U</td>
<td>Effluent of WWTP, Milli-Q water, influent/effluent sludge of WWTP, sludge</td>
<td>0.11-1.3, 0.04-2.2 ng L(^{-1}); 0.05-4.9 ng g(^{-1})</td>
<td>12-198, 48.5-114.9</td>
<td>Griffith et al. (2014), Zhu et al. (2015)</td>
</tr>
<tr>
<td>E1, E2, E3, EE2</td>
<td>Off-line SPE, NG 1 Hypersep Retain PEP cartridge</td>
<td>LC-MS/MS, LC-ESI-MS/MS</td>
<td>N.U</td>
<td>Influent/effluent of WWTP</td>
<td>0.16-0.67</td>
<td>46.6-117.6</td>
<td>Goh et al. (2016)</td>
</tr>
<tr>
<td>E3, E3-3S, E1, E1-3S, E2-3S, E2-17S, EE2, E2-17G</td>
<td>On-line SPE, BetaBasic column</td>
<td>LC-ESI-MS/MS</td>
<td>N.U</td>
<td>River, drinking and waste water</td>
<td>3.3-27.0</td>
<td>72-117</td>
<td>Naldi et al. (2016)</td>
</tr>
<tr>
<td>E1, E2, E3, EE2</td>
<td>On-line SPE, Oasis HLB</td>
<td>UHPLC-MS/MS</td>
<td>N.U</td>
<td>Effluents of WWTPs</td>
<td>0.9-1.3</td>
<td>72-98</td>
<td>Vega-Morales et al. (2012)</td>
</tr>
<tr>
<td>-----------------</td>
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<td>-------------------------</td>
</tr>
<tr>
<td>E1, E2, E3</td>
<td>On-line SPE, Oasis HLB</td>
<td>UHPLC-MS/MS</td>
<td>N.U</td>
<td>Effluents of WWTPs</td>
<td>4.1-8.5</td>
<td>54.4-126.7</td>
<td>Guedes-Alonso et al. (2015)</td>
</tr>
<tr>
<td>E1, E2, EE2</td>
<td>On-line SPE, Oasis HLB</td>
<td>LC-ESI-MS/MS</td>
<td>Dansyl chloride of WWTP</td>
<td>0.4-0.7</td>
<td>79.7-95.3</td>
<td>Salvador et al. (2007a)</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>Off-line SPE, Oasis HLB</td>
<td>UPLC-MS/MS</td>
<td>Dansyl chloride</td>
<td>Drinking water</td>
<td>0.02-0.05</td>
<td>82-102</td>
<td>Fan et al. (2013)</td>
</tr>
<tr>
<td>E1, E2, E3, EE2</td>
<td>Off-line SPE, Bakerbond C18 disks</td>
<td>UPLC-ESI-MS/MS</td>
<td>Dansyl chloride</td>
<td>River water, effluents of STPs</td>
<td>0.23-0.91</td>
<td>91.3-119.6</td>
<td>Lien et al. (2009)</td>
</tr>
<tr>
<td>E1, E2, E3, EE2</td>
<td>Off-line SPE, PolarPlus C18</td>
<td>LC-APCI-MS/MS</td>
<td>PFBB</td>
<td>River, drinking water and effluents of STPs</td>
<td>0.38-2.9</td>
<td>N.D&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Lin et al. (2007)</td>
</tr>
</tbody>
</table>

<sup>a</sup>, N.U=not used; <sup>b</sup>, N.D=not detected.
2.5 Current treatment methods for removing estrogens

2.5.1 Sorption

Sorption is one of the most important processes employed for alleviating the contamination in an aqueous environment, it works based on getting to the equilibrium between the sorption and desorption in liquid-solid phase (Site, 2001).

Activated carbon (AC) is the product derived from the physical or chemical activation procedure of carbonaceous substances (Haimour and Emeish, 2006, Surkatti et al., 2021). Among diverse functional sorbents, the high surface area and complex porous structure containing macropores, mesopores and micropores allow AC a high adsorption capacity (Beker et al., 2010).

The AC adsorption for organic compounds such as naphthalene, atrazine, pharmaceuticals and phenol was investigated in the past decades (Cabal et al., 2009, Wang and Alben, 1998, Baccar et al., 2012). According to these studies, the AC was found capable of adsorbing a wide range of organic compounds but generally exhibited superior performance for less hydrophilic chemicals (Altmann et al., 2014). Overall, the capability of AC adsorption varied with different characteristics of AC sorbents (e.g. specific surface area, pore size distribution, surface chemistry) (Ebie et al., 2001, Beker et al., 2010, Fukuhara et al., 2006) and physiochemical properties of targeted compounds (e.g. hydrophobicity, molecular size) (Joseph et al., 2013). Additionally, the adsorption affinity was also influenced by the solution chemistry (Fukuhara et al., 2006). For the co-present substances such as NOM, they would mostly weaken the adsorption performance of AC via the competition for adsorption sites (Yan et al., 2013). Moreover, the negative impact was also induced due to the formation of fouling, which was accelerated with the presence of NOM and then blocked AC pores (Meinel et al., 2014). The adsorption kinetic was also prone to the
pH of solution since it not only affected the charges of sorbent surface but also the charges of sorbates (Kilic et al., 2011, Baccar et al., 2012).

In order to increase removal efficiency of contaminants, bulky AC are tailored to granular, powder and even nano-meter level, which is mainly for increasing the contacting area between adsorbents and pollutants. Concerning the removal of estrogens, Snyder et al. (2007) applied GAC and powder activated carbon (PAC) for removing E1, E2, E3 and EE2, and their result showed that both GAC and PAC performed well on removing estrogens, with the removal for all reaching 90%. In line with Zhang and Zhou (2005)’s study, the high adsorption capacity of GAC was also reported for natural estrogens.

Apart from AC, some novel materials such as single wall carbon nanotubes (SWCNTs), graphene nanoplatelets, graphene nanosheet, modified carbonaceous adsorbents and carbon nanotubes were also applied to adsorb estrogens, and their remarkable performance renders them alternative methods (Bisesi et al., 2017, Jiang et al., 2017, Tagliavini et al., 2017, Jiang et al., 2016). However, the application of these novel adsorbents has not been widely adopted due to their high costs (Beltran et al., 2009).

In some cases, the AC adsorption was found to be more cost-effective than membrane filtration and the advanced oxidation process; the latter two techniques required higher energy input (Albatrni et al., 2021, Rad et al., 2015). Furthermore, the non-use of chemical reagent in adsorption process is also effective to limit the production of toxic by-products after treatment processes. Nevertheless, its shortcoming is the disposal of a tremendous amount of solid wastes carrying a high concentration of organic compound after the treatment.
2.5.2 Membrane filtration

Membrane filtration is a highly efficient method to reject contaminants during filtration; micropollutants were eliminated by the membrane filtration via hybrid processes including adsorption, size exclusion and charge repulsion Bolong et al. (2009). The rejection performance was found to be strongly dependent on the physicochemical properties (e.g. molecular weight, octanol-water partition coefficient, water solubility, electrostatic properties) of compounds, the operation parameters (e.g. flux and water quality), characteristics of the membrane (e.g. permeability, pore size, surface chemistry), and solution chemistry such as pH and concentration of NOM (Schäfer et al., 2011). Ultrafiltration, nanofiltration and reverse osmosis are three main membrane techniques developed for controlling water contamination issues. Concerning the removal of compounds, the order of their rejection behaviour followed reverse osmosis (RO)> nanofiltration (NF) > ultrafiltration (UF), while the energy consumption also presented the same tendency (Silva et al., 2012, Liu et al., 2009).

As introduced by Giakoumis et al. (2020) and Oren and Biesheuvel (2018), the UF membrane has an average pore size ranging from 0.005-0.1 µm (diameter), so the more efficient rejection of estrogens should be achieved by the NF (0.001-0.05 µm) and RO membrane (0.66-0.78 nm) due to the Stoke’s radius of estrogens (~0.4 nm). This conclusion was supported by previous studies aimed at investigating the elimination of estrogens using UF, NF and RO membranes. In Yoon et al. (2007)’s work, the removal performance of UF and NF for estrogens E1, E2, E3 and EE2 were compared, the results showed that the retention percentages of targeted estrogens were 0-45% for UF and 38-60% for NF, respectively. The similar removal performance of NF for estrogens was also obtained by Bodzek and Dudziak (2006), being greater than 63%, and higher removal (> 81.5%) was achieved using coagulation-NF
techniques. With respect to RO membranes, the outstanding rejection over 95% for E1 within the pH range of 2 to 10 was observed in the work conducted by Nghiem et al. (2002). Therefore, it is inferred that the pore size of the membrane is of importance for the size sieving mechanism. In addition, according to Alturki et al. (2010), rejection percentage of estrogens using the NF/RO combined technique was over 90%, being a promising method for the removal of estrogens. In the meanwhile, it is also known that these two kinds of membrane materials require high energy consumption and are easy to be damaged due to irreversible contamination and the formation of fouling, especially at the presence of NOM. Specifically, it was reported that the fouling adhesion to the RO membrane led to the significant reduction in the rejection of estrogens (Nghiem et al., 2004b). So, the huge input through the whole operation of membrane filtration system was required for preventing blockage and fouling issues. Thus, even though the application of membrane filtration minimise the process of chemical pretreatment and the generation of byproducts (Jacangelo et al., 1997), a more suitable method with a low cost and less energy is required to attenuate the contamination of estrogens for practical use.

2.5.3 Advanced oxidation process (AOPs)

With the development of treatment technology, the application of AOPs has been confirmed to destroy recalcitrant compounds or, at least, to transform them into biodegradable species as a further removal step (Sarkar et al., 2014, Ribeiro et al., 2015). The prerequisite of AOPs occurrence is the generation of oxidant, hydroxyl radicals (•OH) (Pera-Titus et al., 2004). Specifically, the AOPs could be categorised into chemical, electro-chemical, sono-chemical and photo-chemical processes according to their fundamentals to produce •OH (Babuponnusami and Muthukumar,
2014), where the chemical and photo-chemical processes are the most commonly applied for mineralising estrogen compounds.

Regarding the chemical processes, the removal of estrogens with participation of strong oxidizers such as ozone, Fenton reagent and disinfectants (e.g. chlorine dioxide, hypochlorite) was studied (Pereira et al., 2011, Huber et al., 2005, Nakamura et al., 2007). The average removal percentage of estrogens > 90% was achieved using ozonation (Sun et al., 2019, Bila et al., 2007, Lin et al., 2009) while the estrogenic activity of byproducts was remaining when the solution was neutral and alkaline, so it was further inferred that these byproducts were produced with the presence of •OH (Bila et al., 2007). Using the disinfectants, the reduction of estrogens ranged from 70% to 100% (Westerhoff et al., 2005, Nakamura et al., 2007). However, similar to the addition of ozone, byproducts derived from chlorination reactions also possessed stronger potency than the parent estrogens (Griffith et al., 2014, Bila et al., 2007). Compared to the ozonation and disinfectants, the removal of 40.1% to 98% after 8 h was achieved by a novel Fenton process depending on the different conditions (e.g. pH, amount of hydrogen, illumination condition) (Yaping and Jiangyong, 2008).

For the photon-chemical process, both photolysis and heterogeneous photocatalysis are techniques of great interest for the reduction of contaminants in water. The effective attenuation of estrogens or estrogenic activity under irradiation of diverse light source was reported in previous studies (Coleman et al., 2004, Chen et al., 2013, Chowdhury et al., 2011, Zhang and Li, 2014). According to Ren et al. (2017a), an overlap between the adsorption spectrum of targeted compounds and the wavelengths of radiation was critical for direct photodegradation. The photoactive phenolic moiety in estrogens is significant for direct photolysis (Mazellier et al., 2008). The removal of E1 and E2 was around 95% and 60% under the irradiation of UV light with wavelength
λ=254 nm, respectively, being greater than that observed under a high pressure mercury lamp (λ≥365 nm) (Liu and Liu, 2004). Therefore, it was concluded that E1 was more fragile to UV irradiation and this was also in agreement with the result obtained by Li Puma et al. (2010). Except for the direct photolysis, a portion of removal was also occasionally ascribed to the indirect photodegradation usually initiated by photosensitizers. The reactive oxygen species (ROS) might be generated when the ubiquitous NOM in natural water was irradiated, then the greater photochemical behaviours of E2 were achieved (Leech et al., 2009). Similarly, it was also found that EE2 could be degraded at a greater rate in DOC-containing solutions than in pure water (Canonica et al., 2008). On the contrary, the inhibitory effects of NOM for photolysis performance was also discussed in terms of light filter effect and the competition for •OH in Liu et al. (2018)’s work. Thus, a more detailed investigation of impacts of water constitutions on the photolysis is required.

In the photocatalysis process, the semiconductor TiO$_2$ has been the most common catalyst for water treatment because of its high photocatalytic efficacy. Once the irradiation energy excesses the band gap of TiO$_2$ (3.2 eV), the photocatalysis was initiated and h$^+$ hole was formed in the valence band following ejecting an electron to the conduction band, subsequently hydroxyl radicals were generated and reacted with targeted contaminants according to the following formula (Zhang and Zhou, 2005):

$$TiO_2 + h_v \rightarrow e^- + h^+$$  \hspace{1cm} (2.1)

$$h^+ + H_2O \rightarrow •OH + H^+,$$  \hspace{1cm} (2.2)

$$h^+ + OH^- \rightarrow •OH,$$  \hspace{1cm} (2.3)

$$h^+ + A \rightarrow P$$  \hspace{1cm} (2.4)
Where, after the generation of •OH at the semi-conductor surface, pollutant (A) was adsorbed onto TiO\textsubscript{2} and then was oxidised to a product (P) by •OH. The application of TiO\textsubscript{2} for the removal of estrogens was described in previous studies (Arlos et al., 2016, Coleman et al., 2005, Coleman et al., 2004, Li Puma et al., 2010). Generally, the usage of TiO\textsubscript{2} under irradiation promoted the degradation of estrogens with the presence of •OH. Coleman et al. (2000) showed that the degradation of E1 using photocatalysis was faster than using photolysis only, and the removal percentage up to 98% was achieved after 3h irradiation. In addition, more removal of estrogenic activity was achieved with the addition of TiO\textsubscript{2} (Coleman et al., 2004). It was also noted that the degradation acceleration obtained using photocatalyst for E2 was the greatest, the second greatest for E1, and the poorest under UVA for EE2 (Coleman et al., 2004). The opposite results that degradation of EE2 was accelerated more than E1, E2, E3 were however observed by Li Puma et al. (2010). No explanation accounted for the differences.

AOPs have a great potential for treating estrogen contamination in water rapidly. In addition, AOPs also present advantages such as strong bactericide activity and simplicity for practical use (Oturan and Aaron, 2014). However, it was also found that the existence of natural occurring scavengers including organic matter (e.g. humic substances, protein and carbohydrate) and saline ions (e.g. carbonate, bromide, or chlorinate in some cases) might provoke the inhibitory effect of AOPs due to their competition for the hydroxyl radicals with the targeted pollutants (Ribeiro et al., 2015). Moreover, the reaction products of AOPs likely exhibit stronger potency than parent compounds, so this method is at risk concomitantly. Furthermore, in the AOPs process, either strong oxidant or high intensity of irradiation is required, so cost and energy input are higher than other treatment technologies. But considering their advantages,
AOPs are viable to be a pre-treatment or post-treatment method and coupling with another technique with a low cost such as a biological process, controlling AOPs within less time followed by techniques with a small requirement of chemicals and energy (Ribeiro et al., 2015). For this purpose, the quenching of the strong oxidizers before any biological treatment was needed, as the growth and accumulation of microorganisms were negatively influenced (Oller et al., 2011). Therefore, the photo-chemical process, especially photolysis, was more suitable than the chemical process to be integrated into AOPs-biological hybrid processes.

2.5.4 Biodegradation

Biodegradation is a sustainable and cost-effective method that utilizes the ability of microorganisms to scavenge organic compounds through the specific metabolic or enzymatic effects (Silva et al., 2012, Singh and Borthakur, 2018). According to previous studies, the microbes which are capable of achieving biotransformation of estrogens include bacteria, fungi, and microalgae (Wang et al., 2019, Della Greca et al., 2008, Sei et al., 2008, Zhang et al., 2020, Isabelle et al., 2011). In Isabelle et al. (2011)’s work, estrogen-degrading bacteria strains were isolated from a bioreactor aimed at treating swine waste, and they were identified as the genera of bacteria called *Methylobacterium, Ochrobactrum, Pseudomonas* and *Mycobacterium*, which were within the three classes of *Alphaproteobacteria, Gammaproteobacteria* and *Actinobacteria*. Here, the partial biotransformation of E2 to E1 was noticed in medium containing stains related to genera of *Ochrobactrum, Pseudomonas* and *Mycobacterium* while the reduction of E1 to E2 by *Methylobacterium* related strain was also observed. Apart from that, in river sediments the bacteria *hydrogenophaga* within the *Betaproteobacteria* class, *Bdellovibrio* within the taxon of *Detaproteobacteria* and
Sediminibacterium from the Chitinophagaceae class were discovered to be responsible for transforming E2 to E1 under aerobic condition; however, with varying concentrations of nitrate, ferric, and sulfate, the E2 degraders also changed into other species such as Methylotenera, Geobacter, and Desulfomicrobium, etc. The capacity of Cyanobacteria used for the bioremediation of E1 was also confirmed, here, the laccase produced by Cyanobacteria played an important role in the oxidation of E1 (Sami and Fatma, 2019). Concerning biodegradation of estrogens by fungi, the elimination performance was tightly associated with extracellular enzymes like ligninolytic and laccase produced by fungi (e.g. white-rot fungi strains and T.versicolor) via the catalytical oxidation process (Sei et al., 2008, Suzuki et al., 2003). In addition to bacteria and fungi, microalgal-based technology for eliminating estrogens has also been investigated in past decades. The conversion of E2/EE2 to E1 and further ring cleavage of estrogens were detected when microalgae Selenastrum capricornutum and Chlamydomonas reinhardtii were assayed for biodegradation of E2 and EE2 (Hom-Diaz et al., 2015). In Wang et al. (2019)’s study, good removal of both estrogens (80%-100%) and estrogenicity (40%-80%) were achieved by two common microalgae species S. capricornutum and S. quadricauda after 20 days.

Among biodegradation techniques, the activated sludge (AS) process is the most prevalent method applied for treating sewage all over the world (Clouzot et al., 2010). This technique contains adsorption and biodegradation processes, while it was observed that the portion of removal in sludge adsorption was negligible, the removal of compounds was mostly accounted for by biodegradation (Liu et al., 2009, Andersen et al., 2003).

A significant number of investigations on the removal of estrogens using activated sludge treatment (AS) have been conducted since the 2000s (Andersen et al., 2003,
Suzuki and Maruyama, 2006, Dytczak et al., 2008, Johnson et al., 2000). These studies observed that the removal of estrogens varied over a wide range, from negligible losses to a 90% reduction (Liu et al., 2009). Even in some cases, the higher concentration of E1 in effluent than in influent was observed due to the transformation of E2 to E1 (Johnson and Sumpter, 2001). And as mentioned by Kumar et al. (2012), conjugate estrogens were transformed into free estrogens by AS, then followed by the further biodegradation, which might result in incomplete removal of free estrogens and even a small proportion of sulphate conjugates left in effluents of waste water treatment plants, likely causing continuous contamination of estrogens.

In any scenario, solid retention time (SRT) plays a significant role in the performance of AS. As the ripening and accumulation of specific microorganisms capable of degrading estrogens could just be reached within a long enough SRT (Hashimoto and Murakami, 2009). The dissolved oxygen (DO) is also another pivotal factor to affect the efficiency of estrogen removal (Liu et al., 2009). As the greater removal of estrogens was found in aerobic condition in comparison with anaerobic conditions (Ying et al., 2003, Joss et al., 2004).

In summary, the biodegradation process is susceptible to factors such as the types of treatment, biodegradation agent, water quality, and operation conditions (Silva et al., 2012). The solution to work out this problem is proposed by extending SRT according to practical configuration of treatment plants. Thus, it was concluded that the AS process was time-consuming, and the removal efficiency was not always great. Being consistent with adsorption using AC, the post treatment of sludge is also challenging due to the concentrated pollutant residues and odour of used sludge. According to characteristics of EDCS, the concentration of free estrogens in sludge is far higher than in water environment. If used sludge was used to cover an agricultural field,
estrogens sediment would likely be released from sludge and run into the water cycle again.

2.5.5 Biofiltration (BF)

It is known that BF is one of the most ancient techniques used for the sewage treatment since the first use of it in the UK in 1893, and it is also widely used in the world currently (Silva et al., 2020). Biofiltration is a cost-friendly process, besides the physical retention and adsorption that occurred on the filter medium, it also removed the organic compounds in water via the biological activity of biofilm, which developed and adhered to the surface of packed media (Zhu et al., 2010, Silva et al., 2020). Biofilm are defined as the microbial communities attached in the interface of solid-liquid, in which bacterial or fungi are embedded in the extracellular polymeric substances produced by microorganisms themselves (Coenye, 2013, Flemming et al., 2016). The formation of biofilm is indirectly affected by the concentrations of nutrient (Risse-Buhl et al., 2017). Additionally, the properties of substrate media, contact angle between microorganism and media, the flow pattern of liquid and steric effect are all related to the attachment of biofilm (Srivastava and Majumder, 2008). In order to establish a biofiltration system with satisfactory performance, maintaining the health of biofilm is crucial. It is because the treatment efficiency of biofiltration is primarily associated with the microbial activity conducted by bacteria or fungi (Srivastava and Majumder, 2008). Therefore, the optimisation of factors such as aerobic condition, nutrient concentration, pH, temperature and ions are required for the effective operation of biofiltration (Cohen, 2001).

In terms of the constitution of media material used in BF, the sand filter, GAC filter and dual-media filter are the three main categories in practical use. As one of the most common substrates, sand with a small particle size provides relatively large surface
area and allows more attachment of biofilm (Davidson et al., 2008, Maurya et al., 2020). Moreover, sand particles are biologically inert and affordable due to its prevalence in the natural environment, and thus sand is widely used for the construction of a BF system (Thornhill and Kumar, 2018).

For a biosand filter or slow sand filter (SSF), the slow flow rate of feeding water allows the growth and accumulation of microorganisms on the surface of the medium, then the sticky layer called the Schmutzdecke (Figure 2.3) is maturated after several weeks (Moran, 2018), which consists of bacteria, algae, fungi, protozoa and some larger eukaryotes and their extracellular matrix (Hammes et al., 2011).

![Figure 2.3 The schematic diagrams of SSF and Schmutzdecke.](image)

Because of the biodegradation ability, the removal of trace level organic contaminant using single-media SSF has been reported. Household SSF was proved to be efficient to alleviate the content of the organic compounds diclofenac, naproxen and ibuprofen by 97.4%, 84.7% and 85%, respectively with the continuous system (Erba et al., 2014).
In a study by Escola Casas and Bester (2015), the satisfactory removal of propranolol (94%) and iomerprol (85%) was obtained by the SSF running in a hydraulic loading rate of 0.012 m$^3$ m$^{-2}$ h$^{-1}$, though a relative poorer removal performance (41%-58%) for diclofenac, iopromide and iohexol. The 47.5% reduction of benzophenone-3 and 65.2% for paracetamol was observed in intermittent SSF with the influent spiked at 2 µg L$^{-1}$ (Pompei et al., 2016).

As for the biological activated carbon (BAC) filters, it works for removing organic compounds via the integrated functions of adsorption and biodegradation. Using the GAC, which is inherently porous and has a high specific area as filling medium, the organic compounds could be removed due to its powerful adsorption capacity. In the meantime, the unique structure makes it a good substrate for the growth of microorganisms and offers the sites for the biodegradation process, even though the adsorption capacity of GAC was exhausted (Nemani et al., 2018). In previous studies, BAC was found to effectively remove NOM (Velten et al., 2011), disinfection byproducts (Babi et al., 2007), pharmaceuticals (Rivera-Utrilla et al., 2013) and contaminants of emerging concern (Zhang et al., 2017). However, the drawback of this filtration process is also attributed to the use of GAC fractions as filter medium, the headloss increased quickly due to the blockage of filters, and thus the frequency of backwash was increased accordingly (Meinel et al., 2014).

Over the past years, the dual-media filtration (e.g. GAC-sand, anthracite-sand) has attracted a lot of interests due to its greater removal capacity than single-media SSF and less capital cost than GAC filters. The enhancement of removal is especially remarkable due to the GAC layer (Zhang et al., 2017). However, the layout of GAC particle and sand is also influential to the stability of the filtration system. Being analogous to the GAC filtration, the clogging and quick increase of headloss would be
observed if the GAC was filled as the upper layer bed. Consequently, based on the physical retention and biodegradation of the upper layer sand, the introduction of the GAC layer into the middle of the sand bed (called GAC sandwich biofilter) was expected to simultaneously enhance the removal capability and extend the operation time. GAC sandwich filtration (GSBF) was proposed and tested by Bauer et al. (1996) for mitigating the contamination of pesticides in water for the first time. In the past five years, the application of GSBF for the removal of pharmaceuticals and personal care products (PPCPs) (Li et al., 2018) and antibiotics (Xu et al., 2021) demonstrated its feasibility for water treatment or tertiary wastewater treatment.

There are limited studies about the removal of estrogens using biofiltration. In Haig et al. (2016)’s work, compared to the poor reduction of estrogenic potency obtained in nonaaugmented groups, the removal of E1 could reach up to 79.5% via bioaugmentation with estrogen-degrading strains. However, D’Alessio et al. (2015) found that removal up to 92% was achieved for E2 using a single-media SSF unit ripened with stream water or a mixture of stream water and 1% primary effluent. In addition to SSF, the removal of E2 using BAF and sand-anthracite biofilters was also investigated and a reduction more than 90% was obtained for both filtration systems with empty bed contact times of 10 min and 18 min (Zhang et al., 2017). To date, there is no study being conducted to investigate the removal of estrogens using the GSBF. For any biofiltration setup, their performance was subject to variables such as the media characteristics, water quality of influent, the hydraulic retention time and oxidative pretreatment (Gerrity et al., 2018). Therefore, the optimisation of these parameters needs to be investigated.

The biofiltration process is regarded as a viable technique for removing estrogens because of its low cost and easy maintenance. Moreover, the exemption of other
chemicals used for disinfection or oxidation of pollutants could prevent the extra financial budget and incidence of contamination. In addition, the natural source media materials in the biofilter unit were also helpful for sustainable application in practical cases.

Table 2.5 summarises the techniques used for the estrogen elimination and corresponding advantages and disadvantages.
Table 2. 5 The techniques used for removing estrogens.

<table>
<thead>
<tr>
<th>Estrogens</th>
<th>Techniques</th>
<th>Conclusion</th>
<th>Reference</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1, E2, E3, EE2</td>
<td>GAC, PAC</td>
<td>-All compounds could be removed by over 90% using either GAC or PAC adsorbents</td>
<td>Snyder et al. (2007)</td>
<td>-Cost friendly;</td>
<td>-The disposal and post-treatment of adsorbent waste.</td>
</tr>
<tr>
<td>E1, E2</td>
<td>GAC</td>
<td>-Adsorption constants for E1 (9290 mL g⁻¹) and E2 (12200 mL g⁻¹) were achieved using GAC.</td>
<td>Zhang and Zhou (2005)</td>
<td>-Less energy input;</td>
<td></td>
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<tr>
<td>EE2</td>
<td>Single-walled carbon nanotubes</td>
<td>-The removal of EE2 was associated with the initial concentration of EE2 (2-4000 μg L⁻¹), the highest removal of 95.5% was obtained when the EE2 was dosed at 2 μg L⁻¹.</td>
<td>Bisesi et al. (2017)</td>
<td>-Being free of chemical reagent;</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>Polymer-based spherical activated carbon</td>
<td>-The removal of compounds was kept higher than 90% when the adsorbent was dosed at 1 g L(^{-1}), initial concentration of estrogens was 100 ng L(^{-1}), and the pH value was between 2 and 12.</td>
<td>Tagliavini et al. (2017)</td>
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<tr>
<td>E2, EE2</td>
<td>Graphene material, multi-walled carbon nanotubes, Single-walled carbon nanotubes, PAC, GAC, biochars</td>
<td>-The highest sorption capacity for estrogens was achieved by PAC with the absence or presence of NOM, though the NOM preloading led to the reduction of adsorption capacity by nearly 56%.</td>
<td>Jiang et al. (2017)</td>
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<tr>
<td>Membrane filtration</td>
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<tr>
<td><strong>E1, E2,E3, EE2</strong></td>
<td>UF, NF</td>
<td>-For E1, about 45% retention was achieved by both NF and UF; for E2, less than 5% removal by UF and 40% removal by NF was observed; for E3, retention by NF reached to 38% while negligible removal was achieved by UF; for EE2, retention percentages by NF and UF were 60% and 30%, respectively.</td>
<td>Yoon et al. (2007)</td>
<td>-Efficient retention for pollutants; -Exemption of chemical pre-treatment; -Less by products.</td>
<td>-High energy input; -Irreversible membrane contamination.</td>
</tr>
<tr>
<td><strong>E1, E2, E3, EE2</strong></td>
<td>NF, coagulation-NF</td>
<td>-For all chosen estrogens, the removal percentages greater than 63% and 81.5% were respectively realised by NF and coagulation-NF techniques.</td>
<td>Bodzek and Dudziak (2006)</td>
<td></td>
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<tr>
<td>E1, E2, E3, EE2</td>
<td>NF/RO membrane</td>
<td>-The rejection of selected estrogens was higher than 95% using NF/RO membranes.</td>
<td>Alturki et al. (2010)</td>
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<tr>
<td>E1, E2</td>
<td>NF/RO membrane</td>
<td>-Estrogens E1 and E2 were eliminated by NF/RO membrane with the retention percentage higher than 95%.</td>
<td>Nghiem et al. (2004a)</td>
<td></td>
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</tr>
</tbody>
</table>

**Advance oxidation process**

<p>| E1, E2 | Ozone | -E1 and E2 could be degraded completely after 10 min while the oxidation products were also produced, so that the mineralization efficiency of E1 and E2 only reached to 6.1% and 13.6%. | Lin et al. (2009) | -Rapid and effective removal performance for organic compounds; -Strong bactericide activity; | Be susceptible to natural water matrix; -The formation of by-products with high toxicity; |</p>
<table>
<thead>
<tr>
<th>E1, E2, EE2</th>
<th>Ozone, Ozone-UV, Ozone-H₂O₂</th>
<th>-Almost 100% removal of estrogens were achieved by all three processes. Compared to the single ozonation system, the introduction of UV or H₂O₂ accelerated the removal of E1 and E2 substantially.</th>
<th>Sun et al. (2019)</th>
<th>-Easy to use.</th>
<th>- High cost and energy input.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>Ozone</td>
<td>-Removal efficiency (&gt;99%) for E2 was obtained in solutions with pH 3, 7, and 11. But the estrogenicity was also detected due to the generation of oxidation by-products.</td>
<td>Bila et al. (2007)</td>
<td></td>
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<tr>
<td>E2</td>
<td>Photo-Fenton</td>
<td>-The removal of 40.1% to 98% after 8 h was achieved depending on the different conditions (e.g. pH, amount of H₂O₂, illumination condition)</td>
<td>Yaping and Jiangyong (2008)</td>
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<tr>
<td>Estrogens</td>
<td>Process</td>
<td>Photolysis Details</td>
<td>Ref.</td>
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<tr>
<td>E1, E2</td>
<td>Photolysis</td>
<td>The photolytic degradation of estrogens was greater under the irradiation source with λ=254 nm than that achieved under a high pressure mercury lamp (λ≥365 nm).</td>
<td>Liu and Liu (2004)</td>
<td></td>
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<tr>
<td>E2</td>
<td>Photolysis</td>
<td>Photodegradation of E1 up to 26% was observed under the exposure to simulated sunlight and the presence of 2.0-15.0 mg L⁻¹ dissolved organic carbon (DOC) enhanced the degradation of E2 significantly.</td>
<td>Leech et al. (2009)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1, E2, E3, EE2</td>
<td>UV-LED/TiO₂</td>
<td>Among all selected estrogens, E2 was poorly photodegraded by the UV-LED/TiO₂ process. But the total estrogenicity was reduced remarkably.</td>
<td>Arlos et al. (2016)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1, E2, EE2</td>
<td>UV, UV/TiO₂</td>
<td>-The estrogenic activity results indicated that the introduction of TiO₂ accelerated the degradation rate, being 2.4×10⁻¹ faster times than the single photolysis.</td>
<td>Coleman et al. (2004)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1, E2, E3, EE2</td>
<td>UV, UV/TiO₂</td>
<td>-In comparison with UV photolysis, greater removal of estrogens was realised by the UV/TiO₂ process. Then, the quantum yields of estrogens obtained under the UVC/TiO₂ process was nearly double the quantum yields achieved with UVA/TiO₂ process.</td>
<td>Li Puma et al. (2010)</td>
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</table>

**Biodegradation**

<p>| E1, E2 | AS | -The average removal of 88% for E1 and 74% for E2 were achieved using the AS process. | Johnson et al. (2000) |
| -Sustainable and cost-effective; | -Efficiency of degradation is not always great; |</p>
<table>
<thead>
<tr>
<th>Estrogen(s)</th>
<th>Process</th>
<th>Key Processes</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1, E2, EE2</td>
<td>AS system in STP</td>
<td>-The elimination of E1 and E3 were greater than 98%, EE2 was more recalcitrant with degradation around 90%. Additionally, only 5% of estrogens were removed by the sorption onto AS.</td>
<td>Andersen et al. (2003)</td>
<td>- Being free of chemical reagent; -Less energy input; -Easy to optimise via adjusting factors such as temperature, pH, nutrients, hydraulic conditions and microbial adaption.</td>
</tr>
<tr>
<td>E1, E2</td>
<td>AS</td>
<td>-Estrogens sorbed onto the AS were degraded within 4 h.</td>
<td>Suzuki and Maruyama (2006)</td>
<td></td>
</tr>
<tr>
<td>E1, E2, EE2</td>
<td>AS</td>
<td>-Among studied estrogens, EE2 was the most persistent under both anoxic and aerobic conditions with removal ranging from 5-22%.</td>
<td>Dytczak et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>SSF</td>
<td>-Removal with a range of 11-92% was achieved by two pilot-scale SSF for selected estrogens when the feeding concentration was at 50 μg L⁻¹. The</td>
<td>D’Alessio et al. (2015)</td>
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<td></td>
<td></td>
<td>-Process is time consuming; -Post-treatment of sludge is needed when the AS technique is applied.</td>
</tr>
</tbody>
</table>
presence of feeding organic chemicals was inferred to interfere with the microbial community in *schmutzdecke*.

<table>
<thead>
<tr>
<th>Process</th>
<th>Method</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1, E2, E3</td>
<td>SSF</td>
<td>- Compared to the negligible removal in nonaugmented SSF, the reduction of estrogens was increased substantially via bioaugmentation with estrogen-degrading strains, being up to 79.5%.</td>
<td>Haig et al. (2016)</td>
</tr>
<tr>
<td>E2</td>
<td>BAF with/without ozone, sand-anthracite BF with/without ozone</td>
<td>- Removal of E2 was constantly over 90% in all studied processes with EBCT operated at both 10 min and 18 min.</td>
<td>Zhang et al. (2017)</td>
</tr>
</tbody>
</table>
2.6 The combined UV photolysis pretreatment and biofiltration system

The hybrid processes coupling UV photolysis with biofiltration was assumed to enhance the removal of targeted compounds more than the mono process. The early study applied a UV-biofiltration system for the purification of air containing aromatic volatile organic compounds toluene and oxylene, the combined system showed greater removal than the sum of that contributed by the individual process (Moussavi and Mohseni, 2007).

UV photolysis is an environmentally friendly technique to break organic compounds with the oxidation process, the generated intermediates were found to be more biodegradable than the original form (Mohseni and Zhao, 2006), and might also be more harmful than the parent compounds (Cedat et al., 2016). For the biofiltration process, the removal of compounds being recalcitrant to biodegradation is difficult, however via the integration of UV photolysis and biofiltration, the post-treatment of biofiltration could avoid the direct discharge of the harmful byproducts and the photolysis pre-treatment process promoted the sequential biodegradation of the compound residues.

To our knowledge, there is no systematic investigation carried out for the removal of estrogens in water using the hybrid UV photolysis and biofiltration setup. So, factors such as the amount of GAC media and the hydraulic retention time that may be influential to system performance would be studied specifically.

2.7 Research gaps

This project aims to develop an efficient method for the effective removal of estrogens in water by a hybrid method of photolysis and biofiltration. In addition to photodegrading some estrogens into small pieces, photolysis is also expected to
minimize the interference in water matrix for the subsequent sand filtration process. The subsequent biofiltration may be capable of decreasing the risk of byproducts of AOPs via biodegradation progress, and achieving removal of estrogens thoroughly. Most current treatment methods used to reduce estrogens have proved to be risky due to the remaining estrogenic byproducts, very limited studies suggested adding the post-treatment for mitigating estrogenic activity.

To date, there has not yet been research about removing estrogens by combing photolysis and a biofiltration system, and the removal mechanism of estrogen in a hybrid system is also unclear. Thus, studies conducted to find the answers to these questions were elucidated in the following several chapters.
Chapter 3 General Methodology

3.1 Introduction

In this chapter, the general methodology employed for conducting the whole research is described in detail. It firstly introduces the experimental consumables (e.g. reagents) and equipment used in the studies, as well as their supplier information and corresponding models. Moreover, the physicochemical properties and solution preparation method of targeted compounds are also summarised.

Subsequently, the surface morphology of GAC and sand was captured using the scanning electron microscope. Considering the high adsorption capacity and special porous structure of GAC, the methodology for determining the pore distribution, specific surface area, pH, zero point of charge and functional groups are also included.

Then, following the introduction of the photolysis reactor and biofiltration system in respect of dimension, materials and configuration, the general methodologies for a photolysis experiment (e.g. characterisation of radiance) and biofiltration experiments (e.g. media size distribution, hydraulic retention time and sampling strategies) are presented. Additionally, for investigating the performance of a continuous biofiltration system or photolysis-biofiltration system, the analysis for water quality parameters, microbial characterisation (e.g. total coliforms and *Escherichia coli*), chlorophyll-a, biomass and estrogenic activity was carried out.

In addition, the complementary methodology for investigating the simultaneous detection of free and conjugated estrogens using LC-MS coupling with SPE preconcentration, the adsorption of estrogens using GAC particles, the UV photolysis of estrogens in aqueous solution, the removal of free and conjugated estrogens using
the combined photolysis and biofiltration process is described in Chapters 4, 5, 6, and 7, respectively.

3.2 Reagents and equipment

The analytical grade of four free estrogens estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethyl estradiol (EE2) with purity higher than 98% and estrone-3-sulfate sodium salt (E1-3S) were all purchased from Sigma-Aldrich (Dorset, UK). The remaining compounds of interest including five estrogen conjugates, estrone-3-glucuronide sodium salt (E1-3G), 17β-estradiol-3-sulfate sodium salt (E2-3S), 17β-estradiol-3-(β-D-glucuronide) sodium salt (E2-3G), 17β-estradiol-17-(β-D-glucuronide) sodium salt (E2-17G), and estriol-3-sulfate sodium salt (E3-3S) were obtained from Santa Cruz Biotechnology (California, US). The physicochemical properties and chemical structures of the above estrogens are presented in Table 2.3.

Ammonium formate with purity over 99.0%, ammonia hydroxide solution for analysis (28%), formic acid (FA) with purity higher than 99.0% and reagent grade of sodium carbonate anhydrous and sodium bicarbonate were purchased from Fisher Scientific (Lancashire, UK). Chromogenic Coliform Agar employed for culturing total coliforms and Escherichia coli (E. coli). 1,2-dimethyl-1H-imidazole-5-sulphonyl chloride (DMIS) was obtained from Apollo Scientific Ltd (Cheshire, UK). Sodium hydroxide (NaOH), sulphuric acid (H2SO4), the hydrochloric acid solution with a concentration of 1 M, and humic acid sodium salt (HA) with technical grade were acquired from Sigma-Aldrich (Dorset, UK).

All reagents, HPLC grade solvents including methanol, acetonitrile, acetone, ethyl acetate and ethanol were ordered from Fisher Scientific (Lancashire, UK). Phosphate buffer saline (PBS) solution was supplied by Gibco (Paisley, UK) via the website of
Thermo Fisher Scientific. Milli-Q water (resistivity 18.2 MΩ cm⁻¹) and deionised water applied in the whole study were produced from PURITE Select Analyst 320 system (CAT. L300275, UK).

The corresponding information of all chemicals is presented in Table 3.1.

Table 3.1 Summaries of chemicals consumables applied in experiments described in this thesis and their suppliers.

<table>
<thead>
<tr>
<th>Experimental consumables</th>
<th>Suppliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone (E1)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>17 β-estradiol (E2)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Estriol (E3)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>17α-ethyl estradiol (EE2)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Estrone-3-sulfate sodium salt (E1-3S)</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Sulphuric acid</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Hydrochloric acid solution</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Humic acid sodium salt</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Estrone-3-glucuronide sodium salt (E1-3G)</td>
<td>Santa Cruz biotechnology</td>
</tr>
<tr>
<td>17 β-estradiol-3-sulfate sodium salt (E2-3S)</td>
<td>Santa Cruz biotechnology</td>
</tr>
<tr>
<td>17β-estradiol-3-(β-D-glucuronide) sodium salt (E2-3G)</td>
<td>Santa Cruz biotechnology</td>
</tr>
<tr>
<td>17 β-estradiol-17-(β-D-glucuronide) sodium salt (E2-17G)</td>
<td>Santa Cruz biotechnology</td>
</tr>
<tr>
<td>Estriol-3-sulfate sodium salt (E3-3S)</td>
<td>Santa Cruz biotechnology</td>
</tr>
<tr>
<td>Ammonium format</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Ammonia hydroxide solution</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Formic acid</td>
<td>Fisher Scientific</td>
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<td>Sodium carbonate anhydrous</td>
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</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Methanol</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Item</td>
<td>Brand</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Acetone</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Chromogenic Coliform Agar</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Phosphate buffer solution</td>
<td>Gibco</td>
</tr>
<tr>
<td>1,2-dimethyl-1H-imidazole-5-sulphonyl chloride (DMIS)</td>
<td>Apollo Scientific Ltd</td>
</tr>
<tr>
<td>Mixed cellulose ester (MCE) gridded sterile membrane filter</td>
<td>Gilson</td>
</tr>
<tr>
<td>(0.45µm, 47mm)</td>
<td></td>
</tr>
<tr>
<td>Polytetrafluoroethylene (PTFE) syringe filter 0.22 µm, 13 mm</td>
<td>Gilson</td>
</tr>
<tr>
<td>Type WCN cellulose acetate Membranes (0.45µm, 47mm)</td>
<td>Whatman ™</td>
</tr>
<tr>
<td>Glass microfiber filters (0.7 µm, 47mm)</td>
<td>Whatman ™</td>
</tr>
<tr>
<td>Oasis HLB cartridge</td>
<td>Waters</td>
</tr>
<tr>
<td>Strata-X cartridge</td>
<td>Phenomenex</td>
</tr>
<tr>
<td>Supel™-Select HLB cartridge</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>SPE-12G column processor</td>
<td>J.T. Baker™</td>
</tr>
<tr>
<td>Granular activated carbon</td>
<td>Chemviron Carbon/ DARCO®</td>
</tr>
<tr>
<td>Fine sand</td>
<td>Soil Lab, CEGE</td>
</tr>
<tr>
<td>Micro BCA™ Protein Assay Kit</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>XenoScreen XL YES</td>
<td>Xenometrix</td>
</tr>
<tr>
<td>Sterile silicon tubing (Bore x Wall, 3.2 x 1 mm)</td>
<td>Scientific Laboratory Supplies</td>
</tr>
<tr>
<td>Fisherbrand™ Silicone Tube (Bore x Wall, 8 x 2 mm)</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Sterile Silicone Precision Pump Tubing (Diameter: 4.8 mm)</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Duran™ Keck Hose Clamps (Diameter: 4.5 mm)</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>AlteVin™ Industrial PVC Tube (3 mm)</td>
<td>Altec</td>
</tr>
<tr>
<td>PhthalateFree® PVC Solva 2 Bridge Tubing, 2.29 mm</td>
<td>Gradko Ltd</td>
</tr>
<tr>
<td>Mohr pinch cocks (spinning width x length, 35 x 70 mm)</td>
<td>VWR™</td>
</tr>
<tr>
<td>Ultrasonic bath S100H</td>
<td>Elmasonic</td>
</tr>
</tbody>
</table>
Measurements of the targeted compounds and characterisation of factors involved in each study were performed with equipment listed in Table 3.2, their utilisation is further described in the following chapters.

Table 3.2 Equipment employed in each study.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Brand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accela Liquid chromatography (LC) tandem Finnigan linear ion-trap mass spectrometry</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>Reacti-Vap™ evaporator</td>
<td>Fisher Scientific</td>
</tr>
<tr>
<td>LC tandem single quadrupole mass spectrometry</td>
<td>Shimadzu 2030C 3D Plus</td>
</tr>
<tr>
<td>Solid-Phase Extraction (SPE) Instrument</td>
<td>Dionex™ AutoTrace™ 280</td>
</tr>
<tr>
<td>Rotary extractor/shaker</td>
<td>Designed in CEGE, UCL</td>
</tr>
<tr>
<td>Total Organic Carbon (TOC) analyser</td>
<td>Shimadzu TOC-L</td>
</tr>
<tr>
<td>Ion chromatography (IC)</td>
<td>Dionex ICS 1100</td>
</tr>
<tr>
<td>Brunauer-Emmett-Teller (BET) machine</td>
<td>Quantachrome®</td>
</tr>
<tr>
<td>Focused Ion Beam (FIB) Microscope</td>
<td>Carl Zeiss XB1540</td>
</tr>
<tr>
<td>Scanning Electron Microscopy</td>
<td>JEOL JSM-6700F</td>
</tr>
<tr>
<td>Turbidimeter</td>
<td>Hach 2100AN</td>
</tr>
<tr>
<td>UV-Vis spectrophotometer</td>
<td>Camspec M550/Agilent Cary 60</td>
</tr>
<tr>
<td>Autoclave</td>
<td>Astell Classic</td>
</tr>
<tr>
<td>pH meter/Conductivity analyser</td>
<td>Mettler Toledo SevenMulti</td>
</tr>
<tr>
<td>Dissolved oxygen (DO) meter</td>
<td>Jenway 9200</td>
</tr>
<tr>
<td>Ultra-pure water machine</td>
<td>Ondeo Purite IS</td>
</tr>
<tr>
<td>Deionised water machine</td>
<td>Ondeo Purite Select</td>
</tr>
<tr>
<td>Lab oven</td>
<td>LTE OP250</td>
</tr>
<tr>
<td>Incubator</td>
<td>Stuart S150</td>
</tr>
<tr>
<td>Light intensity meter</td>
<td>Rectifier SKKH 72/20E</td>
</tr>
<tr>
<td>Lux meter</td>
<td>Apogee MQ-100</td>
</tr>
<tr>
<td>Microplate reader</td>
<td>BioTek</td>
</tr>
</tbody>
</table>
3.3 Solution preparation

Individual standard stock solutions of estrogenic chemicals were prepared by dissolving 1mg of E1, E2, E3, EE2, E1-3G, E2-3G and E3-3S, 2mg of E1-3S and E2-3S, 1.5mg E2-17G in 10 mL methanol with a volumetric flask, getting stock solutions at concentrations of 100~200 mg L\(^{-1}\). Then these stock solutions were stored in a -20 °C freezer. Following the method for checking the absolute peak height of each selected estrogens and estrogen conjugates with LC-MS (X. Xu et al., 2007), all stock solutions were verified to be stable for at least 3 months under this temperature. Single working standard solutions of each estrogen with the concentration of 1 mg L\(^{-1}\)~2 mg L\(^{-1}\) were prepared by diluting the stock solutions with methanol when the study proceeded. In addition, a mixed working standard solution of all targeted compounds at a concentration of 10 mg L\(^{-1}\) was prepared weekly by diluting aliquots of each estrogen stock solution together in methanol. Prepared stock and working standard solutions involving either single estrogen or a mixture of targeted compounds were further used in experiments elucidated in Chapters 4, 5, 6, 7, and 8, and related dilution was performed with methanol or water depending on the demand for the specific experiment.
3.4 Characterisation of GAC particles and sand

3.4.1 Morphology of GAC particles

Two commercially available granular activated carbons (GACs) examined for adsorption capacity for estrogens were provided by Chemviron Carbon and DARCO®. Their surface morphology was investigated using the focused ion beam microscope (FIB-SEM, Carl Zeiss XB1540) at 25 kV from London Centre for Nanotechnology (LCN, Bloomsbury) and scanning electron microscopy (SEM, JSM-6700F) at 15 kV from the Chemistry department of UCL, respectively. The captured three-dimensional images for two types of pristine GAC particles were presented at various magnifications.

The distribution of pore sizes and surface areas of examined pristine GAC materials were characterised using Autosorb-iQ2 automated gas sorption analyser (Quantachrome® Instruments version 3.0) by Ph.D. student Zhuojun Li (CEGE) and Dr. Yuchen Yang (Chemistry Department, UCL). The determination was achieved via measuring adsorption and desorption isotherms using liquid-nitrogen at 77 K following outgassing of samples at 180°C for 24 h in total. The surface area ($S_{BET}$) was reckoned using software AsiQwin based on multipoint Brunauer–Emmett–Teller (BET) analysis corresponding to the relative pressure ($P/P_0$) ranged from 0.01 to 0.1 for GAC purchased from both Chemviron Carbon and DARCO®, as examined in a previous study (Fukuhara et al., 2006). The area of nitrogen molecules was assumed as 0.162 nm$^2$ in this calculation.

Likewise, AsiQwin was applied to evaluate pore volume and pore size distribution for two kinds of GAC using desorption branch based on density functional theory (DFT) (Moore et al., 2001). The total pore volume (TPV) of GAC from Chemviron Carbon was simulated as 0.2413 mL g$^{-1}$ for pores smaller than 416.5 nm (in diameter) at $P/P_0=0.99539$, while it was derived as 0.7778 mL g$^{-1}$ for pores smaller than 358.4 nm.
(in diameter) at \( P/P_0 \) equal to 0.99463 for DARCO® GAC. Then, the mean pore size (\( \text{PS}_m \)) (in diameter) was calculated following the equation from publication (Fukuhara et al., 2006):

\[
\text{PS}_m = 4 \times \frac{\text{TPV}}{S_{\text{BET}}},
\]

(3.1)

The distribution of pore size is demonstrated via the relationship between incremental pore volume \( dV(d) \) (under standard temperature and pressure) and pore width, in which they were defined as \( \text{Y-axis} \) and \( \text{X-axis} \), respectively.

### 3.4.2 Analysis of pH and point of zero charge (PZC) of GAC

Analysis of pH and point of zero charge (\( \text{pH}_{\text{PZC}} \)) for GAC samples were carried out using the method described in the previous study (Reffas et al., 2010). The pH of selected GACs was measured using distilled water suspension (12.5 mL) of 0.5 g adsorbent samples after the step of heating them at 90 °C and then cooling them down to ambient temperature. As defined in previous literature, \( \text{pH}_{\text{PZC}} \) is the pH value at which the net charge on the surface of the adsorbent is equal to 0 (Franz et al., 2000).

According to the pH drift method applied by Reffas et al. (2010), pH of 50 mL NaCl solutions (0.01 mol L\(^{-1}\)) was adjusted as the initial values over 2-12 with 2 increments using \( \text{HCl} \) or \( \text{NaOH} \) (0.1 mol L\(^{-1}\)), then 0.15 g GAC was added and the lids of containers were screwed immediately after degassing with nitrogen gas for 2 hours.

Finally, the prepared samples were put in a rotary shaker with 30 rpm for 48h. The obtained final pH (\( \text{pH}_{\text{final}} \)) value was plotted as \( \text{Y-axis} \) versus corresponding initial pH (\( \text{pH}_{\text{initial}} \)) value as \( \text{X-axis} \), within which the \( \text{pH}_{\text{PZC}} \) was estimated at the value where the \( \text{pH}_{\text{final}} = \text{pH}_{\text{initial}} \) (Beltran et al., 2009). A previous study summarised that a negatively charged feature of adsorbents was discovered when the pH of aqueous solution is over \( \text{pH}_{\text{PZC}} \), contrarily GAC exhibited positively charged (Radovic et al., 1997).
3.4.3 Analysis of functional groups for GAC samples

According to the Boehm titrate method proposed by Boehm et al. (1964) and later standardised by Goertzen et al. (2010), qualification and quantification of classes of oxygen functional groups on the surface of GAC were conducted. The principle of this method is utilising bases with different strengths to neutralise oxides which owns various acidities binding to the boundaries of carbon. Thus, the acidic functional groups (AFG) generally include phenols (Ar–OH), carboxyl (R–COOH), lactonic groups (R–OCO) embracing lactone and lactol rings. Then bases such as NaOH, Na₂CO₃, and NaHCO₃ are capable of reacting with them selectively, in which NaOH possesses the strongest basicity and was presumed to achieve neutralisation of all mentioned acidic functional groups, while Na₂CO₃ was able to neutralise carboxyl and lactonic groups and NaHCO₃ was proved to react with carboxylic acid only (Boehm, 2008).

Firstly, 1.5 g of examined GAC samples were added into 50 mL of base solutions (NaOH, Na₂CO₃ or NaHCO₃) with a concentration of 0.05 M, the mixtures were put in the rotary shaker with 30 rpm for 24 h. Then the mixture was taken out and filtered through 0.45 µm membrane filter (Whatman™) and 10 mL filtrate was collected from each sample. Then, the filtrates from the reaction mixtures with base NaHCO₃ and NaOH were acidified via adding 20 mL HCl at a concentration of 0.05 M, while acidification of the filtrate from the reaction mixture with base Na₂CO₃ was achieved by adding 30 mL HCl at 0.05 M concentration. Afterward, the acidified filtrates were transferred into 50 mL borosilicate glass bottles covering the opening with Parafilm and the nitrogen gas was bubbled into the solution via connecting a pipette tip submerged below the liquid surface for 2 h, and a needle was inserted through Parafilm and held in the headspace as outlet exhaust. The degassed solution was
consequently back-titrated with NaOH solution (0.05 M) under continual degasification with N₂, mitigating errors resulted from the dissolution of CO₂ (Goertzen et al., 2010). The endpoint of pH=7 was determined using the pH meter, at which titration volume was recorded. The quantification of acidic functional groups was acquired by the equations (Goertzen et al., 2010):

\[ C_{HCl}V_{HCl} = C_{NaOH}V_{NaOH} + \left( \frac{n_{HCl}}{n_B} C_B V_B - n_{AFG} \right) \frac{V_a}{V_B}, \]  
\[ n_{AFG} = \frac{n_{HCl}}{n_B} C_B V_B - (C_{HCl}V_{HCl} - C_{NaOH}V_{NaOH}) \frac{V_B}{V_a}, \]  

where \( C_{HCl} \) (M) and \( V_{HCl} \) (mL) are the concentration and volume of HCl used to acidify filtrates, then \( C_{NaOH} \) and \( V_{NaOH} \) are the concentration and volume of NaOH used for the neutralisation in back-titrate. Additionally, \( C_B \) and \( V_B \) are the concentration and volume of the alkaline solutions used to add the carbon samples, while \( V_a \) refers to the volume of filtrate collected from \( V_B \), namely 10 mL in this study. Furthermore, \( \frac{n_{HCl}}{n_B} \) is defined as the molar ratio of hydrochloric acid versus selected base in neutralisation reaction, thus in this study \( \frac{n_{HCl}}{n_B} \) is equal to 2 for the base of NaN₂CO₃ and \( \frac{n_{HCl}}{n_B} \) is 1 for bases of both NaNHCO₃ and NaOH, respectively. Finally, the content of individual acidic functional group—\( n_{AFG} \) (meq), was obtained according to the volume of recorded titrant and differences of results derived within various basic solutions.

Likewise, total basic functional groups (TBFG) were measured by using NaOH (0.05 M) to titrate filtrated 10 mL aliquots taken from the mixture of 50 mL HCl solution (0.05 M) and 1.5 g GAC in the rotary extractor for 24 h. The Calculation was conducted using the following equations:

\[ C_{NaOH}V_{NaOH} = (C_A V_A - n_{TBFG}) \frac{V_B}{V_A}, \]
\[ n_{TBFG} = C_A V_A - C_{NaOH} V_{NaOH} \frac{V_A}{V_b}, \]  
\[ (3.5) \]

Where \( C_A \) (M) and \( V_A \) (mL) are concentration and volume of acidic base mixing with GAC samples; \( V_b \) refers to 10 here, then \( C_{NaOH} \) and \( V_{NaOH} \) are concentration and volume of NaOH solution used in the back titration process. Thus, quantification of total basic functional groups—\( n_{TBFG} \) (meq), was carried out using the known parameters.

### 3.4.4 Morphology of fine sand particles

Fine silica sand particles taken from the Soil’s Laboratory of Civil, Environmental and Geomatic Engineering Department (CEGE) of UCL (UCL) were cleaned with distilled water and dried in the oven at 105 °C before use. Morphology of clean fine sand particles was captured using focused ion beam microscope (FIB-SEM, Carl Zeiss XB1540) at 25 kV from the London Centre for Nanotechnology (LCN, Bloomsbury) and the sample particle was analysed at different magnification.

### 3.5 Photoreactor

The photoreactor used for investigating the photolysis of estrogens under direct ultraviolet (UV) light was designed by Kim et al. (2013). The schematic diagram of the photolysis reactor is presented in Figure 3.1. The main part of the photoreactor was made of stainless steel. In the reaction chamber, a UV-C lamp (11W, 240V) with dimensions (diameter: 19 mm, length: 251.8 mm) from Philips (Holland) was installed as the light source of irradiation. The UV-C lamp usually emits light with a wavelength
range of 200-300 nm, in this study, the monochromatic radiation at the wavelength of 253.7 nm was emitted by the used UV-C lamp.

Figure 3. 1 Schematic diagram of photoreactor.

The light intensity in the reactor was determined using a radiometer (Apogee MQ-100, USA), getting a mean photon flux value of 65.87 µmol m⁻² s⁻¹ (21.5 W m⁻²) via averaging the photon flux measured at the surface of the quartz sleeve and the internal wall of the photoreactor. The rubber stopper, which tightly matches the top opening of the reaction chamber, allows to hold the UV lamp and its cover layer of quartz sleeve to be held vertically, protecting the lamp from contacting the solution directly. The reaction chamber was embedded in a cooling-water jacket, and the ongoing cooling water inside is aimed at preventing heat impact induced by the UV lamp and keeping solutions around ambient temperature during the photolysis reaction. For achieving equilibrium of light intensity, the UV lamp was pre-operated for at least 15 minutes before experiments (Kim et al., 2013).

In order to evaluate the impact of the flow mode of fluid on the photolysis performance, the photoreactor was selectively connected or disconnected with the feeding tank
coupled with a peristaltic pump (Watson Marlow 501U), the valves on the inlet and outlet of the reaction chamber were correspondingly controlled and then made available to feed contaminant-containing solutions with continuous flow mode (flow rates of 15, 30, and 45 mL min\(^{-1}\) were tested) from the inlet to the outlet of the chamber or to add the fixed amount of solution from the top opening of the reaction chamber directly for investigating the photolysis performance under stationary mode. The detailed configuration of the photoreactor was described in the experiment design parts of Chapters 6 and 7.

3.6 Biofiltration system

3.6.1 Design of bench-scale system

Investigation into the performance of a gravity biofiltration system on the removal of estrogens was conducted in the Environmental Engineering Laboratory of UCL, at which biofiltration units were wrapped for preventing visible light from the ambient environment and algae growth.

The dimension of this system was designed at laboratory-scale, and then eight polymethyl methacrylate columns with a length of 700 mm, inner diameter (ID) of 38 mm (namely cross-sectional area=0.000361 m\(^2\)) as well as the wall thickness of 3 mm were installed separately and fixed using an 8-channel column holder which was made of polyvinyl chloride (PVC). For sampling media materials packed in the columns, three small stainless steel tubes with an ID of 4 mm were individually embedded into the centre of the columns in a vertical direction, forming sampling ports sitting at a depth of 400 mm, 235 mm and 70 mm along the column wall to the bottom of each column. These sampling ports were connected with 8 cm elastic silicon tubing (3.2 mm ID, Scientific Laboratory Supplies) and two of them were stopped using Mohr pinch cocks (VWR\textsuperscript{TM}) when sampling was suspended, while the remaining one was
connected with PVC tubing via a tubing adapter. The piezometer was used to
determine head loss of the filter bed along operation time via the difference of water
level between the filter and piezometer. An overflow port (9 mm ID) was 50 mm below
the top opening of the column and overflow was sent back to the feeding reservoir via
silicone tubing (8 mm ID), which allowed for constant water level above the media bed.
Additionally, the outlet of effluent with 5 mm of ID was set at the bottom of the columns
and connected with silicone tubing (4.8 mm ID) coupled with a hose clamp for further
adjustment flow rate and collection of treated water. The frontal and lateral
configurations of columns were presented in Figure 3.2. During the operation period
of the filtration system, eight columns were fed source water containing targeted
contaminants using a peristaltic pump (Watson Marlow 520S) with multi-channel pump
head from the feeding reservoir, via a connection system comprising of PVC tubing
(3mm ID) and two-bridge pump tubing (2.29 mm ID).

3.6.2 Media in biofiltration columns

In biofiltration columns, the effective filter bed consisted of merely fine sand or sand-
GAC dual media in the BF layout. The grain analysis of selected fine sand and GAC
was conducted using the ISO sieve set (1.68 mm, 1.18 mm, 1 mm, 0.6 mm, 0.425
mm, 0.3 mm, 0.212 mm, 0.15 mm, 0.063 mm and base) following method ASTM C136.
The sieve set was stacked using the base as the bottom and then the sieve pan was
set in the order from smallest to largest upwardly. After the vibration using the
automatic shaker for 15 min, the particle samples (sand or GAC) retained on each
sieve were transferred and weighed using the scale, getting the weight of each fraction
corresponded to the specific sieve class given above. Accordingly, the accumulative
percentage (by weight) of media samples passing through each sieve class was
calculated. Consequently, the grain size distribution could be further analysed via
graphing the percentage passing data (%) for each given sieve against the grain size (mm) on the x-axis with a reversed logarithmic scale.

Figure 3. Configuration of biofiltration columns. i) front view of the columns and holder, and ii) lateral view of the column. a) overflow port; b) media sampling ports; c) port connected with piezometer; d) drainage outlet.

In addition, there was a 5 cm supporting layer consisting of gravel within grain size range 0.424-2.87 mm on a mesh positioned at the bottom of each column, forming the underdrain to the outlet. Medium materials were washed and soaked in deionised water before being introduced into each unit, wherein GAC was left in water for at least 24 h to expel air in the porous structure. Subsequently, 30 mL of deionised water was pre-loaded into columns and wet media were then inserted for preventing the formation of air bubbles. Detailed composition of media in the biofiltration system is described in Chapter 7.
3.6.3 Hydraulic retention time

Continuous mode of biofiltration has been designated as a plug flow reactor in a previous study (Young-Rojanschi and Madramootoo, 2014, Terin and Sabogal-Paz, 2019). In this study, the residence time distribution (RTD) of fluid within each filter unit was also assessed for confirming flow conditions through media bed.

Prior to the maturation and operation of the biofiltration system for removing estrogens in source water, the tracer test for all types of media-packed filters at a given flow rate of 1 mL min$^{-1}$ (or hydraulic loading rate at 0.053 m h$^{-1}$) was carried out, where the addition of NaCl in dechlorinated tap water was applied as feeding water with conductivity of 1509 µS cm$^{-1}$ to charge into well-packed filters which were initially filled with dechlorinated tap water over the media bed. The variation of conductivity was monitored by collecting 5 mL portions of treated fluid from the outlet of filters every 10 min, and then quantified using a conductivity probe (Mettler Toledo SevenMulti). The hydraulic retention time was estimated by the result expressed by the variation of conductivity versus time (min) so that collection of treated water samples could be done accordingly.

For checking the effect of flow rate on RTD in the filtration system, a tracer test was conducted by holding a constant flow rate for surrogate filters (fine sand bed and 8 cm GAC sandwich bed) at 2 mL min$^{-1}$ (or hydraulic loading rate at 0.1 m h$^{-1}$). In the light of variation of the conductivity and portions of pore volume the liquid travelled, the flow pattern of fluid in the designed filters could be indicated. Therefore, pore volume was derived from measurement of water volume which just filled the filter bed, including the underdrain gravel, completely.
3.6.4 Biofiltration experiments

Prior to investigating the performance of each biofiltration unit, surface water sampled from a lake of Regent's Park (London, UK) was introduced into assembled filters for achieving biological ripening of units. The sampling point was presented in Figure 3.3.

Figure 3.3 Map of the surface water sampling point: 51°31'27.9"N 0°09'13.7"W—a lake of Regent’s Park, London, UK (From Google satellite).

Approximately four weeks would be required for the maturation process, at least 99% removal of *Escherichia coli* (*E.coli*) and total coliform should be observed for all filters at this time (D’Alessio et al., 2015). After the maturation, the schmutzdecke remained undisturbed and unstressed throughout the operation of biofiltration units.

Over the operation period, the two-bridge peristaltic tubing applied for transporting feeding water was replaced monthly to prevent breakage from abrasion with the pump gear, which potentially reduced the risk to drain filters and further decreased a negative impact on the performance of studied filters. Apart from that, PVC and silicone tubing connecting each apparatus of the treatment system were renewed once the build-up of biofilm attached to the tubing wall was discovered, as the biofilm likely
sloughed when it developed to a specific thickness and then flowed into filters along with fluid, covering the indigenous \textit{schmutzdecke} layer. A more detailed set-up of slow sand filter, GAC-sand sandwich filter and their further combination with UV photolysis pre-treatment is described in Chapter 7.

3.6.5 Sampling strategies

During the maturation stage, only influent and effluent of filters with a volume of 200 mL were sampled weekly for characterising the water quality parameters including turbidity, \textit{UV}_{254}, dissolved oxygen (DO), conductivity, pH, dissolved organic carbon (DOC), ion chromatography (IC), and coliforms, whereas the photolysis reactor was suspended here.

Over the consecutive period of operation, dosed natural surface water was introduced into the whole system, 200 mL influent and effluent water samples of both filters and photolysis reactor were collected on a weekly basis for the measurement of water quality parameters, microbial analysis, chlorophyll-a and estrogenic potency determination. Another 600 mL influent and effluent for measuring the concentration of targeted estrogens using LC-MS coupling with SPE preconcentration were sampled. It was worth noting that collection of effluent should be implemented conforming to the calculated hydraulic retention time at a constant flow rate after pumping influent, which was defined by the result of the tracer test.

At the end of the filtration experiment, media samples (sand or GAC particles) at different depth of media bed (e.g. 0-1 cm, 14.8-15.2 cm, 31.3-31.7 cm and 47.8-48.2 cm) were taken for profiling the micro protein binding to them, which was aimed at indicating the density of biomass. To compare the development of biological zone, the media at 0-1 cm depth was taken when the ripening of biofilters was achieved. Media
sample (1.5 mL) collected directly from several random locations at the layer adjacent to the *schmutzdecke* using the sterile plastic pipettes was mixed thoroughly, and then this mixture was representative of the top layer media. The underneath media was taken through the sampling ports setting longitudinally along the column. All media samples were stored in sterile Eppendorf with 2 mL capacity, and then excess liquid inside was removed using a syringe coupling with needle for recording the exact wet weight of media sampled.

3.7 General analysis during experiment stage.

3.7.1 Analysis of water parameters;

3.7.1.1 Determination of DOC and IC

To determine the weekly concentration of dissolved organic carbon (DOC) and characterise ions in the sampled influent and effluent, the pre-treatment of these samples with a volume of 15 mL (for DOC) and 5 mL (for IC) were required via filtration through 0.45 µm WCN cellulose acetate membranes (Whatman™, UK). Then the filtrates were transferred to particular vials matching with auto-samplers of total organic carbon (TOC) analyser (Shimadzu TOC-L, Japan) and ion chromatography (IC) (Dionex ICS 1100, US) for further analysis.

For quantifying DOC for targeted samples, the non-purgeable organic carbon (NPOC) method was loaded. In this method, DOC was determined after completely removing total inorganic carbon. Calibration using standard with a concentration of 10 mg L⁻¹ was conducted periodically before infusing our samples. Moreover, in order to get accurate results, a DOC measurement was carried out shortly after samples were prepared.
The application of IC aimed at measuring the concentration of anions (e.g. nitrite, nitrate and phosphate) and cations (ammonia) in samples. For the determination of anions, analytical column IonPac™ AS23 (4 mm diameter, 250 mm length), guard column IonPac™ AG23 (4 mm diameter, 50 mm length) and corresponding suppressor ACR 500-4mm were installed; then the eluent consisted of Na₂CO₃ at a concentration of 4.5 mmol L⁻¹ and NaHCO₃ at a concentration of 0.8 mmol L⁻¹ was loaded at 1 mL min⁻¹. For cations, the analytical column, guard column and suppressor were changed into IonPac™ CS12A (4 mm diameter, 250 mm length), IonPac™ CG12A (4 mm diameter, 50 mm length) and CMMS 300-4 mm, respectively; accordingly, the eluent was also altered to 20 mmol L⁻¹ at the same flow rate for flushing retained ions. The temperature of the column was set as 30 °C in both methods. Standard over concentration 100, 20, 10, 5 and 1 mg L⁻¹ were run prior to the detection of samples.

3.7.1.2 Determination of pH, conductivity, turbidity, UV₂₅₄ and DO

The determination of pH, conductivity, turbidity, absorbance at a wavelength of 254 nm (UV₂₅₄) and dissolved oxygen (DO) followed standards defined by the Federation and Association (2005) and pre-filtration was exempted for these analyses. For these, Metter Toledo SevenMulti, Hach 2100AN IS turbidimeter, UV-Vis spectrophotometer (Camspec M550 or Agilent Cary 60 UV-Vis) and Jenway 9200 were applied, respectively.

3.7.2 Microbial analysis

Abundance of total coliforms and *Escherichia coli* (*E.coli*) in aqueous samples were determined once a week using the EPA method 1604 (EPA, 2002). Samples (including original sample and its dilutions) with one hundred mL volume or blank-deionised water sterilised in autoclave (Astell Classic) were filtered through the mixed cellulose
ester (MCE) gridded sterile membranes (0.45µm, 47mm) purchased from Gilson using a presterilised vacuum filtration apparatus. Then membrane retaining the bacterial was transferred to a petri dish containing the Chromogenic Coliform Agar (Fisher Scientific, UK) using likewise sterilised forceps and incubated at 35℃ for 24 h. Then dark blue sheen colonies (E. coli) and red sheen colonies (coliforms) were separately counted in colony-forming units (CFU) per 100 mL sample.

3.7.3 Chlorophyll-a determination

Determination of chlorophyll-a was conducted weekly followed the study by (Nusch, 1980). A 100 mL aqueous sample was filtered through a 4.7 mm GF/F glass fibre membrane (Whatman™) with a pore size of 0.7 µm under vacuum, then the used membrane was scrolled and inserted into the glass tube (15 mL) covered by aluminium foil. After that, 10 mL 80% ethanol was added into the tube, making the solution come in contact with the retention side of the filter. The well-prepared tube was put in a pre-heated water bath at 75 ℃ (Elmasonic) for 5 min and the tube was put into a cold bath shortly after the homogenisation of the liquid inside. Settlement of extraction in the tube was allowed by refrigerating for 12 h before measuring its absorbance using UV-vis spectrophotometer-UV 2600 (Shimadzu). At the measurement stage, absorbance of extraction and acidified extraction with one drop 0.4 M HCl in cuvette was read at wavelengths of both 665 nm and 750 nm. Finally, the concentration of chlorophyll-a was calculated as follows (Nusch, 1980),

\[ C = 27.9 \times (A_{\text{non-acidified}} - A_{\text{acidified}}) \times \frac{V_{\text{solvent}}}{V_{\text{sample}}} \times P \]  

(3.6)

where C is the concentration of chlorophyll-a (µg L\(^{-1}\)); \( A_{\text{non-acidified}} \) is equal to the absorbance of non-acidified extraction at 665 nm subtracted the absorbance at 750
nm; $A_{\text{acidified}}$ is the absorbance of acidified extraction at 665 nm subtracted the absorbance at 750 nm; $V_{\text{solvent}}$ (mL) and $V_{\text{sample}}$ (L) are the volume of ethanol used and sample filtered for analysis; $P$ (cm) is the length of a cuvette path, being 1 cm in this study.

3.7.4 Biomass determination

At the end of biofiltration operation, concentration of total protein for media samples collected at different depth of filter bed were measured by an adapted method from (Onesios and Bouwer, 2012, Carpenter and Helbling, 2017). A Commercial Micro BCA™ Protein Assay Kit (Fisher Scientific) was used and the result was taken as a surrogate of biomass. The extraction method for biofilm attached to media was modified according to the yields of protein derived from various sonication times and handling procedures. Approximately 0.5 mL of sampled media in Eppendorf tubes were mixed with 1.5 mL sterilised deionised water, then the capped vials were put onto a vortex mixer (Appleton Woods) for 10 s and sonicated for 5, 15, 25, and 35 min, respectively, following centrifuge at 6500 rpm for 5 min, the supernatant liquid was collected for protein measurement. Compared with the performance of continuous detachment handling, Eppendorf tubes containing sampled media were added 0.5 mL sterilised deionised water and then vortexed for 10 s as well, then the 5, 10, 15 and 20 min ultrasonications for this mixture were proceeded thrice; the supernatant was taken out after centrifuge for 5 min and supplementation of fresh 0.5 mL sterilised deionised water was fulfilled each time, finally gathering the supernatant derived from the procedure being repeated three times (the tandem triplication) into one sample for further protein analysis. As shown in Figure 3.4, the yields of protein for GAC and fine sand under each selected condition were demonstrated.
For GAC particles, three extraction times repeated each 15 min (3x15 min) showed outstanding performance, in comparison with other groups of results by 1-way ANOVA and following Turkey’s test, the protein yield obtained from the three extraction times repeated each 10 min (3x10 min) was not significantly different with p>0.05. Furthermore, this method also worked well for sampled fine sand. Therefore, the 3x10 min method was finally chosen.

Figure 3. 4 Detachment of protein from media sample under variant sonication time and procedures: (i) GAC; (ii) fine sand.

3.7.5 Measurement of estrogenic potency

Genetically modified yeast strains (Saccharomyces cerevisiae) applied for estrogen screening were kindly provided by Dr Edwin Routledge (Brunel University, Uxbridge, UK). The estrogenic activity was quantified with the XenoScreen XL YES kit (Xenometrix, Switzerland). The principle of this bioassay was explained by Routledge and Sumpter (1996) and Sohoni and Sumpter (1998). To achieve this purpose, the DNA sequences of human estrogen receptor (hERα) were integrated with chromosome of yeast cells, and the presence of expression plasmid carrying the reporter gene lac-Z accounted for encoding the enzyme β-galactosidase in cells,
forming a β-galactosidase indicator system when they were exposed to compounds owning estrogenic potency. Then, the breakage of cultured yeast cells occurred when they contacted with the lysis reaction mixture containing 2-mercaptopethanol, lacZ lysis buffer, chlorophenol red-b-D-galactopyranoside and lyticase solution. Consequently, the produced β-galactosidase was released and reacted with chlorophenol red-b-D-galactopyranoside involved in mixture, turning the yellow colour of substrate to red. This colorimetric response can be measured by the absorbance at wavelength of 570 nm according to the kit instruction.

After the incubation at 31 °C for 24 h, the frozen recombinant yeast cells grew to a dense culture with a clearly turbid status, then obtained strains were diluted 1:10 in growth medium. The dilution of culture could be stored at 4 °C for several days. Prior to the start of assay, the yeast cells used for screen test were prepared via the dilution of dense culture in growth medium with a ratio of 1:10 followed by the incubation at 31°C on an orbital shaker overnight. The final yeast culture was inspected using a microscope, and the morphology of strains was presented in Figure 3.5. Then the detailed procedures including preparation of sample, test medium, assay plate and lysis buffer were referred to the kit instruction.
The obtained data was processed according to the equation provided in the manual of XenoScreen XL YES kit. The growth factor (G) and induction ratio (I_R) used to indicate the cell viability and agonistic activities were calculated in the following equations:

\[
\text{Growth factor (G)} = \frac{A_{690,S}}{A_{690,C}}, \tag{3.7}
\]

\[
\text{Induction ratio (I_R)} = \frac{1}{G} \times \frac{OD_{570,S} - OD_{690,S}}{OD_{570,C} - OD_{690,C}}, \tag{3.8}
\]

where \(A_{690,S}\) is the absorbance of sample at 690 nm prior to the addition of lysis buffer; \(A_{690,C}\) is the absorbance of solvent control at 690 nm prior to the addition of lysis buffer; \(OD_{570,S}\) and \(OD_{570,C}\) are the absorbance of sample and solvent control at 570 nm after lysis, respectively; and the \(OD_{690,S}\) and \(OD_{690,C}\) are the absorbance of sample and solvent control at 690 nm after lysis, respectively. Here, before adding the lysis mixture, the growth status of yeast cell was evaluated by the absorbance at wavelength of 690 nm. The colour development of chlorophenol red-b-D-galactopyranoside was quantified at 570 nm. Considering the impacts of diffraction induced by cells and debris, the net values obtained via subtracting absorbance at 690 nm from absorbance at 570 nm.
nm was used for the calculation of agonistic activity. The cytotoxic effect of samples was assessed using the standard curve via plotting the induction ratio against the different concentrations ($3.13 \times 10^{-12}$–$3.13 \times 10^{-7}$ M) of the positive control E2 on an x axis. Then the relative estrogenic activity ($EA/EA_0$) derived from the studied systems could be achieved via equation:

$$\frac{EA}{EA_0} = \frac{I_R, \text{effluent}}{I_R, \text{influent}} \quad (3.9)$$

Where $I_R, \text{effluent}$ and $I_R, \text{influent}$ is the induction ratio of effluent and influent samples of examined treatment system.
Chapter 4 Profiling estrogens using liquid chromatography-mass spectrometry coupled with optimisation of solid phase extraction

4.1 Introduction

Given the properties of inherently poor volatility and weak ionisation, direct detection using gas chromatography tandem mass spectrometry (GC-MS) and liquid chromatography tandem mass spectrometry (LC-MS) for free estrogens is challenging, especially in complicated matrix, signals of compounds are likely suppressed (Li and Franke, 2015, Nie et al., 2009).

As another alternative, derivatization is a common pretreatment method which alters moieties of targeted compounds and enables their ionisation intensity, augmenting signal response of instruments. To date, dansyl chloride is the most common derivatization reagent to esterify estrogens estrone (E1), 17β-estradiol (E2), estriol (E3) and 17α-ethinylestradiol (EE2) (Anari et al., 2002, Nelson et al., 2004, Lin et al., 2007). In the past decades, some novel reagents pyridine-3-sulfonyl (Xu and Spink, 2008), Girard P (Rangiah et al., 2011), N-methyl pyridinium-3-sulfonyl (Wang et al., 2016b, Wang et al., 2015), 4-(dimethylamino)-benzoic acid (Dai et al., 2012) and 1, 2-Dimethylimidazole-4-sulfonyl chloride (Xu and Spink, 2007) are also introduced to enhance ionisation of free estrogens and increase signal of mass spectrometry. However, to our knowledge, very limited research on simultaneous detection of free estrogens and their conjugates including some structural isomers under derivatization pretreatment process has been investigated.

This chapter is aimed at developing a derivatization-based method for monitoring free and conjugated estrogens using LC-MS following optimisation of solid phase extraction (SPE) procedure, and then validating the method using natural surface
water, which allowed the quantification of targeted free and conjugated estrogens in the experiments described in Chapters 5, 6, and 7.

The established method has been published and the relevant information is as follows:


4.2. Materials and Methods

4.2.1 Materials

For evaluating retention capability of SPE cartridges, polymeric reversed sorbent Oasis HLB (Waters, Hertfordshire, UK), Strata-X (Phenomenex, Cheshire, UK) and Supel™-Select HLB (Sigma-Aldrich, Dorset, UK), were compared by the recovery of selected compounds, their characteristics were listed in Table 4.1.

Table 4. 1 Characteristics of cartridges used to optimise recovery of estrogens.

<table>
<thead>
<tr>
<th>Cartridges</th>
<th>Description</th>
<th>Sorbent particle size (µm)</th>
<th>Sorbent weight (mg)</th>
<th>Syringe barrel size (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oasis HLB</td>
<td>polymeric reversed-phase sorbent</td>
<td>30-60</td>
<td>200</td>
<td>6</td>
</tr>
<tr>
<td>Strata-X</td>
<td>Polymeric reversed-phase sorbent</td>
<td>33-100</td>
<td>200</td>
<td>6</td>
</tr>
<tr>
<td>Supel™-Select HLB</td>
<td>Hydrophilic modified styrene polymeric reversed-phase sorbent for polar and hydrophilic compounds</td>
<td>50-70</td>
<td>200</td>
<td>6</td>
</tr>
</tbody>
</table>

Individual and mixed working standard solutions of estrogenic chemicals employed for development and validation of the determination method were prepared following
contents summarised in Section 3.3 and could be stored at -5°C prior to the start of the experiment. The working standard solutions were diluted with aliquots of mobile phase to attain a few concentration points for the calibration curve.

4.2.2 Sample extraction and derivatization

Milli-Q water spiked with multi-level concentrations of estrogen was used to develop the solid phase extraction method. The recovery behaviour was investigated by extracting 10 mL spiked Milli-Q water at a concentration of 1 µg L⁻¹, the value was defined as the ratio between the peak area of extracted samples derived from chromatographic analysis and that of standard solution having a concentration of 10 µg L⁻¹ after infusing into the instrument.

Prior to SPE pre-treatment, pH of water samples was adjusted to ~7 with 0.1 M NaOH or H₂SO₄ solution and then filtered through 0.45µm WCN cellulose acetate membrane (Whatman™, Maidstone, UK). Cartridges held by J.T.Baker® SPE-12G processor were conditioned with 6 mL MeOH and 6 mL Milli-Q water first to activate sorbent. After loading the water samples using Automated Solid-Phase Extraction (Fisher Scientific, Lancashire, UK) at 5 mL min⁻¹, waiting for the equilibrium between analytes and sorbents for 15 min, the SPE cartridge was rinsed with 8 mL washing solution for removing extra interferences and was absolutely dried with air vacuum pump for 1 h. Following elution by 8 mL selected solvent, the elute collected in glass conical tube was evaporated to dryness under a gentle nitrogen gas flow.

After that, the vial containing dry residue was added into buffer solution and 1 mg mL⁻¹ 1,2-dimethyl-1H-imidazole-5-sulphonyl chloride (DMIS) dissolved in acetone with the
same volume. Afterwards, the mixture was put on a vortex mixer for 2 min, and then heated in a water bath to initiate the derivatisation reaction. Here, conditions such as 50 mM derivatisation buffer (ammonium formate and sodium bicarbonate system) with a pH value of 10.5, amount of DMIS used for reacting with estrogens (30, 50, 75, 100 µL), temperature applied for getting the most yield of derivatives (15, 30, 45, 60 and 75 °C) and reaction time (10, 15, 30, 45 and 60 min) were optimised, respectively. The ammonium formate buffer was prepared via dissolving ammonium formate (NH₄HCO₂) in deionised water first and adjusting its pH using ammonia or formic acid. The sodium bicarbonate system was prepared via dissolving 4.2 g anhydrous sodium bicarbonate (NaHCO₃) and 5.3 g sodium carbonate (Na₂CO₃) solid in 1000 mL deionised water respectively and taking 71 mL sodium carbonate solution and 100 mL sodium bicarbonate solution to mix thoroughly. The pH value of 10.5 is within the buffer range owned by these two buffer systems.

After the post-reaction mixture cooled down to ambient temperature, both derivative and non-derivative estrogens were extracted with 2mL of n-hexane twice to remove excessive salt and interferences brought by the derivatization procedure. Then, the organic layer was transferred out and dried under nitrogen gas again, the dry residues were reconstituted in 1 mL of mobile phase (acetonitrile with 0.1% formic acid) waiting for analysis. Prior to infusing into LC-MS, all samples were filtered through 0.22 µm PTFE syringe filter (Gilson, Dunstable, UK).
4.2.3 LC-MS optimization

The capillary LC-MS\textsuperscript{n} analysis was established for derivatised- and conjugated-estrogens using an Accela liquid chromatography system (Thermo Fisher Scientific, Lancashire, UK) coupled to a Finnigan linear ion trap (LTQ) mass spectrometer. 10 µL of sample was injected through 20 µL-loop to a Hypersil GOLD C\textsubscript{18} capillary column (100 mm × 2.1 mm i.d., particle size: 1.9 µm, Thermo Fisher Scientific, Lancashire, UK). Mobile phases were (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid. The flow rate was 180 µL min\textsuperscript{−1}. Gradient programme was as follows: the ratios of solvent A and B were linearly changed to 20% and 80% respectively within 10 min from initial 70% A and 30% B, then solvent B further went up to 90% in the next 8 min. Following a 7 min hold at 10% A and 90% B, a gradient program was run to an initial gradient condition within 1 min and maintained for 5 min. The LC column was kept at the ambient temperature through the LC-MS analysis. The LC effluent was directed into an electrospray ionisation (ESI) source of a LTQ mass spectrometer. The mass spectrometer was operated in MS\textsuperscript{n} mode to monitor analytes. Nitrogen was used as ESI nebulision and as drying, whereas argon as a collision gas. The ESI parameters were set as: capillary temperature 280°C, capillary voltage 33V; ion source voltage 4.5 kV; sheath gas flow 40 L min\textsuperscript{−1}; Aux gas flow, 10 L min\textsuperscript{−1}; and drying gas flow, 15 L min\textsuperscript{−1}. The optimal tuning conditions were obtained by a direct infusion of individual working standard solution prepared at 1 mg L\textsuperscript{−1} with an automatic syringe pump; the sample was injected into the system at a flow rate of 15 µL min\textsuperscript{−1}. The LTQ mass spectrometer was set at ESI Full scan mode at \textit{m}/\textit{z} of 90-750 followed by MS/MS for each estrogen at collision energies 30-45%, representative fragment ions from their dissociation patterns were recorded and summarised in Table 2.
LC-MS analysis were performed to establish the retention times and ESI mass spectra for each analyte. For the quantification of free estrogens-DMIS and E2-17G-DMIS derived from the derivatisation reaction, the ion trap (LTQ) mass spectrometer was set in positive (ESI+) mode with an ESI full scan to measure precursor ions and then MS² event of each precursor [M+H]+ ion with an isolation width of 2 m/z. In contrast, negative (ESI-) mode was employed for monitoring deprotonated molecular ion [M-H]- of authentic conjugated estrogens at full scan mode, excluding E2-17G. Subsequently, events of MS² spectra on these five precursor ions were also created upon chosen collision energies.

The Xcalibur software (Thermo Fisher Scientific) was used for data processing. Specifically, reconstructed ion chromatogram (RIC) was constructed for describing the intensity of target molecules with a given m/z value.

Table 4. 2 Precursor ions and fragment ions of targeted unconjugated and conjugated estrogens under selected collision energy.

<table>
<thead>
<tr>
<th>Estrogens</th>
<th>Molecular Weight (Da)</th>
<th>Precursor ions m/z</th>
<th>Collision energy (%)</th>
<th>Representative MS² fragment ions, m/z (% relative abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-DMIS</td>
<td>428</td>
<td>429</td>
<td>45</td>
<td>365 (100), 161 (33), 270 (8), 251 (6)</td>
</tr>
<tr>
<td>E2-DMIS</td>
<td>430</td>
<td>431</td>
<td>45</td>
<td>367 (100), 253 (46), 161 (27), 272 (13)</td>
</tr>
<tr>
<td>E3-DMIS</td>
<td>446</td>
<td>447</td>
<td>45</td>
<td>383 (100), 251 (35), 161 (24), 269 (11), 288 (10)</td>
</tr>
<tr>
<td>EE2-DMIS</td>
<td>454</td>
<td>455</td>
<td>45</td>
<td>391 (100), 161 (18), 277 (7), 296 (2)</td>
</tr>
<tr>
<td>E2-17G-DMIS</td>
<td>606</td>
<td>607</td>
<td>35</td>
<td>431 (100), 413 (62), 253 (4)</td>
</tr>
</tbody>
</table>
### 4.2.4 Method validation

Method developed in this study was validated subject to the guidance of the Food and Drug Administration (FDA) (Food and Administration, 2016) by evaluating the linearity regression of analytes, limit of detection and quantification with LC-MS, inter-and intra-precision for analysis, matrix effect, and finally recovery performance of established SPE method. Surface water collected from a lake of the Regent’s Park was employed to conduct the validation tests.

Quantification of estrogens was proceeded by the standard addition method using matrix-matched calibration, which was prepared by adding aliquots of estrogen standard solution over a wide concentration spanning from individual instrument quantification limit (IQL) to 1000 µg L⁻¹ into the extraction of 1000 mL natural surface water. The linearity was then evaluated over the chosen concentration points by the correlation coefficient value ($R^2$) given by corresponding calibration curves.

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>E1-3S</td>
<td>350</td>
<td>349</td>
<td>-35</td>
</tr>
<tr>
<td>E1-3G</td>
<td>446</td>
<td>445</td>
<td>-45</td>
</tr>
<tr>
<td>E2-3S</td>
<td>352</td>
<td>351</td>
<td>-40</td>
</tr>
<tr>
<td>E2-3G</td>
<td>448</td>
<td>447</td>
<td>-30</td>
</tr>
<tr>
<td>E3-3S</td>
<td>368</td>
<td>367</td>
<td>-35</td>
</tr>
</tbody>
</table>
According to the literature, the instrument detection limit (IDL) and IQL were experimentally derived as the concentration of analytes when the instrument showed at a signal to noise ratio (S/N) of 3 and 10, respectively (Nie et al., 2009, Ternes et al., 2002), which were established by measuring standards with gradually decreasing concentrations, until getting the closest values which yielded the matching S/N ratios. Then the method detection limit (MDL) and method quantification limit (MQL) were respectively identified using the surface water with spike of estrogens on a given enrichment factor, which were calculated following the equation from (Baker and Kasprzyk-Hordern, 2011):

$$\text{MDL} = \frac{\text{IDL} \times 100}{\text{Re}_{\text{absolute}} \times \text{EF}}$$  \hspace{1cm} (4.1)

Where IDL is the instrument detection limit (ng L$^{-1}$), Re$_{\text{absolute}}$ is the absolute recovery of estrogens (%) in natural surface water matrix, EF is the enrichment factor, specifically being 1000. For the calculation of MQL, IQL was replaced by IDL in Equation 4.1.

For checking repeatability of this analytical method, intra-day precision was obtained by calculating the relative standard deviation (RSD) of samples measured in 6 replicates (n=6) and inter-day precision was assessed by conducting the inter-day procedure among four days (n=4). Both assays used quality control samples (QC) with concentration at 0.1 µg L$^{-1}$ for all chosen compounds.

The presence of matrix effects causes instability of accuracy and reproducibility to the established method, in particular when the ESI source is applied for analysis (Souverain et al., 2004). Thus, to determine matrix effects arising from the surface water, the slope of matrix matched calibration curve was divided by the slope derived
from the neat standard calibration curve, which is expressed as an equation (Pucci et al., 2009):

$$\text{Matrix effect (\%) } = (1 - \frac{S_{\text{matrix}}}{S_{\text{neat}}}) \times 100$$ \hspace{1cm} (4.2),

where $S_{\text{neat}}$ is the calibration curve slope of target compound standards in neat mobile phase, $S_{\text{matrix}}$ is the calibration curve slope corresponding to the same standards spiked into the post-extraction of surface water.

The extraction of the method was validated by triplicate analysis of surface water (1000 mL) with spiked estrogens at three concentration levels of 50, 100, and 1000 ng L$^{-1}$. Non-spiked surface water with the same volume was also extracted in the meantime. The absolute and relative recovery percentages of estrogens were calculated based on the value quantified with the matrix matched calibration and the known addition contents, following Equations 4.3 and 4.4):

$$\text{Re}_{\text{absolute (\%) }} = \frac{(P_{\text{SPE}} - P_{\text{control}})}{(P_{\text{neat standard}})} \times 100\%$$ \hspace{1cm} (4.3)

$$\text{Re}_{\text{relative (\%) }} = \frac{(P_{\text{SPE}} - P_{\text{control}})}{(P_{\text{Matrix standard}} - P_{\text{control}})} \times 100\%$$ \hspace{1cm} (4.4)

Where $\text{Re}$ refers to the recovery of estrogens, $P_{\text{SPE}}$ and $P_{\text{control}}$ are the peak areas of estrogens with a given spiked concentration in surface water and non-spiked control after the extraction procedure, $P_{\text{neat standard}}$ and $P_{\text{Matrix standard}}$ are the peak areas of corresponding standard solutions in neat solvent and in the matrix post-extraction, respectively. It demonstrates the loss of analytes arising from the SPE procedure.
4.3 Results and discussion

4.3.1 Mass Spectra of derivatised estrogens

There was observed poor ESI ionisation of individual E1, E2, E3 and EE2 standard solution prepared at 10 mg L\(^{-1}\) when analytes were directly infused or injected on the column, which is subsequently connected to the LTQ MS or the single quadrupole MS system, which showed neither strong ESI response nor clear chromatographic peaks (Figure A4.1). Previous studies concluded that sensitive response of MS was able to be achieved when the analytes were already ionised in solutions (Van Berkel et al., 1991). As listed in Table 2.3, acid dissociation constant at logarithmic scale (pKa) of E1, E2, E3 and EE2 are 10.25, 10.27, 10.25 and 10.24, respectively (Tomšíková et al., 2012), following equations of calculating percent ionisation for acidic and basic chemicals (Newton and Kluza, 1978):

\[
\text{percent ionisation (\%)} = \frac{100}{1+10^{(pK_a-pH)}} \quad (4.5)
\]

\[
\text{percent ionisation (\%)} = \frac{100}{1+10^{(pH-pK_a)}} \quad (4.6)
\]

Results obtained indicate that ionisation of free estrogens in the range of acid to neutral pH solution is limited. The lack of ionisable groups in unconjugated estrogens, such as carboxy and amine, is the main reason leading to their negligible ionisation (Salvador et al., 2007b). Given less-polar properties for unconjugated estrogens, atmospheric pressure chemical ionisation (APCI) is theoretically more recommendable to obtain a higher signal compared with ESI mode (Robb et al., 2000) while the improvement was found to be minimal in this thesis. Therefore, derivatisation reagent DMIS was employed to incorporate cationic labels into analytes for driving enhancement of detection sensitivity. The nitrogen atom in DMIS made derivatives more ionisable than their parent compounds, which was ascribed to the presence of...
two tertiary amines, owing to easily protonated properties, and the generation of conjugate-acid ammonium ion, owning to low pKa value (Nakao et al., 2019, Anari et al., 2002). From ESI mass spectra and chromatographic result, it is noticeable that the derivatisation reaction by DMIS occurred with estrogens bearing the phenolic hydroxyl group, e.g., four free estrogens and E2-17G, but inactive toward estrogen compounds merely possessing the aliphatic hydroxyl group. The detailed reaction took place as shown in Figure 4.1. This characteristic is consistent with Keski-Rahkonen’s (2015) finding on the reaction between E2 and DMIS. The same selective reactions toward phenolic hydroxyl groups were also observed when using other proposed sulphonyl chloride derivatisation reagents such as dansyl chloride, pyrindine-3-sulphonyl chloride and 1,2-dimethylimidazole-4-sulphonyl chloride (Xu and Spink, 2007, Lampinen-Salomonsson et al., 2006, Salomonsson et al., 2008).

In earlier studies, dansyl chloride was the most common derivatisation reagent for measuring estrogens and their metabolites (Anari et al., 2002, Nelson et al., 2004, Xu et al., 2007, Salvador et al., 2007b). In all of these publications, it was observed that the fragment ion showing critical abundance was 5-(dimethylamino)-naphthalene moiety, which was presumed to be solely from dansyl label dissociated from the parent ions in the light of molecular structure (Anari et al., 2002). Here, it was not diagnosed adequately to monitor compound transition and susceptibility to interferences in complicated matrices. Likewise, the positional isomer of DMIS, 1,2-dimethylimidazole-4-sulfonyl chloride, also allowed the improvement of detection for E2 or other phenolic compounds by forming dimethylimidazole sulfonyl derivatives via a nucleophilic substitution reaction. However the fragment ions at $m/z$ 159, $m/z$ 144 and $m/z$ 96 found dominantly were almost entirely from the dimethylimidazole sulfonyl and dimethylimidazole moieties (Xu and Spink, 2008, Salomonsson et al., 2008).
Nevertheless, DMIS tagged estrogens in this study displayed more peculiar cleavage schemes by generating characteristic fragment ions containing estrogen moiety. The different dissociation schemes might originate from the relative positions of chargeable groups (e.g. amine and imine groups) in precursor ions.

As confirmed by the linear ion trap MS, DMIS derivatives produced stable protonated ions at m/z 429, 431, 447, 455, 607, namely [M+159]+ under positive electrospray (Figure 4.1). Under MS² monitoring of each precursor ion, representative product ions subject to MS/MS were identified and their relative abundance was described in Table 4.2. It was observed that derivatives of unconjugated estrogens exhibited analogous cleavage pathways activated by collision energy. Undergoing dissociation of the precursor ion at m/z 429 for E1, charged fragment ions m/z 365 and m/z 161...
dominated relying on their high relative abundance. The former is assigned as 
$[\text{C}_{23}\text{H}_{31}\text{N}_2\text{O}_2]^+$, arising from the loss of SO$_2$ from E1 derivatives as $[\text{C}_{23}\text{H}_{31}\text{N}_2\text{O}_2]^+$

arising from the loss of SO$_2$ from E1 derivatives and rearrangement consequently. This resembling dissociation mechanism was also elucidated in previous studies analysing DMIS, pyridine-3-sulfonyl chloride and dansyl chloride derivatives of estrogens (Keski-Rahkonen et al., 2015, Xu and Spink, 2008). A fragment ion at $m/z$ 161 corresponding to $[\text{C}_5\text{H}_9\text{N}_2\text{O}_2\text{S}]^+$ with a structure of dimethylimidazole moiety affixing SO$_2$ was noticed, which has been stated before (Keski-Rahkonen et al., 2015, Lampinen Salomonsson et al., 2009). The dissociation ions at $m/z$ 270 and $m/z$ 251 corresponded to a radical E1 cation and a cyclic compound transformed from E1 ion after loss of angular methyl and bond transition in ring moiety, which has a formula of $[\text{C}_{17}\text{H}_{15}\text{O}_2]^+$. The less prominent fragmentations at $m/z$ 337 and $m/z$ 309 were designated as rearranged compounds attaching dimethylimidazole moiety to dissociation intermediates of estrogen nucleus after ring cleavage. The similar cleavage schemes were discussed before (Djerassi et al., 1962, Xu and Spink, 2008). According to the informative spectra, primary suggested fragmentation schemes for E1-DMIS are proposed in Figure 4.2.
Figure 4. 2 MS² spectra of E1-DMIS acquired at the collision energy 45%, and proposed dissociation scheme from its precursor ion at m/z 429.

For the other free estrogens, i.e. E2, E3 and EE2, the dissociation patterns for their derivatives showed analogous to E1. Their mass spectra demonstrated an increment by +2, +18 and +26 to m/z of E1 derivatives and its partial corresponding fragment ions. However, peaks at m/z 429 and m/z 251 were noticed under profiling E3-DMIS at m/z 447 upon collision activation. These two fragment ions were also observed in positive fragmentation spectrum of E1 derivatives. It is assumed that they originated from a loss of H₂O from precursor ion m/z 447 and product ion m/z 269 of E3-DMIS respectively, undergoing rearrangement analogous to pinacol reaction. An ion at m/z 161 was found in fragmentations of all derivatised estrogens, making it possible to confirm that this product ion is indeed from labelling reagent. The dissociation
pathways of the remaining derivatised estrogens were portrayed in Figure A4.2 in the Appendix-Chapter 4.

The application of chemical derivatisation also enables differentiation between positional isomers of E2-3G and E2-17G by its selective reaction with phenolic hydroxyl groups. In their native forms, it is found that their full ESI mass spectra not only show their precursor ions \([M-H]^-\) at \(m/z\) 447 but also product ions \(m/z\) at 271 with high abundance are entirely the same (Reddy et al., 2005a, Kuehnbaum and Britz-Mckibbin, 2011), which makes it difficult to achieve identification of E2-3G and E2-17G especially in complex matrix. Upon derivatisation with DMIS label, a positive ESI mass spectrum shows a peak at \(m/z\) 607 corresponding to E2-17G-DMIS. MS\(^2\) spectra on \(m/z\) 607 shows the fragment ions at \(m/z\) 431 and \(m/z\) 413 for E2-17G-DMIS; the former was originated from the loss of monodehydrated glucuronic acid with 176 Da, which was also demonstrated for the derivatisation of propranolol metabolites (Lampinen Salomonsson et al., 2009); the latter was presumed to form after further dehydration at C-17 position. Less prominent fragment ions at \(m/z\) 543 corresponded to the loss of \(SO_2\) from E2-17G-DMIS as E1-DMIS did. Additionally, E2-containing specific ions at \(m/z\) 367 and \(m/z\) 253 were both discovered as seen in MS\(^2\) spectrum of E2-DMIS.

4.3.2 Mass Spectra of non-derivatised estrogen conjugates

The deprotonated \([M-H]^-\) ions were observed as precursor ions for conjugated forms of estrogens E1-3S, E1-3G, E2-3S, E2-3G and E3-3S. These precursor ions were subjected to MS\(^2\). MS\(^2\) spectra showed their unique fragment ions, which were investigated in order to identify these molecules of interest in samples containing complicated interferences. The first and most abundant fragment ions of both glucuronide and sulphate conjugates were their corresponding free estrogens, E1 (\(m/z\)
269), E2 (m/z 271) and E3 (m/z 287), and this is attributed to the loss of their sulphate/glucuronide group during MS/MS. Additionally, a fragment ion [C_{10}H_{9}O]^{-} at m/z 145 was also present in sulphate estrogens except for E3-3S, the latter has a peak at m/z 80 with a presumed structure of \([SO_3]^{-}\) originated from fragment of sulphate moiety. For E1-3G and E2-3G, the glucuronide ring is brittle and yielded fragment [C_{5}H_{5}O_{3}]^{-} ion at m/z 113. These dissociation patterns were aligned with observations in previous a study (Nieto et al., 2008, Naldi et al., 2016b, Gentili et al., 2002). The typical fragment ions for all underivatized estrogens are summarised in Table 4.2.

Mixed solution containing all estrogens was tested to investigate the loss of estrogen conjugates excluding E2-17G caused by derivatisation procedure. Comparison with their absolute standards dissolving in neat organic solvent at the same concentration level (1 µg L^{-1}) (n=3), the recoveries of E1-3S, E1-3G, E2-3S, E2-3G and E3-3S were 100.3%, 97.2%, 103.6%, 94.0% and 96.2%, respectively (Figure A4.3). From this result, it was confirmed that conjugated estrogens without phenolic hydroxyl groups were inactive to DMIS and they were not affected on account of the mild reaction conditions.

4.3.3 Chromatographic profiling of estrogens

Given the basic characteristics of estrogen derivatives incurred by the structure of cationic moiety, positive polarity was preferable, as observed for detection of dansyl estrogens (Lien et al., 2009). On the contrary, analysis of intact estrogen conjugates was more suitable to be undertaken under negative ESI due to their acidic nature (Reddy et al., 2005b).
To achieve chromatographic separation and symmetric peak shapes for estrogens, organic solvents acetonitrile and methanol were compared. The same gradient program was utilised and a sharper peak shape for analytes was found by using acetonitrile-water mobile phases instead of the methanol-water mobile phase. Additionally, estrogens especially in their conjugated forms, were found to yield higher intensity abundance when the acetonitrile-water binary mobile phase was used, this was also observed by Reddy et al. (2005b). The disparity in response was possibly attributed to the lower viscosity of the acetonitrile/water mixture than that of a methanol-containing mixture, which may accelerate the formation of droplets with a desirable size for subsequent evaporation of ions (Benijts et al., 2002, Yao et al., 2019).

Furthermore, the addition of 0.1% formic acid in the mobile phase enhanced ESI response of derivatised estrogens more than in its absence, but intact estrogens were less affected. This may be due to the fact that the presence of an acidic modifier facilitates the ionisation of estrogen derivatives by helping protonation of analytes on their basic amine groups under positive polarity mode (Anari et al., 2002). However, in contrast to the use of formic acid, it has been stated that the involvement of alkaline buffer in the mobile phase facilitates the ionisation of acid compounds such as intact conjugated estrogens for greater resolution (Kuehnbaum and Britz-Mckibbin, 2011). As indicated in the literature, the mobile phase under basic condition often leads to premature failure of the reverse-phase column arising from the increased solvability of silica with increasing pH (Atwood et al., 1979). Therefore, formic acid was more suitable to be added into the mobile phase.

The analytes were injected on a capillary C18 column and LC-MS run was 30 min. Figure 4.3 shows LC chromatograms of targeted compounds spiked in post-
extraction of 1000 mL surface water at concentration of 0.1 µg L\(^{-1}\). All compounds of interest were eluted between 8.99 and 14.83 min.
Figure 4. 3 Chromatograms of selective compounds spiked in extraction of surface water samples at concentration of 0.1 µg L⁻¹. i) estrogen derivatives after reacting with DMIS under the positive ESI mode; ii) intact conjugated estrogens under the negative ESI mode.
4.3.4 Optimisation of derivatisation process

Considering the novelty of the derivatisation method for the co-presence of free and conjugated estrogens, optimisation of derivatisation for estrogens was investigated following the development of LC-MS method to achieve high and stable derivatisation yields. In this section, not only conditions for derivatisation reaction were compared but also the loss of conjugated estrogens excluding E2-17G was discussed.

4.3.4.1 Selection of derivatisation buffer

The yields of estrogen derivatives with participation of buffer solutions, ammonium formate or sodium bicarbonate system with a pH value at 10.5 were compared. In previous study, the mixture consisting of NaHCO₃ and Na₂CO₃ was the most common alkaline buffer to be employed in derivatisation reaction between phenolic compounds and dansyl chloride or sulfonyl chlorides (Xu and Spink, 2008, Xu and Spink, 2007, Maas et al., 2017). Nevertheless, owing to its non-volatile property, its presence in solution sample is likely to result in the damage of capillary columns and blockage of mass spectrometry. As another alternative with a buffer capacity ranged close to pH 10.5, ammonium formate was tested. However, the yields of corresponding derivatives shown significant decline though its easily-volatile feature was suitable for the apparatus of LC-MS (Figure 4.4).
Figure 4. The yields of estrogen derivatives in reaction using sodium bicarbonate or ammonium formate as buffer.

It was assumed that its volatility issued in unstable state during heating process, affecting the performance of derivatisation. Hence, sodium bicarbonate was more appropriate in this study according. Moreover, the liquid-liquid extraction using 2 mL of n-hexane for twice to remove salt in derivative solution according to method described by Maas et al. (2017) with some modification, reducing the risk of breakage of instrument and the interference to signal of MS in further analysis.
4.3.4.2 Optimisation of derivatisation reaction

Investigation on the dose of derivatisation reagent DMIS prepared at 1 mg mL\(^{-1}\) was proceeded by testing the following doses: 30, 50, 75, 100 µL. The relationship between the peak areas of derived products and dose of DMIS was indicated via the measurement with LC-MS and then displayed in Figure 4.5. According to Turkey comparison on the basis of ANOVA analysis, the difference of tested volumes for E1, E2, EE2 and E2-17G was not significant (p>0.05), while 75 and 100 µL worked better for E3 in comparison with 30 and 50 µL with p < 0.05.

![Figure 4.5 Various amount of derivatisation reagent used for generating estrogen derivatives.](image)

As applied by Keski-Rahkonen et al. (2015), 20 µL of 1 mg mL\(^{-1}\) DMIS in acetone were sufficient for the derivatisation procedure. Whereas 80 or 100 µL derivatisation reagent with a concentration at 1 mg mL\(^{-1}\) were applied in other studies (Xu and Spink, 2008, Xu and Spink, 2007). It was consequently concluded that the volume of derivatisation reagent used should match with the amount of analytes in accordance with the reaction formula. Furthermore, it was observed that the addition of over 50 µL derivatisation reagent plus the same volume of buffer solution facilitated the
dissolvability and homogenisation of all chemicals in 3 mL Reacti-vials. Therefore, 75 µL was suitable for the following application.

Further optimisation concerning the reaction temperature (15, 30, 45, 60 and 75°C) and time (10, 15, 30, 45 and 60 min) required for the derivatisation procedure was carried out. The former was compared with a fixed reaction time of 15 min and the latter was conducted under a nominated temperature of 60 °C. Finally, the optimal parameters were obtained as 60 °C for 15 min regarding the trend demonstrated by Figure 4.6.

![Figure 4.6 Optimisation of a) reaction temperature, and b) reaction time for the derivatisation procedure.](image)

4.3.5 Optimisation of extraction method

SPE is the most common technique used for a pre-concentration of endocrine disrupting chemicals from water or other more complex matrix. The factors possibly
affecting recovery performance of extraction were evaluated for constructing a feasible method with satisfied accuracy and reproducibility.

4.3.5.1 Adsorbent for extraction

The selection of SPE cartridges is the inception prior to proceeding with further optimisation. Adsorption capability mainly depended upon the materials of sorbents and properties of the interested chemicals. Free estrogens are weakly acidic and possess pKa around 10, which is much higher than that of sulphate and glucuronide estrogens. According to previous literature, the pKa of glucuronide conjugate was approximately 3, and sulphate steroids are more acidic with pKa value \(\sim -1.7\) (Qin et al., 2008, He and Aga, 2019). Due to the great difference between lipophilic free estrogens and hydrophilic estrogen conjugates, and the highly broad range of polar and nonpolar property of them, reverse-phased absorbents were suitable for retaining interested compounds due to the polymeric fillings (Griffith et al., 2014). An Oasis HLB, Strata-X and SupeI\textsuperscript{TM}-Select HLB were selected and studied further by extracting 10 mL spiked Milli-Q water at concentration of 1 \(\mu\)g L\(^{-1}\). The result (Table 4.3) showed that Oasis HLB achieved satisfactory recoveries (89.3%-102.8%) for chosen compounds. Reproducibility was with a relative standard deviation (RSD) value less than 9.72%, having better performance than Stata-X (65.8%-110.3%) and SupeI\textsuperscript{TM}-Select HLB cartridges (74.5%-103.3%). The versatility of Oasis HLB originates from its hydrophilic-lipophilic balance design in polymeric particles. Therefore, it is capable to affix moderately polar free estrogens owing to high Log \(P\) value and much more hydrophilic estrogen conjugates which exhibit high polarity simultaneously. Considering the stability, the Oasis HLB cartridge was selected for this study. Similarly,
the extraction of free estrogens in influent and effluent of a waste treatment plant was also carried out using Oasis HLB cartridges in (Salvador et al., 2007b)’s study.

Table 4. 3 Recoveries rate (± RSD %) (n=3) of MiliQ-water spiked 1 µg L\(^{-1}\) estrogen observed with three different chosen cartridges.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Oasis HLB</th>
<th>Strata-X</th>
<th>Supel\textsuperscript{TM}.-Select HLB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery, % (RSD, %)</td>
<td>Recovery, % (RSD, %)</td>
<td>Recovery, % (RSD, %)</td>
</tr>
<tr>
<td>E1</td>
<td>96.4 (2.3)</td>
<td>93.8 (7.74)</td>
<td>89.4 (6.06)</td>
</tr>
<tr>
<td>E2</td>
<td>98.9 (2.01)</td>
<td>75.2 (5.61)</td>
<td>74.5 (4.11)</td>
</tr>
<tr>
<td>E3</td>
<td>92.3 (1.88)</td>
<td>68.3 (1.79)</td>
<td>87.6 (1.93)</td>
</tr>
<tr>
<td>EE2</td>
<td>102.8 (5.15)</td>
<td>110.2 (3.70)</td>
<td>76.9 (7.40)</td>
</tr>
<tr>
<td>E1-3S</td>
<td>96.1 (2.65)</td>
<td>87.1 (6.86)</td>
<td>94.5 (4.15)</td>
</tr>
<tr>
<td>E1-3G</td>
<td>89.3 (8.81)</td>
<td>65.8 (6.61)</td>
<td>82.9 (6.34)</td>
</tr>
<tr>
<td>E2-3S</td>
<td>95.8 (4.87)</td>
<td>91.7 (4.33)</td>
<td>93.1 (2.88)</td>
</tr>
<tr>
<td>E2-3G</td>
<td>99.6 (7.36)</td>
<td>87.4 (5.89)</td>
<td>103.4 (5.03)</td>
</tr>
<tr>
<td>E2-17G</td>
<td>99.0 (8.99)</td>
<td>73.3 (10.74)</td>
<td>98.5 (8.39)</td>
</tr>
<tr>
<td>E3-3S</td>
<td>91.2 (9.72)</td>
<td>88.9 (1.45)</td>
<td>95.8 (6.60)</td>
</tr>
</tbody>
</table>

RSD: relative standard deviation (should be less than 20%)(Ben et al., 2008).

4.3.5.2 pH and loading flow of water samples

The pH value of sample solution is known to be critical for the enrichment of analytes as their acidic and alkaline properties. Ten mL Milli-Q water with spike of 1 µg L\(^{-1}\) estrogens was adjusted to pH values ranging over pH 2.97 to pH 8.96 with 0.1 M
NaOH or H₂SO₄ solution for evaluating the impacts of pH on recoveries of selected compounds. We observed that improved recoveries of free estrogens were achieved in samples with a pH range of 2.97 to 7.01, especially under the neutral condition. A further increase of alkalinity in solutions yielded poor recoveries for E1, E2 and EE2. The estrogen conjugates were given recoveries matched with requirement 80%∼120% generally with the pH between 2.97 and 8.96, but the losses of them was also noticed at pH 8.96, in which the recovery of E3-3S had a remarkable reduction. It was possibly because the alkaline solution induced the dissociation of acidic estrogens and hinder the binding to the SPE sorbents, leading to limited extraction efficiency (Liu et al., 2004). The recoveries of all estrogens at pH 7.01 had a range of 81.3%-106.0% with acceptable coefficients of variation not over 10% excluding E3 at 13.0% and E3-3S at
11.2%, showing the best performance among representative pH value chosen as demonstrated in Figure 4.7.

![Figure 4.7 Influence of pH of water samples on the recovery percentage of estrogens.](image)

Moreover, there is a study reporting that the co-extraction of humic acids in SPE sorbents is restricted substantially under neutral aqueous condition, cleaner elutes with less interferences could be obtained (Hennion, 1999).

The influence of water samples through the SPE cartridges at load flow rate (3 mL min\(^{-1}\)–5 mL min\(^{-1}\)) on the concentration efficiencies was studied. Abstractly, a slower flow rate allows adequate contact between the compounds and SPE sorbents, so a higher recovery percentage could be obtained. However, the test results demonstrated that negligible improvement was discovered on the recovery of targeted compounds over the chosen flow rate 3 mL–min\(^{-1}\)-5 mL min\(^{-1}\). Consequently, the adoption of 5 mL
min⁻¹ was finally decided in order to increase throughput. The analogous result was also reported by Rodriguez-Mozaz et al. (2004) for the enrichment of natural water with the loading rate of 5 and 8 mL min⁻¹, better recoveries were obtained with the flow rate of 5 mL min⁻¹.

4.3.5.3 Optimisation of the elution solvents

As another crucial factor decides the optimisation of the SPE procedure, the evaluation of eluent solvents was carried out with 10 mL Milli-Q water with a spike of 1 µg L⁻¹ estrogens, 8 mL of organic solvents [methanol, acetone, ethyl acetate, a 5:1 ethyl acetate/methanol mixture, a 3:2:5 (v: v: v) dichloromethane (DCM)/acetone/methanol ternary mixture and methanol containing 5% acetonitrile] were assayed. Results revealed that conjugated estrogens were poorly recovered using either ethyl acetate or 5:1 ethyl acetate/methanol binary solvent, especially E3-3S, which exhibited low recovery percentage from below 10% to 65.4%. This was mainly attributed to the high polarity of conjugated estrogens, the elution strength of ethyl acetate dominant elutes may not be adequate to release them from the HLB sorbents. Significantly improved recoveries with a span of 83.2% to 118.9% were obtained when eluted by methanol, mixed solution of 3:2:5 DCM/acetone/methanol and methanol including 5% acetonitrile for both free and conjugated estrogens. Therefore, the final decision of superior elute was made by further testing the recovery of targeted chemicals in water samples by increasing the volume to 500 mL and 1,000 mL.

The extraction efficiencies of estrogens in aqueous solution with various loading volume using these three elution solvents were summarised in Figure 4.8. The results revealed that using 100% methanol achieved the optimum pre-concentration
performance in all three volume levels of water samples with the absolute recoveries of 83.0~111.1%, except for EE2 in the 1,000 mL sample, which yielded a relatively poor extraction efficiency of 75.9% with the RSDs less than 15% overall. Therefore, methanol was employed to elute estrogens off the cartridges in this study.

4.3.5.4 Washing solution

It is known that interferences originated from natural samples retained in SPE sorbents and are potentially co-eluted together with targeted compounds, leading to ion suppression to some extent. Therefore, the washing step following loading samples closely was tested using spiked natural lake water at the concentration level of 1 µg L⁻¹ in triplicate (n=3). The composition of methanol in Milli-Q water from 0% to 10% with an increment of 5% was studied for eliminating matrix but not compromising extraction performance. The results show that there was a reduction of recoveries of estrogens when there was an increasing percentage of methanol in the washing solution. Thus, 8 mL Millii-Q water was used for cleaning up interferences.
Figure 4. 8 Recovery efficiencies (%) and RSD (%) of estrogens in aqueous solution with various loading volume (10, 500 and 1000 ml) using these three elution solvents A) methanol; B) DCM: acetone: methanol, 3:2:5 (v: v: v); C) methanol with 5% acetonitrile.

4.3.6 Method validation

The validation of the established method for determining both free and conjugated estrogens using natural surface water was summarised in Table 4.4.

To investigate linearity of the established method, calibration curves for all estrogens were constructed by using matrix matched solutions over a wide concentration to 1,000 µg L\(^{-1}\), covering their instrumental quantification limit (IQL). The correlation coefficient R\(^2\), for each analyte greater than 0.99, was obtained in both standard diluents and surface water.
The method intra-day and inter-day repeatability was evaluated by the relative standard deviations (RSDs) of intra-day and inter-day were between 2.84% and 11.73%, the good repeatability and stability of the established method was accounted for, meeting the requirements for the following analysis.

As previously reported, the components in natural matrix are prone to result in either ion suppression or enhancement of response for analytes under electrospray conditions, which potentially could affect an accuracy and reproducibility of results (Salvador et al., 2007b). Consequently, it is pivotal to take matrix effects into account, especially when quantification is undertaken using an external calibration curve. Ciofi et al. (2013) evaluated the matrix effect in river and lake waters by the comparison of peak areas for selective compounds in spiked water samples with those acquired from spiked Milli-Q water. In this thesis, matrix effects were estimated based on the post-addition method (Kang et al., 2007), using the ratio of the calibration curve slopes for estrogens standard solutions spiked into extraction of surface water matrix and neat dilution solvent. The occurrence of ion suppression was evidenced as the matrix effect >0. However, it was also found that the matrix effect was at a low level between 8.32% and 22.69%. According to the results obtained in other studies, the matrix effect was noticed to be related to the values of dissolved organic carbon (DOC) and chemical oxygen demand (COD) for samples, the higher matrix effect was attained within samples owing higher DOC or COD (Ciofi et al., 2013). In addition to the aqueous chemical condition of samples, the types of ionisation source used also contributes to the discrepancy of signals caused by matrix (Lien et al., 2009). Through comparison between analytes, it was found that the glucuronide form of estrogens suffered the most severe matrix effects in comparison to other analytes, which is possibly due to their high hydrophilicity and results in restricted separation from the co-eluting matrix.
along with the LC column. The similar observation was also achieved for investigating the impact of waste water matrix on glucuronide estrogens, while higher loss of signal was derived due to the presence of more complicated interferences in wastewater usually (Griffith et al., 2014).

The low (50 ng L$^{-1}$), middle (100 ng L$^{-1}$) and high (1000 ng L$^{-1}$) concentration levels were spiked into natural surface water for checking the recovery performance of selected estrogens. The average recovery of estrogens in surface water are presented in Table 4. Satisfactory relative recoveries were still obtained for the majority of selected compounds, which met the requirement from 80% to 120% with RSD < 10.8%, except for EE2 and E1-3G, having an extraction percentage of 78.6% and 76.4%, respectively.

The IDLs, IQLs, MDLs and MQLs for all chosen estrogens are presented in Table A4.1 and Table 4.4. The highest response was observed for sulphate estrogens, especially for E1-3S and E2-3S with low IDLs of 2.5 ng L$^{-1}$ and 1.8 ng L$^{-1}$, respectively. However, another sulphate compound E3-3S yielded poor signal, owing an IDL at 16.8 ng L$^{-1}$. Derivatives of free estrogens also produced high signal, whose IDLs ranged from 4.6 ng L$^{-1}$ to 5.8 ng L$^{-1}$, except for 21.4 ng L$^{-1}$ of E3. Glucuronide-containing estrogens had the poorest sensitivity and their IDLs varied from 14 ng L$^{-1}$ to 29 ng L$^{-1}$. Their corresponding IQLs were calculated and the lowest concentration point of the calibration curve was settled accordingly. MQLs based on an enrichment factor of 1,000 were found at low picogram to nanogram per litre levels and in a range of 7-132.3 pg L$^{-1}$ for analytes, which were comparable to the results reported by (Reddy et al., 2005c) with a MDLs range of 0.04-0.28 ng L$^{-1}$ using Milli-Q water as matrix.
Table 4. Linear range and validation results of estrogens in surface water using LC-MS.

<table>
<thead>
<tr>
<th>Estrogens</th>
<th>Surface water</th>
<th>Linear range (µg L(^{-1}))</th>
<th>R(^2)</th>
<th>MDL (pg L(^{-1}))</th>
<th>MQL (pg L(^{-1}))</th>
<th>Intra-day precision (%), n=6</th>
<th>Inter-day precision (%), n=4</th>
<th>Matrix effect (%)</th>
<th>Extraction performance (^a)</th>
<th>Absolute RE, % (RSD, %)</th>
<th>Relative RE, % (RSD, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>0.01-1,000</td>
<td>0.9985</td>
<td>5.8</td>
<td>19.4</td>
<td>7.76</td>
<td>4.53</td>
<td>11.21</td>
<td>78.4±5.2</td>
<td>86.3±4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>0.01-1,000</td>
<td>0.9995</td>
<td>7.5</td>
<td>25.1</td>
<td>5.11</td>
<td>6.17</td>
<td>15.01</td>
<td>76.5±1.8</td>
<td>83.5±3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>0.05-1,000</td>
<td>0.9986</td>
<td>31.8</td>
<td>105.9</td>
<td>5.72</td>
<td>8.37</td>
<td>15.23</td>
<td>67.4±8.0</td>
<td>85.2±5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EE2</td>
<td>0.01-1,000</td>
<td>0.9982</td>
<td>6.6</td>
<td>22.0</td>
<td>11.73</td>
<td>6.80</td>
<td>13.87</td>
<td>70.9±7.7</td>
<td>78.6±4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2-17G</td>
<td>0.02-1,000</td>
<td>0.9979</td>
<td>18.6</td>
<td>62.1</td>
<td>7.68</td>
<td>9.04</td>
<td>19.11</td>
<td>75.2±5.2</td>
<td>95.4±7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1-3S</td>
<td>0.005-1,000</td>
<td>0.9994</td>
<td>3.1</td>
<td>10.4</td>
<td>5.47</td>
<td>4.96</td>
<td>17.22</td>
<td>81.4±2.8</td>
<td>90.8±6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1-3G</td>
<td>0.05-1,000</td>
<td>0.9959</td>
<td>39.7</td>
<td>132.3</td>
<td>4.28</td>
<td>3.82</td>
<td>22.69</td>
<td>68.1±2.1</td>
<td>82.5±3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2-3S</td>
<td>0.005-1,000</td>
<td>0.9988</td>
<td>2.1</td>
<td>7.0</td>
<td>3.97</td>
<td>4.47</td>
<td>8.32</td>
<td>84.7±5.4</td>
<td>91.7±8.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2-3G</td>
<td>0.05-1,000</td>
<td>0.9982</td>
<td>38.7</td>
<td>129.1</td>
<td>4.98</td>
<td>2.84</td>
<td>15.16</td>
<td>74.8±6.0</td>
<td>85.6±7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E3-3S</td>
<td>0.05-1,000</td>
<td>0.9965</td>
<td>22.6</td>
<td>75.3</td>
<td>6.58</td>
<td>7.66</td>
<td>20.55</td>
<td>74.1±2.03</td>
<td>76.4±10.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Average recovery of estrogens in 1,000 mL natural surface water (spiked at three levels concentration: 50, 100 and 1,000 ng L\(^{-1}\).
4.3.7 Application to environmental samples

An optimised/validated method was employed to determine the content of free and conjugated estrogens in natural water collected from the Regent’s Park lake in London, UK. Using the standard addition method, known amounts of estrogen standards were added into 1,000 mL water samples for calculating the original concentrations of analytes by calculating an X-intercept value of corresponding calibration curve.

Table 4.5 summarises the maximum and average concentrations of free and conjugated estrogens measured in 7 water samples. Notably, the contents of free estrogens were significantly higher than their conjugated compounds, in which E1 was found the most abundant compared with E2 and E3, having a maximum concentration at 0.86 ng L$^{-1}$ and average concentration at 0.21 ng L$^{-1}$ in 7 samples. These concentrations are consistent with those reported in previous studies for surface water. Williams et al. (2003) detected E1 at a concentration of <0.4-2.5 ng L$^{-1}$ and E2 of <0.4-0.8 ng L$^{-1}$ in River Nene and River Lea. Owing to a great population of birds dwelling in Regent’s Park, the occurrence of estrogens in the lake water was expected as a result of possibly receiving the excretion from birds. Moreover, it was hypothesised that the residue of estrogens could reach the sampling sites via aquifers connecting to the run-off since the previous reported presence of E1, E2 and E1-3S profiled at concentrations (0.4-120, 0.2-45 and 1-4 ng L$^{-1}$, respectively) in ground water (Swartz et al., 2006). As the precursors of free estrogens, conjugated estrogens are hydrolysed gradually with the presence of micro-organisms (Ma et al., 2016). Furthermore, it has been evidenced before that a majority of free estrogens are biodegraded via E1 (Bai et al., 2013). The amount of EE2 in the sample was found to be less than its quantification limit. This is because, as a synthetic constituent mainly applied for birth control tablets, EE2 is hardly found in excreta of birds, and consequently it was not
detected in the nearby sampling sites. Glucuronides were not detectable in natural water. In contrast, the prevalence of sulphates was observed, which was attributed to the lower MDLs the sulphates possessed; additionally, the transformation of glucuronides to free estrogens was inferred in natural water while the sulphates were more recalcitrant to biodegradation induced by microorganisms, as reported prior to this study (Ben et al., 2017).

Table 4. 5 Concentration of free and conjugated estrogens in natural water collected from Regent's Park lake.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Surface water (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration Max (ng L⁻¹)</td>
</tr>
<tr>
<td>E1</td>
<td>0.86</td>
</tr>
<tr>
<td>E2</td>
<td>0.55</td>
</tr>
<tr>
<td>E3</td>
<td>0.48</td>
</tr>
<tr>
<td>EE2</td>
<td>&lt;MQL</td>
</tr>
<tr>
<td>E2-17G</td>
<td>&lt;MQL</td>
</tr>
<tr>
<td>E1-3S</td>
<td>0.17</td>
</tr>
<tr>
<td>E1-3G</td>
<td>&lt;MQL</td>
</tr>
<tr>
<td>E2-3S</td>
<td>0.09</td>
</tr>
<tr>
<td>E2-3G</td>
<td>&lt;MQL</td>
</tr>
<tr>
<td>E3-3S</td>
<td>&lt;MQL</td>
</tr>
</tbody>
</table>
4.4 Conclusion

In this Chapter, a novel and sensitive LC-MS method based on derivatisation with DMIS was developed to achieve one-pot detection of free and conjugated estrogens at trace level. The occurrence of derivatisation reaction was favourable for enhancing mass spectrometric signal under ESI conditions of free estrogens significantly. Derivatives affixing DMIS label were capable of providing specific structural characterisation for the confirmation of chosen compounds using MS/MS, of which structural isomers E2-17G and E2-3G were identified due to the selectivity of derivatisation reaction, alleviating the susceptibility of determination in presence of interferences. Following a feasible SPE procedure, the enrichment of analytes from samples was achieved using Oasis HLB sorbent, and impurities were washed away with Milli-Q water followed by elution using 8 mL of methanol. Method quantification limits for all estrogens ranging from 7.0 to 132.3 pg L\(^{-1}\) in surface water were acquired by validation of the established methodology.

The practicability of this method was demonstrated by profiling estrogens in surface water collected from a lake of Regent’s Park, EE2, E2-17G, E1-3G, E2-3G and E3-3S were not found in samples, but E1, E2, E3, E1-3S and E2-3S were detected. However, their concentration levels were differential, for example sulphates were significantly more prevalent than glucuronide estrogens. These results confirmed the capability of quantifying free and conjugated estrogens using this established methodology (Figure 4.9) in the following Chapters 5, 6, and 7.
Method development

**Water sample pre-treatment**
- Filtration through 0.45 μm membrane filter;
- Impacts of water sample pH (3, 5, 7, 9, 11) were investigated

**Optimisation of SPE**
- Assessment of cartridges (Oasis HLB, Strata-X, Supelco - Select HLB)
- Loading rate of water samples (3-5 mL/min)
- Selection of elution (MeOH, acetone, ethyl acetate, a 5:1 ethyl acetate/MeOH mixture, a 3:2:5 (v:v:v) DCM/acetone/MeOH ternary mixture, and MeOH containing 5% acetonitrile)
- Choice of washing solution (MilliQ-water, MilliQ-water with 5% MeOH, and MilliQ-water with 10% MeOH)

**Development of derivatisation**
- Selection of derivatisation buffer with a pH of 10.5 (ammonium formate and sodium bicarbonate)
- Volume of derivatisation reagent (30, 50, 75, and 100 μL)
- Reaction temperature (15, 30, 45, 60, and 75°C)

Final method

**Water sample pre-treatment**
- Filtration through 0.45 μm membrane filter
- Adjusting pH value of water sample to 7

**Established SPE**
- Condition Oasis HLB cartridges with 6 mL MeOH and 6 mL Milli-Q water
- Loading water sample with flow rate of 5 mL min⁻¹
- Washing impurities with 8 mL Milli-Q water
- Drying cartridges with air vacuum pump for 1 h
- 8 mL MeOH was applied to elute estrogens from cartridges
- Evaporating the eluent to dryness under N₂ flow

**Derivatization**
- Dry residue mixed with 75 μL DMIS and 75 μL sodium bicarbonate (pH 10.5)
- The micro-reactor containing mixture was put in 60 °C water bath for 15 min
- The derivatised mixture was extracted with 2×2 mL n-hexane

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(continued)

Figure 4. Developed methodology for measuring estrogens using LC-MS/MS.

Analysis using LC-MS/MS

i. Sample pre-treatment
   - Filtration through 0.22 µm PTFE syringe filter

ii. LC method
   - Hypersil GOLD C₁₈ capillary column (100 mm x 2.1 mm i.d., particle size: 1.9 µm) was used
   - Mobile phase with flow rate of 180 µL min⁻¹: A) water containing 0.1% formic acid; B) acetonitrile containing 0.1% formic acid.
   - Gradient program: 0 min: 70% A, 30% B; 10 min: 20% A, 80% B; 18 min: 10% A, 90% B; 25 min: 10% A, 90% B; 26 min: 70% A, 30% B; 30 min: 70% A, 30% B
   - Column temperature: ambient temperature
   - Injection volume: 10 µL

iii. MS method
   - Ion source: ESI mode: 1) positive polarity for estrogen derivatives; 2) negative polarity for estrogen conjugates excluding E2-17G
   - Capillary temperature 280°C, capillary voltage 33V;
   - Ion source voltage: 4.5 kV; sheath gas flow: 40 L min⁻¹, Aux gas flow: 10 L min⁻¹ and drying gas flow: 15 L min⁻¹
Chapter 5 The adsorption mechanism of estrogens using granular activated carbon and the influence of NOM on the adsorption capacity

5.1 Introduction

For better understanding the degradation behaviours that occurred in the GSBF system, the sorption process between the targeted estrogens and GAC particle could not be neglected and a more detailed investigation on the adsorption mechanisms was required.

As one of the most common non-specific adsorption materials, activated carbons (ACs) principally comprised of micropores (< 2 nm in size), were employed to remove emerging contaminants in water in numerous projects due to the simplicity of operation and environmental friendly character (Rossner et al., 2009, Li et al., 2019, Ji et al., 2010, Erdem et al., 2020, Jiang et al., 2017b, Mestre et al., 2014). It is well known that the physical properties of adsorbents (e.g. specific area, total pore volume, pore size distribution and nature of material) and chemical properties (e.g. molecular size, surface functional groups) are likely to influence the adsorption of organic contaminants (Bunmahotama et al., 2017). Apart from that, the adsorptive performance is also affected, to some extent, by characteristics of aqueous solution, which involves pH, temperature and the substances co-presented in (Bunmahotama et al., 2017, Aldegs et al., 2008, Rossner et al., 2009), wherein, natural organic matter (NOM) in water matrix was noticed as a primary factor to influence adsorption ability (Newcombe et al., 1997).
NOM is prevalent in natural water and this highly heterogeneous mixture mainly consists of humic and fulvic acids-like substances with a high molecular weight higher than 10 kDa (Erdem et al., 2020); its presence leads to the wide spanning of dissolved organic matter (DOM) concentration in natural water over 0.5~100 mg L$^{-1}$ (Sgroi et al., 2018, Frimmel, 1998). It was noted that the presence of NOM in aqueous solution resulted in significant loss of sorption capability of targeted compounds, which was attributed to reasons: 1) the acceleration of fouling and blockage of adsorbent pores; or 2) the competition between NOM and compounds of interest for adsorption sites (Sbardella et al., 2018, Chang et al., 2004, Erdem et al., 2020, Jiang et al., 2017b, Newcombe et al., 1997, Jiang et al., 2017a). While the opposite behaviour, the enhancement of sorption for some organic compounds on carbon nanotubes, was observed as the increased dispersion of adsorbent was invoked by the dissolved humic acid in the solution (Pan et al., 2013, Newcombe et al., 1997).

To date, the elimination of free estrogens, such as E1 and E2, by the sorption of ACs has been documented in previous work (Rossner et al., 2009, Fuerhacker et al., 2001, Fukuhara et al., 2006, Jiang et al., 2017b, Chang et al., 2004, Song et al., 2009, Grover et al., 2011, Joseph et al., 2013, Zhang and Zhou, 2005). However, the sorption mechanism of other free estrogens such as E3 and EE2 using GAC has not been studied simultaneously yet. Moreover, the investigation into impacts of co-present NOM and preloading GAC on adsorbility of the expansive estrogenic chemicals is also required for more comprehensive understanding in practical treatment condition.

In this chapter, a systematic strategy was designed to investigate: 1) the adsorption capability of two different kinds of commercial pristine GAC; 2) the impacts of humic acid on the adsorption capacity of GAC for targeted compounds; 3) the sorption
behaviour of targeted compounds onto selected GAC within the natural water matrix which contains naturally occurring NOM.

5.2 Methodology

5.2.1 Materials and chemicals

The carbon adsorbents assayed in this chapter were granular activated carbon (GAC) purchased from Chemviron Carbon (activated coal) and DARCO® (20-40 mesh size, activated charcoal); and, for the purpose of the thesis, their names have been abbreviated as AC-i and AC-ii, respectively. The methodologies used for characterising GAC and corresponding properties, such as morphology captured by scanning electron microscope (SEM), pore size distribution, surface area, pH, point of zero charge (PZC) and functional groups on their surface, were summarised in Sections 3.4.1., 3.4.2 and 3.4.3. Pristine GAC sorbents were washed with Milli-Q water for cleaning fines away and oven-dried with a temperature of 105 °C, then stored in a desiccator prior to the experiments.

Humic acid sodium salt (HA) purchased from Sigma-Aldrich (Dorset, UK) was taken as a surrogate of natural organic matter (NOM) in this study. It was documented that the fraction of HA in NOM from the natural surface water was up to 50-90% (Wang et al., 2012), and it was also considered as the primary extractable component in humic substances (Kim et al., 2013). As described by Jiang et al. (2017b), the alkaline condition was discovered to foster the dissolution of HA in the solution and mitigating the precipitation of solid phase. Modifying the method from Peng et al. (2015)’s preparation procedure, 2 g HA was weighed and dissolved in 10 mL NaOH solution (with a concentration of 0.1 M), then the mixture was stirred for homogenisation and transferred into a 200 mL volumetric flask for obtaining 200 mL of solution with a concentration of 10 g L⁻¹ after adding MilliQ-water to the graduated line. Subsequently,
the adjustment to pH around 7 was carried out using 0.1 M H$_2$SO$_4$ solution. The prepared dissolved HA solution was finally filtered through 0.45 µm WCN cellulose acetate membrane (Whatman$^{TM}$, UK).

5.2.3 Batch adsorption experiments

Aqueous solutions involving targeted estrogens (e.g. E1, E2, E3, EE2) with a concentration of 10 µg L$^{-1}$ were employed for investigating adsorption mechanism with GAC through this study. In order to minimize the cosolvent effect, the mixed estrogen stock solution (100 mg L$^{-1}$) was diluted using Milli-Q water to obtain 1 mg L$^{-1}$ working solution, then the final assayed solution was prepared via diluting aliquots of this working standard estrogen solution into each water matrix prior to tests. In this process, less than 0.1 vol% methanol presenting in the final solution was assumed not to cause any inhibition of ACs adsorption capacity (Jiang et al., 2017b). It was also implied by Giusti et al. (1974)'s finding that a small amount of methanol (i.e. 0.007 g) was adsorbed by 1 g AC when the equilibrium concentration of methanol was 0.964 g L$^{-1}$. Herein, Milli-Q water, synthetic water involving a concentration of HA at 30 mg L$^{-1}$ which is within the usual level for surface water from the terrestrial source as described by Black et al. (1996). Surface water collected from a lake of Regent’s Park was the main water matrix used to evaluate the adsorbility of the targeted compounds onto GAC.

Batch experiments for adsorption measurements were performed under ambient temperature as follows. 1) Adsorption of estrogens in MilliQ-water onto different ACs; 2) adsorption of estrogens spiked in synthetic water with HA concentration of 30 mg L$^{-1}$ on GAC; 3) adsorption of estrogens spiked in natural surface water on GAC.
The equilibrium test was first carried out via vertically putting caps-screwed vials embracing 600 mL Milli-Q water (under minimal headspace condition) with a spike of all chosen estrogens at 10 µg L\(^{-1}\) and 120 mg GAC into the rotary mixer at a speed of 30 rpm for different contact time (0, 2, 6, 10, 30 min, 1, 2, 3, 5, 8, 12, 24, 48 h and 5 days). Based on the result shown in Figure 5A.3, the content of estrogens adsorbed by GAC increased with the contact time, but after a specific time point, the amount of estrogens adsorbed by the solid phase changed little with time. Accordingly, 24 h was selected as the end point of the equilibrium time for the following isotherm study.

For comparing two commercialised GAC, the isotherm studies were conducted using MilliQ-water spiked with 10 µg L\(^{-1}\) estrogens. The dose concentration of GAC ranging from 0\textendash 1000 mg L\(^{-1}\) (0, 10, 20, 100, 200, 500 and 1000 mg L\(^{-1}\)) was added into aqueous sample and allowed the 24 h contact time in a rotary mixer at a speed of 30 rpm. For evaluating the effect of NOM on adsorptive ability to estrogens, Milli-Q water dosed 30 mg L\(^{-1}\) HA and surface water was applied for the isotherm study. For controlling the effects of solution chemistry, the pH value of aqueous solution prepared using Milli-Q water and humic acid-contained synthesized water was adjusted to ~7 using 0.2 M NaOH and H\(_2\)SO\(_4\) before adding into adsorbents. All samples were prepared in triplicates for accounting for the experimental errors and vials for adsorption experiments were wrapped with aluminium foil to be free from the interferences of light.

The mass of targeted compounds adsorbed on the GAC was calculated following the Equation (1) (Joseph et al., 2011, Fukuhara et al., 2006):

\[
q_t = (C_0 - C_t) \frac{V}{m}
\]  

(5.1)
Where $q_t$ refers to the targeted compounds adsorbed by GAC at time (mg g$^{-1}$); $C_0$ (µg L$^{-1}$) and $C_t$ (µg L$^{-1}$) are the initial concentration of compounds and their concentration after a given contact time, respectively; $V$ is the volume of the aqueous solution (L); and $m$ is the weight of GAC added in solution (mg).

5.2.4 Analytical method
The concentration of HA was determined with Cary 60 UV-Vis spectroscopy (Agilent Technologies) throughout the whole study. Each sample was loaded into quartz cuvette and measured against a Milli-Q water electrolyte background. Though TOC analyser (Shimadzu TOC-L) was also available to quantify the concentration of dissolved organic carbon (DOC), the presence of estrogens and organic solvent (e.g. methanol) in solutions results in overestimation to some extent, thus the application of UV-Vis spectrometry is more specific for determination of HA. Then the specific ultraviolet adsorbance (SUVA$_{254}$) was obtained by dividing UV$_{254}$ by DOC. The DOC analysis showed that the humic acid consisted of approximately 40% DOC as reported by Joseph et al. (2013).

After finishing adsorption experiments, aqueous samples were filtered through 0.45 µm membrane filter, then the filtered solutions were concentrated using the SPE procedure and subsequently analysed with LC-MS following the method described in Chapter 4.

5.2.5. Kinetics of adsorption
For investigating the effect of contact time on adsorption of estrogens, as the most common used kinetics (Simonin, 2016), the pseudo-first-order model and pseudo-second-order model with their rearrangement forms were applied to analyse the kinetic parameters for free estrogens.
The pseudo-first-order model proposed by Lagergren (1898) is presented as follows:

\[
\frac{dq_t}{dt} = k_1(q_e - q_t)
\]  

(5.2)

It is indicated that the sorption rate is proportionate to the difference of adsorbate adsorbed by adsorbent at equilibrium \(q_e, \) mg g\(^{-1}\) and at given time \(q_t, \) mg g\(^{-1}\). Its rearranged form is expressed as:

\[
\ln(q_e - q_t) = \ln q_e - k_1 t
\]  

(5.3)

where \(k_1, \) (min\(^{-1}\)) is the pseudo-first-order rate constant. The linear tendency was profiled via plotting ln \((q_e - q_t)\) against \(t\) (time, min).

The pseudo-second order model was established by Ho and McKay (1999) and it is expressed by:

\[
\frac{dq_t}{dt} = k_1(q_e - q_t)^2
\]  

(5.4)

And it has a rearranged form of:

\[
\frac{t}{q_t} = \frac{1}{k_2q_e^2} + \frac{1}{q_e t}
\]  

(5.5)

where \(k_2, \) (g*(mg*min)\(^{-1}\)) is the pseudo-second-order rate constant. The linear regression was plotted \(\frac{t}{q_t}\) against \(t\) (time, min). The obtained \(R^2\) is able to indicate the
involvement of chemical interaction in the adsorption process (Yu et al., 2009). Moreover, $k_2 q e^2$ or $k_{initial}$ (mg*(g*min)$^{-1}$) refers to the initial adsorption rate.

Both pseudo-first-order model and pseudo-second-order model are applied for evaluating whether the adsorption mechanism is the rate limiting step (Hameed and Rahman, 2008).

Additionally, for further identifying rate controlling factors in the adsorption process, an intra-particle diffusion model proposed by Weber Jr and Morris (1964) was also used to evaluate the diffusion mechanism as follows:

$$q_t = k_i t^{1/2} + C$$  \hspace{1cm} (5.6)

where $k_i$ (mg (g*min$^{1/2}$)$^{-1}$) is the intra-particle diffusion constant, the larger value is assumed as a faster adsorption process. Intra-particle diffusion is considered as the sole rate controlling step if the fitted straight line passes through origin, otherwise, other steps will be involved in the adsorption process if multi-linearity is fitted based on this model (Boparai et al., 2011). For the constant $C$ (mg g$^{-1}$) in this equation, namely the intercept obtained in the linear portion via plotting, $q_t$ against $t^{1/2}$ is discovered proportional to the boundary layer effect (Mckay et al., 1980).

5.2.6 Isotherm modelling

Isotherm modelling explains the association between the amount of adsorbed adsorbate and the concentration of adsorbate in the solution. The data obtained from the adsorption experiments were applied to fit the Freundlich, Langmuir models and their rearranged forms as (Lerman et al., 2013):

Freundlich: $q_e = k_f C_e^{1/n}$  \hspace{1cm} (5.7), which is rearranged to $\log q_e = \log k_f + \frac{1}{n} \log C_e$  \hspace{1cm} (5.8);

Langmuir: $q_e = \frac{Q k_f C_e}{1 + k_f C_e}$  \hspace{1cm} (5.9), which is rearranged to $\frac{C_e}{q_e} = \frac{1}{k_f Q} + \frac{C_e}{Q}$  \hspace{1cm} (5.10);
where $q_e$ is estrogens adsorbed at equilibrium (mg g$^{-1}$); $k_f$ (mg g$^{-1}$) (L mg$^{-1}$)$^{1/n}$ is the Freundlich adsorption constant; $C_e$ is the estrogen concentration in solution when equilibrium is reached (mg L$^{-1}$); $1/n$ refers to the empirical parameter indicating the sorption intensity and heterogeneity of adsorbents; $Q$ is the saturation adsorption capacity (mg g$^{-1}$), and $k_l$ is the Langmuir adsorption constant (L mg$^{-1}$) (Dong et al., 2018).

Subsequently, the adsorption affinity between the four free estrogens and AC-i and AC-ii were compared via calculating $k'_f$ (mg m$^{-2}$) (L mg$^{-1}$)$^{1/n}$, which are the normalised $k_f$ by the specific surface area (SSA, m$^2$ g$^{-1}$) of adsorbents. The equation was as follows:

$$k'_f = \frac{k_f}{SSA} \quad (5.11)$$

5.3 Results and discussions

5.3.1 Properties of adsorbents

The morphology of AC-i and AC-ii were captured at different magnification as shown in Figure 5.1, in which rugged features of selected carbon adsorbents were observed. Being consistent with the previous finding, the GAC particles also embrace heterogeneous pores (Li et al., 2021). The morphology of AC-i was captured using a Focused Ion Beam (FIB-SEM, Carl Zeiss XB1540) Microscope in the London Centre for Nanotechnology (London), however, due to the suspension of this instrument; the image of AC-ii was taken using the scanning electron microscopy (SEM, JSM-6700F) at 15 kV from the UCL Chemistry Department with the help from Dr Yuchen Yang. Compared to SEM, FIB-SEM has higher resolution, so the scales of AC-i and AC-ii shown in Figure 5.1 are different.
Figure 5. 1 Three-dimensional images of GACs captured using SEM at different magnifications. (A) GAC purchased from Chemviron Carbon; (B) GAC purchased from DARCO®.

The adsorption-desorption isotherms of both GAC adsorbents was presented in Figure 5A.1 (i). The concave shapes and hysteresis loop were discovered to match up with the combination of Type I and Type IV isotherms according to the International Union of Pure and Applied Chemistry (IUPAC) classification scheme (Thommes et al., 2015), indicating the co-existence of micropores and mesopores on selected adsorbents (Schneider, 1995). The presence of hysteresis loops in both isotherms around p/p₀=0.4 were noticed, where type H4 and H3 were designated to AC-i and AC-ii,
respectively. Both loop shapes are usually induced by the slit-shaped pores in lamellar particles, while the H4 loop was found to be more associated to micropores (Bizi, 2019). Additionally, the increase of isotherm slope was assumed to imply the higher mesoporosity in previous studies (Lladó Valero, 2016). Therefore, a more mesoporous structure of AC-ii was speculated, which was further evidenced by the profile of pore size distribution shown in Figure 5A.1 (ii).

The characterisation information and surface functionalities of the GAC materials are given in Table 5.1. It was noticeable that AC-ii has a larger surface area and total pore volume with a domination of mesopores. On the contrary, AC-i was more microporous. But overall, it was noticeable that the examined GACs were hybrid in micro- and mesopores.

Table 5.1 The characteristics of examined GAC adsorbents.

<table>
<thead>
<tr>
<th>Brand of GAC</th>
<th>S BET (m² g⁻¹)</th>
<th>TPV (mL g⁻¹)</th>
<th>PS m (nm)</th>
<th>Peak qualities of pore</th>
<th>pH pzc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R micro (&lt;2 nm*)</td>
<td>R meso (2-40 nm*)</td>
</tr>
<tr>
<td>AC-i</td>
<td>557.1</td>
<td>0.241</td>
<td>1.73</td>
<td>&lt; 2 nm</td>
<td>2-3 nm</td>
</tr>
<tr>
<td>AC-ii</td>
<td>642.8</td>
<td>0.778</td>
<td>4.84</td>
<td>&lt; 2 nm</td>
<td>2-4 nm; 5-10 nm</td>
</tr>
</tbody>
</table>

* in diameter

The amount of dissociable functionalities on GACs with their relative standard deviation (RSD) is presented in Table 5.2, where the main constitution of acidic functionalities is phenolic, lactonic and carboxylic groups. For the total basic functional groups, there would be ketones, pyrones, chromones and π-π bonds if the specific detection was conducted based on previous publication (Shannon et al., 2018). It was assumed that the pH values of adsorbents were impacted by the functionalities on the surface; apart from the difference between total acidic groups and total basic groups
of two examined GACs, the dominance of the phenolic group with higher pKa value (~10) might also explain that AC-i displayed slightly more basic than AC-ii which was predominated by carboxylic groups with pKa (4−5) (Reffas et al., 2010). This was further confirmed by the pH values of 7.64 and 6.35 determined for AC-i and AC-ii (Table 5.1), respectively.

Table 5.2 The surface functionalities of examined GAC adsorbents.

<table>
<thead>
<tr>
<th>Brand of GAC</th>
<th>Amount of functional groups (meq g⁻¹)± RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenolic Lactonic Carboxylic Total acidic Total basic</td>
</tr>
<tr>
<td>AC-i</td>
<td>0.262±6.28 0.029±7.06 0.027±12.58 0.318±4.24 0.357±3.91</td>
</tr>
<tr>
<td>AC-ii</td>
<td>0.021±13.61 0.023±6.90 0.255±6.15 0.299±3.70 0.272±2.52</td>
</tr>
</tbody>
</table>

To some extent, the discrepancy of the surface functional groups resulted in the difference of pH_pzc value derived from Figure 5A.2 for two sorption materials, being 7.39 for AC-i and 6.47 for AC-ii the same phenomenon was also explained by Baccar et al. (2012) and Becker et al. (2017) prior to this study.

5.3.2 Adsorption kinetics of estrogens

The adsorption kinetics experiment was conducted for demonstrating the association of contact time with adsorption of estrogens onto GAC. As illustrated by Figure 5A.3, the adsorption of four kinds of free estrogens could reach to the equilibration within 24h with the addition of 200 mg L⁻¹ GAC particles. It was clearly observed that the amount of compounds absorbed by both GAC samples increased with time, but finally it remained constant after a specific time. This is because the desorption rate is equal to the adsorption rate after this point (Baccar et al., 2012).
On the basis of the time dependent results, the pseudo-first order model, the pseudo-second order model and the intra-particle diffusion model were applied to investigate the adsorption behaviour of E1, E2, E3 and EE2. The calculated parameters and correlation coefficient $R^2$ for each model were summarised in Table 5.3.

Table 5.3 The Parameters and correlation coefficient $R^2$ of the pseudo-first order, the pseudo-second order and the intra-particle diffusion model.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>EE2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AC-i **</td>
<td>AC-ii **</td>
<td>AC-i</td>
<td>AC-ii</td>
</tr>
<tr>
<td>Pseudo-first order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_1$ (1/min)</td>
<td>0.0062</td>
<td>0.0084</td>
<td>0.0125</td>
<td>0.0077</td>
</tr>
<tr>
<td>$q_{e, \text{exp}}$ (mg g$^{-1}$)</td>
<td>0.0410</td>
<td>0.0441</td>
<td>0.0483</td>
<td>0.0478</td>
</tr>
<tr>
<td>$q_{e, \text{calc}}$ (mg g$^{-1}$)</td>
<td>0.0230</td>
<td>0.0143</td>
<td>0.0322</td>
<td>0.0322</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.907</td>
<td>0.741</td>
<td>0.869</td>
<td>0.813</td>
</tr>
<tr>
<td>Pseudo-second order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_2$ (g*(mg*min)$^{-1}$)</td>
<td>1.41</td>
<td>7.02</td>
<td>3.61</td>
<td>8.36</td>
</tr>
<tr>
<td>$k_{\text{initial}} \times 10^3$ (mg*(g*min)$^{-1}$)</td>
<td>2.41</td>
<td>13.43</td>
<td>8.31</td>
<td>18.99</td>
</tr>
<tr>
<td>$q_{e, \text{exp}}$ (mg g$^{-1}$)</td>
<td>0.0410</td>
<td>0.0441</td>
<td>0.0483</td>
<td>0.0478</td>
</tr>
<tr>
<td>$q_{e, \text{calc}}$ (mg g$^{-1}$)</td>
<td>0.0414</td>
<td>0.0437</td>
<td>0.0479</td>
<td>0.0476</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.999</td>
<td>0.996</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Intra-particle diffusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_1 \times 10^4$ (mg (g*min$^{1/2}$)$^{-1}$)</td>
<td>38.4</td>
<td>75.3</td>
<td>77.9</td>
<td>82.7</td>
</tr>
<tr>
<td>$k_2 \times 10^4$ (mg (g*min$^{1/2}$)$^{-1}$)</td>
<td>4.47</td>
<td>2.56</td>
<td>23.5</td>
<td>4.93</td>
</tr>
<tr>
<td>$C_2$ (mg g$^{-1}$)</td>
<td>0.0308</td>
<td>0.040</td>
<td>0.018</td>
<td>0.041</td>
</tr>
<tr>
<td>$R_i^2$</td>
<td>0.960</td>
<td>0.714</td>
<td>0.942</td>
<td>0.935</td>
</tr>
</tbody>
</table>

* GAC from Chemviron Carbon; ** GAC from DARCO®.
The applicability of kinetic models was evaluated according to their \( R^2 \) values. Compared with the pseudo-first order model, adsorption of estrogens was discovered to be better fitted with the pseudo-second order model \( (R^2>0.99) \) than the pseudo-first order model. Furthermore, the amount of selected estrogens adsorbed by GACs at the point of equilibrium \( (q_{e, \text{calc}}) \) obtained via the calculation using the pseudo-second order model was in close accordance with the results derived from experiments \( (q_{e, \text{exp}}) \), while the underestimation of \( q_{e, \text{calc}} \) calculated from the pseudo-first order model was observed. Therefore, it was concluded that the adsorption of all four free estrogens onto GAC was suitable to be described by the pseudo-second kinetics, which further indicated the involvement of chemical interaction (e.g. electronic force, \( \pi-\pi \) bonding and hydro-bonding) for the sorption process (Al-Khateeb et al., 2014, Jung et al., 2013). Similar kinetic tendencies were also reported by Baccar et al. (2012) and Jung et al. (2013) for the adsorption of estrogens or other pharmaceuticals on carbonous adsorbents. In good agreement with what was observed from the experiment, the rate constant \( k_2 \) values for all estrogens when AC-ii was employed were found higher than when AC-i was in use. This difference was found to be more prominent at the initial stage of adsorption according to the \( k_{\text{initial}} \) values. It was inferred that the difference of rate constant values was possibly attributed to the porous characteristic of the selected adsorbents. The GAC with more mesoporosity allows the transferring of adsorbate molecules to the surface-inside boundary of GAC within a short time (Ruiz et al., 2010). Thus, the faster adsorption rate was explained by the less microporous structure of AC-ii.

It is known that adsorption embraces three stages: (1) surface diffusion, where the migration of adsorbates from the solution to the external surface of adsorbents through
the boundary layer occurs; (2) intra-particle diffusion, through this process the adsorbate molecules travel to the inside of adsorbent pores; (3) the adsorption within the adsorbents pores is expected (Chingombe et al., 2006). In this study, experimental data was fitted with the Weber-Morris intra-particle diffusion model for investigating the rate-controlling step in the adsorption process.

As shown in Figure 5.2, the multilinearity in each plot with $q_t$ verse $t^{0.5}$ for describing adsorption kinetics of all free estrogens demonstrated that there should be more than one step involving the adsorption process according to previous studies, and intra-particle diffusion was not the sole rate limiting step (Wu et al., 2009). In this case, the surface diffusion is assumed to take place first, then the step of intra-particle diffusion occurred, followed by the equilibrium finally being reached. The same phases were reported by Al-Khateeb et al. (2014). In the meantime, the parameters for the intra-particle diffusion model summarised in Table 5.3 were calculated based on the linearity of the second region. The positive intercepts were acquired for the interaction between all estrogens and both GACs, giving an indication that the rapid adsorption during a short time occurred according to previous studies (Wu et al., 2009). In addition, it was noticed that the C values were greater when AC-ii was applied to the kinetic study. Since it was found that the intercept value $C$ associated with the thickness of the boundary layer (Mckay et al., 1980), the conclusion that the boundary layer effect was more remarkable for AC-ii, which decelerated the intra-particle diffusion of adsorbate molecules and then lower diffusion constant $k_{i2}$ was observed correspondingly. Considering the overall higher adsorption rate given by pseudo-second constant $k_2$, it was presumed that the initial phase, surface diffusion stage was more important for controlling the rate on the basis of the far higher $k_{i1}$ values.
summarised in Table 5.3. From the porous structure of selected adsorbents, the higher dominance of micropores in AC-i should inhibit the accessibility of free estrogen molecules to more inner pores and a thicker boundary layer was expected, theoretically. However, the opposite fitting parameters were attained. It was inferred that the impact of the surface chemistry of adsorbents resulted in this phenomena. A little more functionality from the per unit mass of AC-i (Table 5.2) made it more hydrophilic in nature and this feature facilitated the migration of the water solution containing dissolved estrogens in pores of adsorbents as described prior to this study (Vargas et al., 2015, Ruiz et al., 2010).
Figure 5. 2 Intra-particle diffusion models for describing the sorption processes of free estrogens onto two commercial GACs: (a) E1; (b) E2; (c) E3 and (d) EE2.
5.3.3 Adsorption isotherms of estrogens

The adsorption isotherms of E1, E2, E3 and EE2 on two assayed GACs spiked with different concentrations were obtained with a contact time of 24 h. The results were then analysed with the Freundlich and Langmuir models. In the Langmuir model, the surface of the adsorbent is assumed to be homogeneous and a monolayer of adsorbate molecules is formed without other interactions; for the Freundlich model, the heterogeneity of adsorbent surface and the interactions between adsorbate molecules are taken into account (Anbia and Ghaffari, 2009). As a surrogate, the adsorption of E1 on A-i and A-ii was fitted as shown in Figure 5.3. The corresponding parameters and correlation coefficients are summarised in Table 5.4. The slightly greater correlation coefficient $R^2$ of the Freundlich model was observed, therefore its general suitability for investigating the adsorption processes of free estrogens on selected carbon adsorbents was illustrated.
Figure 5.3 The fitting plots of Freundlich and Langmuir isotherm models for the sorption processes of E1 on two commercial GACs AC-i and AC-ii.

In the Freundlich model, $k_f$ and $1/n$ are the adsorption constants, which are indicative of the adsorption capacity and the intensity of adsorption force between the surface of adsorbents and adsorbates, respectively (Fukuhara et al., 2006). The $k_f$ values for the sorption of all chosen estrogens using AC-ii were obviously found higher than what were derived when AC-i was employed. In the meantime, the close, but slightly higher $1/n$ of AC-ii also implied a bit higher sorption intensity with free estrogens (Kumar et al., 2008).

Table 5.4 The parameters and coefficients of Freundlich and Langmuir models fitted with the isotherm experiment of free estrogens with AC-i and AC-ii.
Moreover, the adsorption affinity was further explained via comparing the specific surface area normalised $k_i$, namely $k_i'$ of estrogens on both GAC adsorbents for investigating the factors affecting the uptake process. As shown in Table 5.5, in comparison with $k_i$, the difference on $k_i'$ values between AC-i and AC-ii was attenuated but still pronounced. It was inferred that the surface area of adsorbents was not the sole factor acting on the sorption of estrogens. The higher $k_i$ and $k_i'$ values were both noticed for AC-ii, the more hydrophobic surface was expected due to the lower content of oxygen-containing functional groups than AC-i, which possibly accounted for its superior adsorption performance as a result of weakening the adsorption competition with water clusters on the surface in comparison with more hydrophilic AC-i (Apul et al., 2013). The same observation was also reported for uptake of phenol using carbon materials by Moreno-Castilla (2004) early. Likewise, the same trend was also discovered for single point distribution coefficients $k_d$ and their homologous specific surface area normalised value $k_d'$ summarised in Table 5A.1. Notably, the disparity between AC-i and AC-ii decreased along with the gradually increasing equilibrium concentration of solution. It was presumed that the adsorbents were surrounded by

<table>
<thead>
<tr>
<th>Adsorbents</th>
<th>Freundlich model</th>
<th>Langmuir model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_i$ (mg g$^{-1}$) (L mg$^{-1}$)$^{1/n}$</td>
<td>$Q$ (mg g$^{-1}$)</td>
</tr>
<tr>
<td>AC-i</td>
<td>3.34 3.56 1.79 2.88</td>
<td>0.217 0.228 0.145 0.160</td>
</tr>
<tr>
<td></td>
<td>0.655 0.641 0.624 0.657</td>
<td>k_i (L mg$^{-1}$)</td>
</tr>
<tr>
<td></td>
<td>0.992 0.982 0.991 0.965</td>
<td>R$^2$</td>
</tr>
<tr>
<td>AC-ii</td>
<td>5.20 5.98 2.84 4.74</td>
<td>0.424 0.433 0.228 0.343</td>
</tr>
<tr>
<td></td>
<td>0.668 0.632 0.663 0.688</td>
<td>k_i (L mg$^{-1}$)</td>
</tr>
<tr>
<td></td>
<td>0.982 0.998 0.964 0.976</td>
<td>R$^2$</td>
</tr>
</tbody>
</table>
more estrogen molecules in high equilibrium concentration in which the water cluster was under low equilibrium condition, hence the difference resulting from the hydrophobic interaction was alleviated, as discussed by Jiang et al. (2017b).

Apart from the features of the adsorbents, the physiochemical properties of the adsorbate molecules (e.g. molecular size, hydrophobicity, pKa) also play an important role during the adhesion process (Baccar et al., 2012). Given the similar pKa values around 10 and molecular size characterised by diameter (Table 5A.2) for all studied compounds, the condition of their accessibility to pores of adsorbents and the dissociation pattern within the same solution chemistry was consistent. However, the order of E2>E1>EE2>E3 was observed for both GAC particles, conforming to the order of their octanol-water partition coefficients log P shown in Table 2.3. Herein, the log P values are indicative of the hydrophilicity of compounds. Less hydrophilic compounds have the greater log P value (Ruiz et al., 2010). In previous literature, it was reported that the P value was proportional to the adsorption performance directly (Baccar et al., 2012, Nam et al., 2014).

Table 5.5 The specific surface area normalisation of adsorption constant (ki) derived from the Freundlich model.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>k’ *100</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>EE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC-i</td>
<td>0.60</td>
<td>0.64</td>
<td>0.32</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>AC-ii</td>
<td>0.81</td>
<td>0.93</td>
<td>0.44</td>
<td>0.74</td>
<td></td>
</tr>
</tbody>
</table>

* RACs: the ratio of k or k’ values of ACi and AC-ii.
5.3.4 Influence of NOM

NOM was ubiquitous in water from the natural sources; its impact on adsorption performance of studied adsorbents was investigated using synthetic solution spiked with known 30 mg L\(^{-1}\) humic acid and natural water sample collected from the Regent's Park lake whose location was described in Figure 3.3, the corresponding characteristic was summarised in Table 5A.3.

From the isotherm simulation (Figure 5.4), Freundlich constants \(k_f\)-HA or \(k_f\)-natural derived from different water matrix for targeted estrogens as well as the percent reduction values, \(R_{\text{HA}}\) or \(R_{\text{natural}}\), namely the ration of \(k_f\)-HA or \(k_f\)-natural to \(k_i\) which was obtained in pure water (Table 5.6), were obtained for quantifying the influence of NOM. The lower percent reduction value indicated severe deterioration of adsorption capacity. Within both humic solution and natural surface water, the impairment of adsorption ability was observed. After analysing using the Turkey method of one-way ANOVA, the disparity of adsorption capacity in the three kinds of water matrix that were studied was statistically significant at the 0.05 level.
Table 5. Freundlich adsorption constants of estrogens for AC-i and AC-ii in humic acid solution and natural water. AC-i and AC-ii.

<table>
<thead>
<tr>
<th></th>
<th>Humic acid solution</th>
<th>Natural water matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k_{f-HA} (mg g^{-1}) (L mg^{-1})^{1/n}</td>
<td>1/n</td>
</tr>
<tr>
<td>AC-i</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>3.03</td>
<td>0.674</td>
</tr>
<tr>
<td>E2</td>
<td>3.05</td>
<td>0.691</td>
</tr>
<tr>
<td>E3</td>
<td>1.22</td>
<td>0.707</td>
</tr>
<tr>
<td>EE2</td>
<td>2.37</td>
<td>0.690</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Humic acid solution</th>
<th>Natural water matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k_{f-HA} (mg g^{-1}) (L mg^{-1})^{1/n}</td>
<td>1/n</td>
</tr>
<tr>
<td>AC-ii</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>2.85</td>
<td>0.693</td>
</tr>
<tr>
<td>E2</td>
<td>3.14</td>
<td>0.675</td>
</tr>
<tr>
<td>E3</td>
<td>1.42</td>
<td>0.681</td>
</tr>
<tr>
<td>EE2</td>
<td>2.27</td>
<td>0.694</td>
</tr>
</tbody>
</table>

*K_{f-HA} and K_{f-natural} are the k_f values derived from the humic acid solution and natural surface water;

**R_{HA} and R_{natural} are the ratio of k_{f-HA} to k_f and k_{f-natural} to k_f, respectively.
The rich aromatic content and lower polarity property of NOM suggested that their adsorption on carbon adsorbents was through π-π interaction and hydrophobic effect (Newcombe et al., 2002, Niederer et al., 2007), as the same as the driven force for the sorption of estrogens onto AC. Moreover, the concentrations of NOM in both synthetic humic acid solution and natural surface water were 2~3 orders of magnitude higher than the concentration of compounds of interest. Herein, the reduction of adsorption ability of adsorbents was further specifically ascribed to the competition of active sites and blockage of pores with the presence of NOM (Li et al., 2019).

Contrary to previous observation that ACs with a high proportion of small pores were more susceptible to impacts of NOM (Ebie et al., 2001), in the synthetic humic acid solution, the lower $R_{HA}$ values of all estrogens for AC-ii was noticed, ranging from 47.9% to 54.8%. However, a value over 82%, except for E3 with a $R_{HA}$ of 68.3% when AC-i was in use, indicated that the greater interference of humic acid acted on the AC-ii which has more mesoporous structure. To some extent, the result was ascribed to the size of humic acid and the pore structure of adsorbents. Compounds were most likely adsorbed to pores owning the similar size (Pan et al., 2008), the pores with a size ranging from 30 to 100 Å were preferred for the adsorption of humic acid (Ebie, 1995). So, AC-ii with larger mean pore size was prone to adsorb more humic acid and the blockage problem arose, while the adsorption of bulky humic acid was hampered by microporous AC-i due to the size exclusion effect. Therefore, more active adsorption sites were available on AC-i for uptake of estrogens. In addition to the pore structure, adsorbents with lower pH$_{pzc}$ value were favoured for capturing humic acid (Kołodziej et al., 2014). Consequently, as humic acid, which has a pKa value of 2-4, was adsorbed on ACs and was negatively charged when the solution has a pH value of 7-8 (which is the pH value of studied matrix), then it makes the adsorbents wrapped by
humic acid more hydrophilic and probably weakens the hydrophobic affinity between estrogens and GAC (Tagliavini et al., 2017).

In comparison with humic acid, nature induced NOM in surface water exhibited a more pronounced impact on deteriorating adsorption ability of AC-i and AC-ii, which was demonstrated by the $R_{\text{nature}}$ values less than 10.2% for the uptake of estrogen onto both GAC particles. The remarkable reduction was provoked by the hybrid fractions of NOM occurring in a natural environment. Humic acid is merely one fraction and has a relatively larger size than other components of NOM (Murray and Ormeci, 2018), while natural occurring NOM contains massive substances capable of getting access to smaller pores, which may also be suitable for adsorbing estrogens in the meantime. It was known that the total micropore volume of AC-i and AC-ii was close, so it also explained why their isotherms were similar in natural water matrix. According to the water characterasition determined for humic acid solution and natural surface water (Table 5A.3), the SUVA$_{254}$ of 6.7 L mg$^{-1}$ m$^{-1}$ for synthetic acid solution and 2.1 L mg$^{-1}$ m$^{-1}$ for surface water applied in this study was reported. Based on previous literature, the water sample with SUVA$_{254}$ values greater than 4 L mg$^{-1}$ m$^{-1}$ was indicative of its relatively high contents of hydrophobic, aromatic and high molecular NOM fractions, while water samples having a SUVA$_{254}$ value less than 3 L mg$^{-1}$ m$^{-1}$ was found to contain more hydrophilic, non-humic and low molecular weight NOM fractions (Ates et al., 2007; Han et al., 2013). Thus, the assumption that the natural water has a smaller NOM fraction was evidenced to some extent.

In the previous study, it was discovered that the more intensive competition arose with smaller fulvic acid (Hertzing et al., 1977). From the Freudlich parameters, it was discovered that $1/n$ values increased with the presence of NOM and rising complicated level of matrix, revealing a decline in heterogenity of adsrobert surface and being also
indicative of the reduction of high energy adsorption sites on account of the occupation of NOM molecules (Zhang et al., 2011). Additionally, the impact of equilibrium concentration of estrogens was evaluated by calculating $k_d$ and their percent reduction values ($R_{AC-i}$ or $R_{AC-ii}$) at two types of equilibrium concentration in humic solution and natural surface water (Table 5A.4). Superior adsorption performance for both adsorbents was observed at higher adsorbate equilibrium concentration with the presence of NOM, which it was hypothesised that a decreased ratio of NOM to targeted estrogens would inhibit the competition of NOM. Given the overall results, both AC-i and AC-ii could effectively achieve removal of estrogens from an aqueous environment. However, pre-treatment for removing the NOM is suggested for minimising the impairment of adsorption capacity due to the interferences from water matrix (Sbardella et al., 2018). In previous studies, a UV photolysis process has been successfully employed in removing NOM from natural water (Wang et al., 2000, Goslan et al., 2006b). Therefore, in the future work, the pretreatment of photolysis before the participation of GAC adsorption would be tested.
5.4 Conclusion

In this chapter, the batch adsorption experiments were conducted for studying the adsorption behaviour of estrogens onto two kinds of commercial GAC adsorbents. From the time-dependent investigation, the pseudo-second-order kinetic model was found to describe experimental data well, based on the correlation coefficient $R^2 > 0.99$. 

Figure 5. 4 Adsorption of isotherms in different water matrix: (A) for E1; (B) for E2; (C) for E3 and (D) for EE2.
and the agreement between the adsorption capacity derived from calculation and lab work, respectively; furthermore, the experimental data was found well fitted with an intra-particle diffusion model, and the rate-limiting step in the removal process was mainly in the surface diffusion stage. According to the GAC dosage experiment, the adsorption pattern of estrogens with both AC-i and AC-ii was better explained with the Freundlich isotherm model than the Langmuir isotherm model with excellent correlation coefficient. The Freundlich constants for the sorption of four estrogens on the two selected GACs in pure water ranged from 1.79–5.98 (mg g⁻¹) (L mg⁻¹)¹/n when the initial spiked concentration was 10 µg L⁻¹.

With the presence of NOM, the reduction in adsorption capacity of examined GACs was observed due to the site competition and pore blockage problems. For adsorbent A-i, the Freundlich adsorption constants dropped to 1.22–3.05 (mg g⁻¹) (L mg⁻¹)¹/n for the synthetic humic solution and 0.15 to 0.36 (mg g⁻¹) (L mg⁻¹)¹/n for natural water matrix. For adsorbent A-ii, the Freundlich adsorption constants dropped to 1.42–3.14 (mg g⁻¹) (L mg⁻¹)¹/n for the synthetic humic solution and 0.12 to 0.26 (mg g⁻¹) (L mg⁻¹)¹/n for natural water matrix.

After further specialising the impact of humic acid and natural surface water matrix, factors such as the size distribution of NOM fraction, pore structure and surface chemistry most likely induced the reduction of estrogen uptake to a different extent. Compared with natural occurring NOM, the adsorption ability of estrogens onto AC-i and AC-ii was less influenced by uniform humic acid molecules. That's ascribed to the loss of active adsorption site due to the occupancy of a small fraction of NOM components. Considering the lower reduction of adsorption ability induced by the natural water matrix, adsorbent AC-i was selected for further investigation of the performance of GAC sandwich biofiltration (GSBF) in Chapter 7.
Chapter 6 Photodegradation of free estrogens from water under irradiation of UV light

The results for Chapter 5 showed that the adsorption of estrogens onto GAC was subject to the NOM in the solution. Consequently, the setup of pretreatment such as photolysis before the BF system was suggested. For the primary aim at removing estrogens, the investigation into the photodegradation of estrogens under irradiation of UV irradiation was conducted in this chapter.

6.1 Introduction

Except for acting as an experienced application for being against waterborne pathogens (Kim et al., 2016), the photolysis reaction under the UV region was also studied for achieving mineralisation of organic compounds (Chen et al., 2013, Li et al., 2017b, Xu et al., 2017a). Herein, the photodegradation of hormones has also been carried out in previous studies and the destruction of surrogate estrogens was observed (Frontistis et al., 2015, Liu et al., 2003, Cedat et al., 2016, Li Puma et al., 2010, Pereira et al., 2012). In comparison with UV-based photocatalysis, the exemption of post-treatment such as the requirement to remove photocatalyst from treated water renders the photolysis process more suitable for practical application (Sanches et al., 2013).

Photolytic removal of organic compounds in aqueous solution was mainly achieved via direct and indirect photolysis processes (Oliveira et al., 2016). For the direct photolysis, the cleavage of chemical bonds occurred after the compound molecules absorbed the photon emitted by the light source (Liu and Liu, 2004). Nevertheless, the indirect photolysis was initiated via producing photoreactants in the presence of
photosensitisers and light irradiation, and then the obtained reactive species could further oxidize the examined pollutants (Chen et al., 2013).

The solution chemistry was one of the most important factors to affect the photodegradation performance. In order to scale up relevant techniques for practical use, the impacts of solution properties should be taken into account. Especially for the natural water matrix, the most common constituents such as NOM, nitrate and nitrite were considered as the most important photosensitisers (Lin and Reinhard, 2005a).

In the on-going research, there have not been investigations carried out for studying the photolysis mechanisms of E1, E2, E3 and EE2 simultaneously. Furthermore, most of the current studies evaluated the photolysis behaviour of pollutants in stationary installation but did not consider the influence of flow-through condition (Frontistitis et al., 2015, Rosenfeldt and Linden, 2004, Carlson et al., 2015), while the latter was more prevalent in practical treatment facilities for the huge demand of treated water.

In this chapter, the photolysis of estrogens under the irradiation of UV-light was studied. The photodegradation rate constants and the related parameters such as molar absorption coefficients and corresponding quantum yields were examined for depicting the photochemical degradation in detail. As a novelty, the installed system in terms of the operation mode was evaluated in this study for the first time. Besides, the photolytic degradation of chosen estrogens was also assessed in natural surface water with the aim to elucidate the relationship between water matrix and photolysis performance.
6.2 Methodology

6.2.1 Chemicals and installation of reactor

Aliquots (10 mL) of a mixed working standard solution of E1, E2, E3 and EE2 (1 mg L\(^{-1}\)), which was prepared in methanol following the method described in Section 3.3, was added into an empty 1 L volumetric flask. To be free of solvent effect, the methanol was evaporated before adding Milli-Q water (18 MΩ resistance) to the etched graduation marking. Then the free estrogen solution with a nominal concentration at 10 µg L\(^{-1}\) was finally obtained. The spiked concentration of targeted compounds were slightly higher than their natural occurring level (ng L\(^{-1}\)– µg L\(^{-1}\)) (Souissi et al., 2012) for allowing an explanation for their removal considering the limitation of the analytical instrument. The pH of Milli-Q water was determined as 6.58 (Chowdhury et al., 2011), which was also around the pH of estrogen solutions prepared using Milli-Q water. Consequently, 600 mL of prepared solution was taken and transferred into the pre-cleaned photoreactor whose dimension, structure and UV-C lamp information have been described in Section 3.5.

6.2.2 Irradiation experiments

The irradiation experiments were conducted in the Environmental Engineering Lab, Civil, Environmental and Geomatic Engineering Department (CEGE) of UCL. The stationary and up-flow modes of the estrogen solution were applied for investigating the percentage removal of estrogens under the irradiation of UV-C lamp. For the stationary mode, the inlet and outlet of the photolysis chamber, whose schematic diagram and dimension were presented in Section 3.5, was closed before adding the prepared 600 mL solution containing four model free estrogens with a concentration of 10 µg L\(^{-1}\), and the irradiation time was set as 15, 30, 40, 60, 120, 180 and 240 min; for up-flow mode, the peristaltic pump, whose flow rate has been calibrated,
connected to the inlet of the photolysis chamber. Then the solution was treated using flow rates of 15, 30, 45, 60 and 75 mL min\(^{-1}\) for evaluating the effect of flow rate on photodegradation of targeted estrogens, and consequently the residence times of 40, 20, 13.3, 10 and 8 min in the photolysis chamber were also verified based on the corresponding flow rate, respectively.

The impacts of naturally occurring NOM on photochemical degradation of estrogens was studied using the same experimental design described above but with spiked natural surface water collected from a lake of Regent’s Park, London (Figure 3.3). To avoid the impact of different pH values, the pH of spiked surface water was adjusted to 6.58 using NaOH and HCl. Additionally, based on the method developed by Shi et al. (2010), the surface water sample was sterilized by combining filtration through a 0.45 µm membrane filter and radiation under ultraviolet light for 30 min prior to the photolysis experiment for exempting overestimation of removal due to the presence of the enzymatic and microbial activity.

The spiked Milli-Q water and natural surface water with the same concentration of estrogens was put under dark condition (via being wrapped with aluminium foil) as a control group for verifying if there was a loss of compounds due to the ambient background.

Cooling water (16.3±0.7°C) was introduced into the outer jacket for preventing thermolysis as a result of overheating over the irradiation time, thus keeping the temperature of the liquid in the reaction chamber ranging from 34.5°C to 38.6°C.

All experiments in this chapter were conducted in triplicates.
6.2.3 Analysis of the samples

A sample with 500 mL volume was collected after the photolysis experiment and then filtered through a 0.45 µm membrane filter using the vacuum filtration apparatus. The filtered sample was concentrated and analysed according to the SPE protocol, developed derivatization and LC-MS method described in Section 4.2.2 and 4.2.3.

In addition, the water samples adopted for investigating the matrix influences were characterised in respect to water quality parameters (e.g. DOC, UV$_{254}$ and IC) following the method reported in Section 3.6.1). The transmittance of UV-light (UVT) at 254 nm to solution was calculated by (Zhang et al., 2021):

\[
\% \text{ UVT} = 10^{-A}
\]  

(6.1)

where A (cm$^{-1}$) is the UV$_{254}$ absorbance of solutions.

6.2.4 Rate constants, molar absorption coefficient and quantum yields

Rate constants for the photolysis of estrogens under the irradiance at wavelength 254 nm were determined using the pseudo-first order reaction kinetics which was commonly applied to describe the rate of photolytic removal of estrogen on the basis of published literature (Nasuhoglu et al., 2012, Whidbey et al., 2012).

The pseudo-first-order rate constants were calculated by the linear regression of natural logarithmic concentration of compounds against irradiation time as follows:

\[
\ln \frac{C}{C_0} = -k_1 t
\]  

(6.2)

Where $C_0$ (µg L$^{-1}$) and C (µg L$^{-1}$) are the concentration of estrogen at the beginning and concentration after exposure to irradiance for time t (min), respectively; $k_1$ is the pseudo-first-order rate constant (min$^{-1}$). The half-life ($t_{1/2}$) of compound in the photolysis process could be obtained via equation:
\[ t_{1/2} = \frac{\ln 2}{k_1} \]  \hspace{1cm} (6.3)

The absorption spectra of chosen estrogen were profiled over the range of 200-400 nm wavelength using the Agilent Cary 60 UV-Vis (Cheshire, UK) coupled with Cary WinUV software. The estrogen single solution with a concentration of 100, 50, 10, 5 and 1 mg L\(^{-1}\), was obtained via diluting each of their stock solutions and 5\% methanol was contained in final solutions. Each sample was loaded into a 1 cm path length quartz cuvette and its absorbance was measured against with the corresponding background solvent which contained methanol at the same concentration for correcting their respective baselines. The equation for calculating the molar absorption coefficient \(\varepsilon(\lambda)\) (M\(^{-1}\) cm\(^{-1}\)) of individual estrogen was as follows (Bonneau et al., 1991):

\[ a(\lambda) = \varepsilon(\lambda) \times M_{\text{estrogen}} \times l \]  \hspace{1cm} (6.4)

Where \(a(\lambda)\) (cm\(^{-1}\)) is the absorbance of solutions containing nominated molar concentrations \((M_{\text{estrogen}}, \text{mol L}^{-1})\) of individual estrogen with a cell path length \((l)\), being 1 cm in this study.
Figure 6. 1 the simplified schematic for calculating \( z \)—the length of path solution penetrated by the irradiance. (a) the simplified structure of photolysis chamber; (b) top view of photolysis chamber; (c) side view of photolysis chamber.

Apart from the molar absorption coefficient, quantum yield, \( \Phi (\lambda) \) (E mol\(^{-1}\)) was also determined to describe the mechanism of photodegradation using the equation proposed by (Schwarzenbach et al., 2016):

\[
\Phi (\lambda) = \frac{k_1(\lambda)}{k_s(\lambda)}, \tag{6.5}
\]

\[
k_s(\lambda) = \frac{E_p^0 a(\lambda)z}{a(\lambda)z} \tag{6.6}
\]

Where, \( k_1 \) (s\(^{-1}\)) is the pseudo-first-order rate coefficient obtained from the irradiance exposure at a wavelength of 254 nm; \( k_s \) value with a unit (E mol\(^{-1}\) s\(^{-1}\)) refers to the specific photon absorption rate of studied compound under the radiation region of \( \lambda \).

In equation 6.6, \( E_p^0(\lambda) \) in a unit of mE cm\(^{-2}\) s\(^{-1}\) is the photon fluence rate determined on the surface of solution; \( z \) is the path length (cm) solution penetrated by the
irradiance, according to the dimension information of photolysis reactor and diameter of lamp depicted in Section 3.5, it was assumed that the emission from the UV-C lamp was perpendicular to the surface of solution in the photolysis chamber without deviation. As presented in Figure 6.1, in the cylindrical chamber, the optical path length of solution travelled by the light ray as \( z \), which was also confirmed via the top view and side view of the system, being verified as 1.95 cm in this study.

6.2.5 Statistical analysis

All experiments in this study were conducted in triplicate for checking the repeatability. The statistical analysis was carried out using OriginPro 2019 software. The availability of pseudo-first-order kinetic in each irradiation experiment was evaluated via the linear fit option of the analysis method. The statistically significant differences on the operation mode (stationary and up-flow mode) and water matrix (MilliQ-water and surface water) accounting for the photolysis performance of examined estrogens were estimated using the one-way ANOVA method (\( \alpha=0.05 \)).

6.3 Results and discussion

6.3.1 Photolysis of estrogens in Milli-Q water

For dark controls of both stationary and up-flow operation mode for up to 4 h, no significant loss of estrogens was illustrated via the minor difference (\(-2.90 \% \) to \(1.45 \%\)) between the concentrations before and after reaction under the dark condition, which specifically indicated that the participation of hydrolytic and thermolytic destruction, biodegradation, and adhesion of estrogen to the inner wall of the photolysis chamber could be ignored.

The photolytic removal of E1, E2, E3 and EE2 in Milli-Q water matrix by the irradiance of UV-C lamp was indicated via the pseudo-first-order kinetics derived via plotting
\[ \ln(C/C_0) \text{ against irradiation time (Figure 6.2), the corresponding rate constants } (k_1), \text{ photodegradation half-life } (t_{1/2}) \text{ and correlation coefficients } (R^2) \text{ were reported in Table 6.1. The significant linear correlation coefficients (0.980-0.994) demonstrated that the obtained data fit well with the pseudo-first-order kinetics.} \]

Figure 6. 2 The photolytic removal of chosen estrogens in Milli-Q water matrix under different operational modes of the photolysis reactor. A) running in stationary mode; B) running in up-flow mode

For the photolysis of four representative estrogens, their removal performances were found to follow the order E1>E2> EE2> E3 in terms of the steepness of linear models, which was further evidenced by their pseudo-first-order rate constants and degradation half-life. E1 was degraded under irradiation at \( \lambda=254 \) nm with the rate constants 0.0177 min\(^{-1}\) for stationary mode, which corresponded to the degradation half-life of 39.8 min. In contrast to E1, E3 was discovered far more resistant to the direct photolysis with the rate constants 0.0023 min\(^{-1}\) under the identical exposure.
condition and furthermore approximately 7.6 times longer photodegradation half-life than that of E1 was shown. The acquired degradation rate constants of E2 and EE2 were similar and slightly higher than E3.

Table 6.1 Time-based pseudo-first-order rate constants ($k_1$), correlation factor ($R^2$), photodegradation half-life ($t_{1/2}$), quantum yields ($\Phi$) for the photolysis experiment of estrogens in Milli-Q water with stationary and up-flow mode under the irradiation of UV-C lamp.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Stationary mode</th>
<th>Up-flow mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_1$ (min$^{-1}$)</td>
<td>$t_{1/2}$ (min)</td>
</tr>
<tr>
<td>E1</td>
<td>0.0177 (0.994)</td>
<td>39.8</td>
</tr>
<tr>
<td>E2</td>
<td>0.0043 (0.993)</td>
<td>161.2</td>
</tr>
<tr>
<td>E3</td>
<td>0.0023 (0.980)</td>
<td>301.4</td>
</tr>
<tr>
<td>EE2</td>
<td>0.004 (0.988)</td>
<td>173.3</td>
</tr>
</tbody>
</table>

It was confirmed from previous studies that the photolytic removal of compounds was mainly associated with their decadic molar absorption coefficients and quantum yields.
at a given range of \( \lambda \) (Pereira et al., 2012). Therefore, these two parameters were investigated for explaining the differences in the photochemical destruction of selected estrogens in this study.

The spectra of studied estrogens within a range of 200-400 nm wavelength were detected, then the obtained molar absorption coefficient data implied the capacity of surrogate compounds absorbing the photon delivered by the light from installed UV-C lamp and played an important role in the photolysis reaction (Yuan et al., 2009). According to published work, the photon absorbed by targeted molecules could be capable of driving their photochemical reaction (Wells, 1972). As indicated by Figure 6.3, among the available irradiation sources including UVA, UVB and UVC in our lab, the absorbance of radiation for estrogen molecules was more abundant in the UVC section (100-290 nm) than regions UVA (320-400 nm) and UVB (290-320 nm) (Dale Wilson et al., 2012, Lindblad et al., 2020). Moreover, the negligible absorption probability in the visible light region was also noticed. Therefore, the irradiation used for investigating photolysis of estrogens was selected as UVC. As the most common commercially accessible UVC lamp emitting UV light with specific wavelengths of 253.7 nm was applied correspondingly. Similarly, the photolysis of estrogens under the irradiation of UVC light at 253.7 nm was also conducted in previous studies (Li Puma et al., 2010, Liu and Liu, 2004, Nasuhoglu et al., 2012, Trudeau et al., 2011, Zhang et al., 2010), and some of the results indicated the better photolysis performance achieved by UVC compared to UVB or UVA irradiation.
Figure 6. 3 Molar absorption coefficients of estrogens over a wavelength range of 200-400 nm.

The molar absorption coefficients of four selected estrogens at a given wavelength (λ) of 253.7 nm were presented in Table 6.2, the value of 402.4 M cm\(^{-1}\) for E1, 378.0 M cm\(^{-1}\) for E2, 212.7 M cm\(^{-1}\) for E3 and 315.6 M cm\(^{-1}\) for EE2 were summarised.

Table 6. 2 Molar absorption coefficients of targeted estrogens at λ=253.7 nm.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\varepsilon)(_{253.7,\text{nm}}) (M(^{-1}) cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>402.4</td>
</tr>
<tr>
<td>E2</td>
<td>378.0</td>
</tr>
<tr>
<td>E3</td>
<td>212.7</td>
</tr>
<tr>
<td>EE2</td>
<td>315.6</td>
</tr>
</tbody>
</table>
As another important parameter governing photolytic degradation rate, the quantum yield of chosen estrogens under UV-C lamp with \( \lambda = 253.7 \text{nm} \) was ranged from 0.013 to 0.056 mol E\(^{-1}\) under the stationary mode, where E1 exhibited the highest quantum yield while the values of the remaining three compounds were nearly the same, ranging from 0.013 to 0.016 mol E\(^{-1}\). Overall, the quantum yield values determined in this study are within the range obtained in other studies. For example, as reported by Lin and Reinhard (2005a), the quantum yields of E1, E2, E3 and EE2 under irradiation by the monochromatic light source with a wavelength of 254 nm were 0.0296, 0.0048, 0.0048 and 0.0048, respectively. Rosenfeldt and Linden (2004) and Mazellier et al. (2008) found 0.043/0.067 mol E\(^{-1}\) for E2 and 0.026/0.062 mol E\(^{-1}\) for EE2 under the exposure to the same wavelength. Although similar values were expected for the four chosen estrogens owing to their similar chemical structures, the differences observed in previous work and this study were surprising actually. It was assumed that the variation of functional groups on the C-17 position was the main reason. As emphasized by Ahmad et al. (2016) and Lin and Reinhard (2005a), the carbonyl group, which linked to the C-17 position of E1, was considered as one of the most important chemical groups for initiating the photochemical reactions.

In summary, as a result of both relative greater molar absorption coefficient and quantum yields achieved for E1, the considerable removal of E1 could be explained.

6.3.2 Influence of operation mode

The influence of the photolysis reactor operation mode on the degradation of estrogens was estimated by irradiating the same spiked Milli-Q water solution under the same condition besides changing a stationary mode to an up-flow mode. The degradation of the chosen estrogen in up-flow mode were closely analogous to that observed in stationary mode, following the same order E1>E2> EE2> E3. Regarding
the residence time as the time-span of irradiation, the photolysis rate constants obtained from the pseudo-first-order kinetics with significant correlation coefficients (0.981-0.995) for E1, E2, E3 and EE2 were 0.0233, 0.0088, 0.0039 and 0.0069 min⁻¹, respectively. Accordingly, their t₁/₂ values were over a range of 29.7-177.7 min. In addition, the quantum yields increased to 0.107 mol E⁻¹ for E1, 0.035 mol E⁻¹ for E2, 0.029 mol E⁻¹ for E3 and 0.034 mol E⁻¹ for EE2.

It was noteworthy that both larger photolysis rate constant and higher quantum yields for all four estrogens were determined in an up-flow mode in comparison with what derived from a stationary mode according to the data presented in Table 6.1. Therefore, it was indicated that the shorter irradiation time was required for reaching the same photochemical removal of targeted compounds in up-flow mode. Particularly, after the analysis using the one-way ANOVA method, the difference of k₁ between the stationary and up-flow mode for each estrogen was significant (p< 0.05).

With respect to the impact of stationary and flow mode, there has not been a relevant discussion reported on before. Herein, the flowing operation mode was assumed to increase the dissolved oxygen concentration of solution in the photolysis reactor compared with the stationary status of fluid. Subsequently, the presence of oxygen probably promoted the oxidation of organic compounds with UV exposure.

This assumption was also mentioned in Pailthorpe et al. (1973)’s study. Since in the presence of dissolved oxygen, hydroxyl radicals -OH could be produced by water molecules upon absorption of photons emitted by the UV lamp (Zhang and Li, 2014). The detailed reaction occurred as follows:

\[ H_2O + hv \rightarrow e_{aq}^- + H + \cdot OH + H^+ \]  \hspace{5cm} (6.7)
The oxidation potential of 2.80V owned by -OH was capable of increasing the decomposition percentage of estrogens via oxidation reaction via -OH (Buxton et al., 1988). The similar acceleration of photolytic removal was also observed in a study of mineralising of diafenthiuron, CGA-140408 and CGA-177960 under aerated condition (Keum et al., 2002). Additionally, the removal of organic compounds (e.g. 4-chlorophenol and aniline) was also found to be elevated in the presence of dissolved oxygen (Zhao et al., 2013, Du et al., 2011).

6.3.3 The impact of solution matrix

To understand the impacts of solution matrix on the photolytic decomposition of estrogens, the irradiation experiment with up-flow mode was conducted using the surface water spiked with the same 10 µg L⁻¹ mixed estrogens. Based on the characterisation of water samples (Table 6.3) in terms of the DOC, UV₂₅₄, SUV₂₅₄ and IC, the results indicated that the natural water matrix had DOC content of 3.75±0.25 mg L⁻¹, UV₂₅₄ of 0.067±0.002 cm⁻¹ and SUVA₂₅₄ of 1.79±0.14 L mg⁻¹ m⁻¹. The higher absorbance illustrated that the greater content of chromophore was contained in natural surface water, which further indicated that natural surface water was more sensitive to photon at λ=253.7 nm (Silva et al., 2016a, Silva et al., 2016b). From another aspect, the higher absorbance was likely indicative of the existence of inner filter effect of surface water matrix (Oliveira et al., 2016). Additionally, the relatively rich ion composition in the surface water matrix was also profiled. SUVA₂₅₄ was employed here to indicate the amount of organic compounds, the value obtained via dividing UV₂₅₄ absorbance by the DOC concentration could illustrate the dissolved aromatic carbon content in water samples (Weishaar et al., 2003). The higher SUVA₂₅₄ values refers to the greater molecular complexity such as higher aromaticity, on the other hand, it also demonstrated the decreased biodegradability (Hozalski et al., 1995).
Table 6.3 The water quality parameters of surface water in terms of DOC, \( UV_{254} \) and representative ions characterised by IC with the standard deviation.

<table>
<thead>
<tr>
<th>Water quality parameters</th>
<th>Surface water sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC (mg L(^{-1}))</td>
<td>3.75±0.23</td>
</tr>
<tr>
<td>( UV_{254} ) (cm(^{-1}))</td>
<td>0.0671±0.002</td>
</tr>
<tr>
<td>( SUVA_{254} ) (L mg(^{-1}) m(^{-1}))</td>
<td>1.79±0.14</td>
</tr>
<tr>
<td>Nitrite, NO(_2^−) (mg L(^{-1}))</td>
<td>N.A</td>
</tr>
<tr>
<td>Nitrate, NO(_3^−) (mg L(^{-1}))</td>
<td>6.63±0.61</td>
</tr>
<tr>
<td>Chloride, Cl(^−) (mg L(^{-1}))</td>
<td>74.23±5.68</td>
</tr>
</tbody>
</table>

Figure 6.4 described the time-based pseudo-first-order rate constants of the four surrogate estrogens in Milli-Q water and natural surface water matrix when the flow rate was fixed as 15 mL min\(^{-1}\). Contrary to the decline of photolysis removal for methyltriclosan presented by Liu et al. (2018), as a result of the drastically decreased transmittance of UV light (namely light-shielding effects) with the presence of NOM, the higher content of DOC and dissolved solids in natural water matrix did not impede the decomposition of estrogens in this study, instead a higher removal rate for all chosen estrogens was noticed. However, there were no statistically significant differences between MilliQ- water and surface water for E2 and E3 (\( p > 0.05 \)), while the significant enhancement was found for E1 (\( p < 0.05 \)) only at a 95% confidence interval. The degradation rate constants, correlation coefficients and \( t_{1/2} \) were calculated using the pseudo-first-order kinetics (Table 6.4). The contribution of solution
matrix to the acceleration of photodegradation rate could be calculated in accordance with:

\[
\text{Contribution of solution matrix (\%) = } \frac{(k_{1,\text{matrix}}-k_{1,\text{MilliQ water}})}{k_{1,\text{matrix}}} \times 100
\]  

(6.8)

Compared to the photolytic degradation in MilliQ-water, the degradation rate constants for E1, E2, E3 and EE2 were 0.03, 0.0098, 0.0055 and 0.0085 min\(^{-1}\), respectively; as a result, their \(t_{1/2}\) values were accordingly shortened to 23.1, 70.3, 126.8 and 81.2 min, respectively. The acceleration was attributed to the replacement of water matrix, and its contribution to the change of rate constants for targeted estrogens ranged from 12.8% to 40.8%. Similarly, the enhancement of photodegradation for E1, E2, and E3 in the presence of humic acids or fulvic acids, which were the most common constituents of NOM in water, has also been reported by Zhang et al. (2007), Chen et al. (2013), Oliveira et al. (2016) and Caupos et al. (2011). Furthermore, the great increase in phototransformation of EE2 in natural water under UV-light irradiation was also described by Canonica et al. (2008).
Figure 6.4 The time-based photolytic degradation rate constants of estrogens in Milli-Q water and natural surface water.

The NOM in water samples have dual effects on the behaviour of photochemical destruction: 1) the inhibitory effect. NOM is well-known as an absorber of UV-light, so that the available light for phototransformation of estrogens was diminished due to the photon competition of NOM or scattering of radiation; 2) the promotion effect. The transformation of NOM took place once they absorbed light and the property of acting as a photosensitiser allowed the further occurrence of indirect photolysis (Atkinson et al., 2011, Nasuhoglu et al., 2012, Canonica et al., 2008).

In this study, even though the occurrence of light-screening was proven on the basis of $\text{UV}_{254}$ value, the calculated UVT of 85.7% for spiked surface water and 94.5% for spiked Milli-Q water showed that the loss of available light was not massive from the difference of transmittance values less than 10%. Consequently, it was inferred that the inhibitory impact was limited to some extent.
Table 6. The pseudo-first-order parameters for photochemical removal of estrogens in surface water matrix.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Natural water</th>
<th>Contribution of solution matrix (%)</th>
<th>p-value, $k_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_1$ (min$^{-1}$)</td>
<td>$R^2$</td>
<td>$t_{1/2}$ (min)</td>
</tr>
<tr>
<td>E1</td>
<td>0.030</td>
<td>0.996</td>
<td>23.1</td>
</tr>
<tr>
<td>E2</td>
<td>0.0098</td>
<td>0.989</td>
<td>70.3</td>
</tr>
<tr>
<td>E3</td>
<td>0.0055</td>
<td>0.995</td>
<td>126.8</td>
</tr>
<tr>
<td>EE2</td>
<td>0.0085</td>
<td>0.990</td>
<td>81.2</td>
</tr>
</tbody>
</table>

As for the promotion effect, upon the absorption of photon, NOM worked as a photosensitiser due to the involvement of aromatic, carboxyl groups and carbonyl-type chromophores in its complex structure, and then the photo-induced transformation occurred with the production of reactive oxygen species (ROS), which specifically contained singlet oxygen($^{1}$O$_2$), superoxide radical (O$_2$•−), hydroperoxyl radicals (HO$_2$•) and hydroxyl radicals (•OH) (Alegria et al., 1999, Liu et al., 2018, Atkinson et al., 2011, Caupos et al., 2011). The emerging ROS subsequently participated in the oxidation reaction with targeted compounds (Leech et al., 2009):

$$\text{DOC} + O_2 + h\nu \rightarrow \text{ROS}_{\text{DOC}}$$  \hspace*{1cm} (6.9)

$$\text{ROS}_{\text{ss}} = \text{ROS}_{\text{DOC}} + \text{ROS}_{\text{estroge}}$$  \hspace*{1cm} (6.10)
ROS_{ss} + estrogen \rightarrow \text{products}. \quad (6.11)

The opposite impacts in terms of the presence of NOM, enhancement or hindrance observed in different studies might be ascribed to the types and content of the NOM used in studies (Atkinson et al., 2011, Ge et al., 2009, Zhang and Li, 2014). For example, the inverse correlation between aromaticity of humic substances and the impact on the photolysis removal rate constant was observed by Silva et al. (2016b), which meant that the humic substances with more aromatic or chromophoric groups might induce more suppression on the rate of photodegradation.

Apart from NOM, the nitrate and nitrite were also regarded as important photosensitisers due to their capacity to produce the photoreactants after exposure to the light, and then affect the photolysis of organic compounds (Oliveira et al., 2016, Chowdhury et al., 2011, Hao et al., 2019). In assayed surface water sample, the concentration of nitrite was negligible while the average concentration of nitrate was characterised as 6.63 mg L^{-1}. As discussed by Chen et al. (2013), once nitrate absorbed the light within the UV range (\lambda< 350 nm), its photolysis reaction took place and further •OH was formed according to the following reactions (Machado and Boule, 1995):

\begin{align}
\text{NO}_3^- + h\nu &\rightarrow \text{NO}_2^- + O \quad (6.12) \\
\text{NO}_3^- + h\nu &\rightarrow \text{NO}_2 \cdot + O \cdot^- \quad (6.13) \\
O \cdot^- + \text{H}_2\text{O} &\rightarrow \text{OH} + \text{OH}^- \quad (6.14)
\end{align}
The low molar extinction coefficient of nitrate within the UV–visible wavelength range (the maximum value was determined as 7.2 M$^{-1}$ cm$^{-1}$ at $\lambda$=302 nm) indicated that the light-shielding impact could be neglected (Machado and Boule, 1995). Hence, the influence of nitrate on photodegradation was simpler compared with the dual effect of NOM.

Additionally, being one of the most prevalent constituents in studied water, the chloride ion (Cl$^-$) was also discovered to behave with dual effects in previous publications (Zhai et al., 2008, Mihas et al., 2007, Oliveira et al., 2016). Specifically, under irradiation of light with $\lambda$ >200 nm, the Cl$^-$ in solutions was found capable of inducing the formation of single oxygen ($^{1}$O$_{2}$) and then increased the photolysis rate of florfenicol and thiamphenicol (Ge et al., 2009). However, in some other research, the chloride ion could scavenge hydroxyl radicals in solutions and exhibit the suppression of the indirect photolysis process (Sornalingam et al., 2016). In the light of experiments conducted by Ge et al. (2009), the effects of Cl$^-$ on the photolysis process was light source-dependent.

In this study, it was difficult to calculate the detailed positive or negative impacts on the direct and indirect photolysis of estrogens induced by each component in natural water matrix due to its complicated constitution. Thus, more investigations considering this issue in the future are required for illustrating the mechanisms of photochemical destruction of estrogens comprehensively. Therefore, it could be concluded that the enhancement effect on photolysis of estrogens resulted from the surface water matrix was more remarkable than its inhibitory effects in this study.

In the UV photolysis process, though the removal of estrogens was observed in both Milli-Q water and more complicated natural water matrix, the degradation percentage
of E1, E2, E3 and EE2 was still limited if the irradiation time would not be prolonged, being 69.6%, 38.8%, 24.5% and 33.6% under UV-C irradiation for 40 min. However, the extension of irradiation time was deemed to increase the financial budget and energy cost drastically. So, it may be more appropriate to add an extra barrier with low cost demand such as biofiltration process to achieve effective removal of estrogen. In a previous study, the integration of UV pretreatment with biofilter was found to provide 60% extra removal compared with the sum of performance behaved by the single process of UV photolysis or biofilter.

6.4 Conclusion
In this chapter, the removal results of estrogens E1, E2, E3 and EE2 under irradiation of UV-C lamp were well-described by the pseudo-first-order kinetics with correlation factors $R^2 > 0.981$. According to their photolysis rate constants and $t_{1/2}$, the order of recalcitrance to the employed UV-light followed E3>EE2>E2>E1. Out of all studied estrogens, E1 had the largest molar absorption coefficient of 402.4 M$^{-1}$ cm$^{-1}$ and quantum field of 0.065 mol E$^{-1}$ at $\lambda$=253.7, which further explained that E1 was more prone to photodegradation than the other three estrogens under the identical exposure conditions.

The operation mode of the photolysis reactor (stationary and up-flow mode) was found to induce the significant difference on the photolytic performance of all selected compounds, whose $t_{1/2}$ values were respectively shortened to 29.7 min, 78.8, 177.7 and 100.5 min for E1, E2, E3 and EE2 using the up-flow mode. Compared with the $t_{1/2}$ calculated for the stationary mode (39.8 min for E1, 161.2 min for E2, 301.4 min for E3 and 173.3 min for EE2), the increased time-based rate constants in the flow-through system were mainly attributed to higher content of dissolved oxygen, which promoted the oxidation of estrogens.
Simultaneously, the impact of surface water matrix on the photolysis of estrogens was also assessed comparing the rate constants derived in the use of spiked Milli-Q water. The obtained short $t_{1/2}$ ranging over 23.1 to 126.8 min indicated the enhancement of estrogen photodegradation with involvement of surface water matrix, even though the statistically significant difference was only attained for E1. It was assumed that the most common constituents such as DOM, nitrate and chloride ion worked together under the UV-C irradiation to achieve the overall positive impact on the photolysis performance.

However, it was also noticed that the degradation percentage of E1, E2, E3 and EE2 was still limited if the irradiation time would not be prolonged, being 69.6%, 38.8%, 24.5% and 33.6% under UV-C irradiation for 40 min. Therefore, in the next chapter, the installation of the sequent BF system was proposed for improving the removal of estrogens.
Chapter 7 Removal of free estrogens using BF or photolysis-BF hybrid system

7.1 Introduction

According to the results obtained in Chapter 5, the elimination of studied free estrogens via the adsorption onto GAC particles purchased from Chemvrion and Darco was over 90% in pure water with a GAC dose amount of over 500 mg L\(^{-1}\) after contacting for 24 h. As described in previous literature, the application of GAC was also evidenced to be promising for the removal of pharmaceuticals and endocrine disrupting chemicals (Park et al., 2020, Fuerhacker et al., 2001, Jiang et al., 2017b, Hu et al., 2016). However, there are also a few drawbacks for the direct usage of GAC in practical treatment plants: 1) expensive regeneration of used GAC adsorbents; 2) the negative impact of NOM in water matrix on its adsorption capacity (Weng and Hsu, 2008, Sbardella et al., 2018).

Slow sand filtration (SSF) is one of the most ancient treatment technologies and is still utilised for improving water qualities on account of its easy operation, simple maintenance and cost-effective characteristics (Campos et al., 2002, Li et al., 2018). The mechanisms of attenuating pollutants for SSF is mainly ascribed to the biological, physicochemical and physical interaction which occurs during the operation of system (Anderson et al., 2009). The biodegradation process primarily takes place on the surface of media bed and within the upper part, close to the solution-bed boundary, at which point the reddish brown colour and gelatinous layer called Schmutzdecke is developed at the beginning after a few weeks and provides the site for biological activity (Ranjan and Prem, 2018, Huisman and Wood, 1974b). The ripened cake layer usually consists of alluvial mud, organic matter, bacterial, diatoms, zooplankton and
thread-like algae (Ranjan and Prem, 2018), accounting for the attachment and degradation of pollutants. The detailed dominant phyla of microorganisms can be identified via sequencing technology based on the extracted DNA from samples.

In previous studies, SSF has been applied for the removal of pharmaceuticals (D’Alessio et al., 2015, Pompei et al., 2016, Li et al., 2018), organic micro-pollutants (Escola Casas and Bester, 2015) and pathogens (Seeger et al., 2016) and its removal performance varied dramatically. Regarding estrogen compounds, their limited removal in SSF was improved significantly via augmentation with the isolated estrogen-degrading bacteria or by adding household bleach, while the percentage of removal not exceeding 80% was also observed (Haig et al., 2016, Kennedy et al., 2013). Therefore, the SSF-based technique is in need of further optimisation for achieving better performance of organics.

Since the GAC-sand dual media SSF was proposed by Bauer et al. (1996) for removing pesticides, the GAC-containing biofiltration (BF) was also reported as a promising method attributing to the excellent removal of PPCPs and antibiotics in recent years (Li et al., 2018, Altmann et al., 2016, Xu et al., 2021). Furthermore, the integration of GAC with natural source medium as filter bed was also expected to diminish the capital cost effectively. The involvement of GAC layer in dual-media filtration provides suitable sites for microbial colonization and promotes the biodegradation process; adsorption function is also introduced for capturing biodegradation recalcitrant compounds (Li et al., 2018, Shirey et al., 2012).

Additionally, the degradation of estrogens under UV-C light was achieved in Chapter 6, though the poor removal of E2 (38.8%), E3 (24.5%) and EE2 (33.6%) was also observed, thus the sequent BF was designed as an extra barrier to promote the
elimination of targeted estrogens and also scavenged the intermediates produced from the photolysis process, which might still exhibit estrogenic potency (Olmez-Hanci et al., 2015, Whidbey et al., 2012).

In this chapter, the modified continuous down-flow SSF with addition of GAC as a middle layer of media bed, also called GAC sandwich biofiltration (GSBF), was operated solely, or in combination with, photolysis pre-treatment at the laboratory scale simultaneously, to investigate the elimination of free estrogens E1, E2, E3 and EE2 in natural surface water matrix. For comparison, the BF units were comprised of one mono-sand filter and three GAC-sand sandwich filters respectively containing 8 cm, 16 cm and 24 cm thickness of GAC media layer. Specifically, aside from the removal of estrogen compounds, which were spiked at a constant concentration in influent, the water quality parameters, variation of head loss and accumulation of biomass, estrogenic activity were also investigated systematically for evaluating the feasibility of designed systems.

7.2 Methodology

7.2.1 Configuration and composition of system

The whole system primarily consisted of two feeding reservoirs (I&II) with volume capacity of 15 L, one photolysis reactor, eight filter columns, three peristaltic pumps, one outflow tank and each apparatus was connected with PVC tubing (3 mm ID).

The photolysis reactor with UVC irradiation source and bench-scale PVC filter columns embedding sampling ports and piezometers were designed following the descriptions in Sections 3.5 and 3.6.1, in which their material and dimension information were introduced in detail. As shown in Figure 7.1, two groups of four filter columns dedicated
to different composition of media were setup, and here the photolysis reactor was installed as the pre-treatment site coupling with one of the BF groups, for examining the performance of a photolysis-BF hybrid system. The filters were wrapped during the whole operation time to prevent alterations from light as shown in Figure 3.2.

In each filter unit, the media bed with a total of 55 cm depth, including 5 cm of a fine gravel support layer, was packed into each PVC column, being abbreviated as F1, F2, F3, F4, F5, F6, F7 and F8, respectively. Thereinto, the detailed media constitution of each filter from the upper layer to the drainage channel was as follows:

F1 & F5: 50 cm sand+5 cm gravel support;
F2 & F6: 15 cm sand + 8 cm GAC+27 cm sand+5 cm gravel support;
F3 & F7: 15 cm sand+ 16 cm GAC+19 cm sand+5 cm gravel support;
F4 & F8: 15 cm sand+ 24 cm GAC+11 cm sand+ 5 cm gravel support.
The schematic cross section of the designed BF system with and without photolysis pre-treatment process for removing free estrogens. F1 & F5: SSF; F2 & F6, F3 & F7 and F4 & F8: GSBF with 8, 16 and 24 cm thickness of GAC layers. (Flow rate of photolysis process: 15 mL min\(^{-1}\); flow rate of BF effluents: 1 mL min\(^{-1}\)).

Filter columns F1, F2, F3 and F4 were set for testing the sole BF system with different amount of GAC, while the treated water out from the drainage outlet of columns F5, F6, F7 and F8 went through the system combing photolysis reactor and the same BF units as F1, F2, F3 and F4 together.

The fine sand was taken from the Soil’s Laboratory (Civil, Environmental and Geomatic Engineering department, UCL) and GAC was purchased from Chemviron Carbon. Their surface morphology was captured using a focused ion beam microscope (FIB-SEM, Carl Zeiss XB1540) at 25 kV from London Centre for Nanotechnology (LCN, Bloomsbury) at different magnifications. Moreover, GAC
particles were also characterised in terms of pore size distribution, specific surface area ($S_{\text{BET}}$), pH value, point of zero charge ($pH_{\text{PZC}}$) and functional groups on their surface, the detailed methods were summarised in Sections 3.4.1, 3.4.2 and 3.4.3. As another important factor for the performance of BF, the grain size distribution of fine sand and GAC was analysed according to the methodology described in Section 3.6.2.

The fine sand and GAC were washed clean using deionised water prior to assembly of filters. The overflow port (Figure 3.2) located 50 mm below the top opening of the filter column allows excessive water to flow back to the feeding reservoir I or II, keeping 10 cm of water level above the filter bed. The drainage outlet was set at the bottom of columns and connected with silicone tubing (4.8 mm ID) coupled with a hose clamp for adjusting the flow rate and collection of treated water.

7.2.2 The operation of treatment system

The treatment system involving the photolysis process and BF units were operated continuously in the Environmental Engineering Laboratory of CEGE, UCL from July 2018 to October 2018 with a constant ambient temperature ($22.4 \pm 3 \, ^{\circ}\text{C}$). Through the operation of system, the flow rate of effluent was constantly fixed at 1 mL min$^{-1}$, which corresponded to the filtration rate of 0.053 m h$^{-1}$, being within the typical rate of 0.04–0.4 m h$^{-1}$ applied for investigating the treatment behaviour of SSF (Li et al., 2018, Xu et al., 2021, LeChevallier and Au, 2004, Cleasby and Logsdon, 1999). The flow rate was monitored three times a day by measuring the volume of effluent in a given time using a graduated cylinder. The calibration of flow rate back to 1 mL min$^{-1}$ was carried out if an alteration of flow rate was observed.

The whole operation period was divided into two stages: 1) maturation; 2) operation with estrogen spike. For the maturation stage, photolysis process was suspended and natural surface water from a lake of Regent’s Park, whose location was presented in
Figure 3.3, was used as the ripen agent of BF. At this stage, estrogens were not spiked in the feeding water. To check if the maturation was reached, the turbidity and removal of total coliforms and *E. coli* abundance were determined weekly. Once the BF was ripened, the turbidity of effluent of SSF should be less than 1 NTU and the removal of total coliforms and *E. coli* could be over 99% (Tebbutt, 1997).

After the maturation of SSF and GSBF, the photoreactor was switched on and connected with BF, the effluent undergoing the photolysis process with a flow rate of 15 mL min\(^{-1}\) was sent to the feeding water reservoir II, acting as the influent of sequential BF units in the combined system, namely influent 2 in the following contents of this chapter. To differentiate this, the estrogens spiked surface water delivered to the single BF unit was marked as influent 1. Due to the sensitivity limitation of detection method, the surface water dosed with the free estrogens E1, E2, E3 and EE2 at a concentration of 10 µg L\(^{-1}\) was employed as the influent water and was delivered to the system using the peristaltic pump. This spiked concentration was within the range of 0.3–50 µg L\(^{-1}\) for removal of estrogens reported by previous studies (D’Alessio et al., 2015, Zhang et al., 2017). In order to reduce the influence of methanol, the mix stock solution of E1, E2, E3 and EE2 with a concentration of 1000 mg L\(^{-1}\) was diluted to the working solution at 1 mg L\(^{-1}\) with Milli-Q water and then diluted the targeted concentration in surface water with a volume of methanol less than 0.1 vol %. In the second operation stage with spike of estrogens, the BF systems with and without photolysis pretreatment were continuously run for 2 months, and the fresh water dosed with estrogens was recharged into the influent reservoir depending on the sampling schemes and the travel time of the liquid in the filters.

During the course of operation, in order to guarantee the concordance of influent and effluent sample obtained from the BF, the sampling schemes were carried out
following the hydraulic retention time (HRT ≈13.6 h) estimated via the tracer test and the sampling strategies for sample analysis described in Sections 3.6.3 and 3.6.5, respectively. More specifically, 200 mL influents and effluents of all filters and photolysis reactor were collected on a weekly basis for the measurement of water quality parameters (e.g. turbidity, UV254, DO, conductivity, pH, DOC and IC), microbial analysis, chlorophyll-a and estrogenic potency determination. Another 600 mL of influents and effluents for measuring the concentration of targeted estrogens using LC-MS coupling with SPE preconcentration were sampled. For the BF system, the collection of effluents should be implemented after taking influents for at least 13.6 h prior to the recharge of fresh water in feeding reservoirs.

7.2.3 Analytical methods

The analysis of influent and effluent of the treatment system (BF, photolysis and photolysis-BF combined system) was proceeded in terms of water quality parameters including the concentration of most common ions (e.g. nitrite, nitrate, phosphate and ammonium), DOC, pH, conductivity, turbidity, UV_{254} and DO according to the methodology summarised in Section 3.7.1. Then, the microbial abundance (e.g. total coliforms and *E.coli*), concentration of chlorophyll a, estrogenic potency (EP) of influent and effluent as well as the biomass binding to media indicated by the total protein concentration were determined following the method described in Sections 3.7.2., 3.7.3 and 3.7.4.

Additionally, the influent and effluent of system with the volume of 600 mL were taken and the concentration of estrogens was profiled according to the method provided in Chapter 4.
7.2.4 Statistical analysis

The removal data with respect to water quality parameters, microbial abundance (e.g. total coliforms and *E. coli*), concentration of chlorophyll and estrogenic potency was processed and calculated using Excel 2016. The figures representing the results in this chapter, one-way ANOVA tests accounting for the significance of difference between results and Pearson correlation analysis indicating the correlation between removal performance and factors influencing the performance of the system were all conducted using Origin 2019b.

7.3 Results and discussion

7.3.1 The characterisation of media

The surface morphology and physicochemical properties (e.g. $S_{\text{BET}}$, pore characterisation, pH, $pH_{\text{pzc}}$ and surface functionalities) of GAC, which was purchased from Chemviron Carbon, were detailedly provided in Section 5.3.1. Similarly, the surface morphology of fine sand particles was also captured using the focused ion beam microscope (FIB-SEM, Carl Zeiss XB1540). As presented in Figure 7.2, sand particles are smooth and without porous structure, showing a higher level of roundness and sphericity by comparison with GAC.
Subsequently, according to the grain analysis derived from the sieving test (Figure 7A.1), the size range 0.15-0.60 mm for fine sand and 0.30-1.60 mm for GAC particles were obtained after the shaking process. Based on each sieve size and percentage (by weight) of media samples passing through them, grain size distribution for fine sand and GAC are presented in Figure 7A.1. Then, the characteristics of media grain were calculated and summarised in Table 7.1.

Figure 7. 2 Morphology of fine sand particles under various magnifications.
Table 7. 1 Grain characteristics of fine sand and GAC selected for the BF system.

<table>
<thead>
<tr>
<th>Media analysis</th>
<th>GAC Chemviron Carbon</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective size ($d_{10}$, mm)(^1)</td>
<td>0.60</td>
<td>0.24</td>
</tr>
<tr>
<td>$d_{60}$, mm(^1)</td>
<td>1.06</td>
<td>0.38</td>
</tr>
<tr>
<td>Uniformity coefficient(^2)</td>
<td>1.77</td>
<td>1.58</td>
</tr>
<tr>
<td>Size range (mm)</td>
<td>0.30-1.60</td>
<td>0.15-0.60</td>
</tr>
<tr>
<td>Density (kg m(^{-3}))</td>
<td>628</td>
<td>1742</td>
</tr>
</tbody>
</table>

\(^1\) Effective size ($d_{10}$) is defined as the grain size modeled by distribution analysis where 10% media (by weight) pass through; $d_{60}$ is the grain size where 60% media (by weight) could pass through.

\(^2\) Uniformity coefficient is the ratio of $d_{60}$ to $d_{10}$.

The $d_{10}$ and uniformity coefficient of fine sand was 0.24 mm and 1.58, being in line with the typical grain size range from 0.15 mm to 0.30 mm and the uniformity coefficient less than 4 applied to slow sand filter (Huisman and Wood, 1974a). A previous study demonstrated that the internal diameter of the filter column, at least 25 times larger than the average sand grain size, could minify boundary disturbance in down-flow filters (Klotz and Moser, 1974). Apparently, the parameters of media and the filter system in this study also met corresponding requirement. Moreover, the ratio of PVC column ID to the diameter of media used as filter bed was over 50, being effective to prevent the wall and channeling effects (Jiang et al., 2017a, Klotz and Moser, 1974).
7.3.2 Hydraulic retention time

The characterisation of HRT for the examined biofiltration system was proceeded using the NaCl solution prepared in dechlorinated water with an initial conductivity of 1509 µS cm⁻¹ and outlet flow rate of 1 mL min⁻¹, according to the method described in Section 3.6.3. The result of the tracer test showing the variation of conductivity along with time was shown in Figure 7.3 (a). It was noticed that the movement of NaCl solution in the fine sand filter was faster than that in GSBF and the rise of conductivity was first detected in its effluent. It was inferred that the porous structure of GAC made the higher pore volume and longer travel path in media bed than sand, retarding the penetration of NaCl solution. This inference was evidenced as the total pore volume of media bed was respectively measured as 408.0, 392.8, 370.5 and 334.7 cm³ for 24 cm GSBF, 16 cm GSBF, 8 cm GSBF and fine sand filter following the method described in Section 3.6.3. In addition, the occurrence of dramatic rise in conductivity against nearly one pore volume was observed for all examined filters in Figure 7.3 (b), this flow pattern was expected to fit with the plug flow model according to the previous study (Elliott et al., 2008) and allowed the same retention time for all elements in the fluid to travel through the filter system (Sabogal-Paz et al., 2020). It was also demonstrated that the existence of GAC particles did not lead to the formation of tortuous flow in the reactor for this study. Correspondingly, the conductivity of effluents from the sand filter and GSBF reached homogeneity after 815±10 min (≈13.6 h). Therefore, this HRT was confirmed for fulfilling the sampling schemes when the flow rate of effluent was 1 mL min⁻¹.
Figure 7.3 The tracer analysis for the biofiltration units with various compositions. (a) residence time analysis and (b) pre volume analysis.
7.3.3 Operation of the treatment system

After continuous operation using the surface water collected from a lake in Regent’s Park as the ripening agent for four weeks, the maturation of all biofilters was indicated by the removal percentage of Total coliforms and *E.coli* greater than 99%. It was noticeable that the ripening of GSBF was finished by the fourth week, while the shorter time of about three weeks was merely required for fine sand filters. As a result, the biologically active zone including *schmutzdecke* occurring at water-media interface was established and the specialised biofilm was with a sticky texture and reddish-brown as shown in Figure 7.4.

![Figure 7.4](image1.png)

Figure 7.4 The development of schmutzdecke layer during the course of maturation period. (a) Prior to the maturation of filters; (b) during the course of maturation; and (c) completion of maturation.

The estrogen spiked surface water was then applied as the feeding water of the BF or photolysis-BF system continuously for 8 weeks. Over the time of operation period, the pH, conductivity, TDS, concentrations of ammonium, nitrite, nitrate and ammonium, DO, turbidity, head loss, the abundance of total coliform and *E.coli* and biomass were analysed.
7.3.4 Water quality parameters

As presented in Table 7A.1, the pH of influents and effluents for the studies treatment system was determined within the range of 7.91–8.49, being within the suitable range between 6.5 and 8.5 recommended by WHO (Organization, 1993). The average pH results for each filter showed that the increase of alkalinity in effluents was observed, which was analogous to Sabogal-Paz et al. (2020), Andreoli and Sabogal-Paz (2020) and Young-Rojanschi and Madramootoo (2014a)’s finding, and it was assumed that the leaching of filtered media took place after being in contact with the influent water for long HRT such as 14.3 h used in Sabogal-Paz et al. (2020)’s study, leading to the increased pH in filtered water to some extent. Similarly, the HRT of 13.6 h adopted in this study was likely to account for the rise of pH value in treated water.

The TDS and conductivity were usually used to indicate the content of dissolved solids and salinity in water samples (Rusydi, 2018). As shown in Table 7A.2, the conductivity of 936–1070 µs cm⁻¹ and the TDS of 468−537 mg L⁻¹ were respectively determined for the effluents of treatment systems and these two parameters displayed good linearity with the correlation coefficient $R^2 > 0.99$ in this study. The same correlation was also discovered in previous studies (Walton, 1989), and conductivity was generally regarded as a useful indicator of TDS although the relationship might alter due to the complicated solution chemistry (Hayashi, 2004, Thirumalini and Joseph, 2009). The small reduction of both conductivity and TDS in treated water of all filters except for F6 as illustrated (Figure 7.5) according to the difference of conductivity and TDS between the effluents and influents water, however, no significant difference was found between filters and between influents and effluents with $p>0.05$. The same
phenomenon was also profiled in Pompei et al. (2016)’s study using the same source water.

The most common ions such as ammonium, nitrite, nitrate and phosphate were measured and the data is summaried from Table 7A.4 to Table 7A.7. The concentration of ammonium and nitrite in effluents was found below the detection limit of IC chromatography, while the average concentration of nitrate and phosphate in effluents of the treated system ranged from LOD to 5.21 mg L$^{-1}$ and LOD to 11.288 mg L$^{-1}$, respectively.

![Figure 7.5](image)

Figure 7.5 The difference between the effluents and influents of treatment system on the TDS and conductivity over the operation course.

The one-way ANOVA analysis coupling with the Turkey tests showed that no significant difference was observed between the filters with different media compositions and the BF system with or without photolysis pretreatment regarding the aforementioned water quality parameters (p>0.05).
The DO concentration in influents and effluents is listed in Table 7A.8. The reduction of DO concentration in treated water demonstrated the consumption of oxygen in filters and was likely due to the occurrence of biological development and activity in the filter system (Young-Rojanschi and Madramootoo, 2014a). The reduction of DO was 0.24-0.40 mg L\(^{-1}\) for single BF units and 0.086-0.19 mg L\(^{-1}\) for the photolysis-BF hybrid system, a similar range of DO consumption in biofilters for removing antibiotics was reported by Xu et al. (2021). In addition, the decrease of about 0.19 mg L\(^{-1}\) DO content in the photolysis process was also noticed, and it was deduced that a proportion of DO was transformed to oxygen-containing reactive species and participated in the photolysis process under UV irradiation, as reported in the previous study (Varanasi et al., 2018, Lee and Yoon, 2008). At the end of the operation stage, the media sample was respectively collected from the different depths of the media bed (e.g. 47.8-48.2 cm, 31.3-31.7 cm, 14.8-15.2 cm and 0-1cm) for the total protein analysis, which was indicative of the biomass concentration on the longitudinal direction. As shown in Table 7.2, the abundance of total protein at the upper position of media bed implied the relatively higher biomass concentration and was also the backup for the likelihood of DO consumption due to biological activity. Furthermore, the biological zone was found not only confined to the upper layer of sand but formed throughout the studied filters, though the reductive abundance of biomass with the depth of media bed was also observed. This observation was in accordance with the work described by Lee et al. (2014) and Carpenter and Helbling (2017). Overall, the reduction of DO was minimal compared with its initial concentration, and no conversion of nitrate to nitrite was observed in the light of their concentration in the sample, thus the absence of anoxic condition, which was not desired for biofiltration operation, was confirmed (Sabogal-Paz et al., 2020).
Table 7.2 | The concentration of total protein (mg L\(^{-1}\)) at different depth of media bed after the end of system operation.

<table>
<thead>
<tr>
<th>Filters</th>
<th>System configuration</th>
<th>47.8-48.2 cm</th>
<th>31.3-31.7 cm</th>
<th>14.8-15.2 cm</th>
<th>0-1 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total protein</td>
<td>SD</td>
<td>total protein</td>
<td>SD</td>
<td>total protein</td>
</tr>
<tr>
<td></td>
<td>(µ g g(^{-1}))</td>
<td>(µ g g(^{-1}))</td>
<td>(µ g g(^{-1}))</td>
<td>(µ g g(^{-1}))</td>
<td>(µ g g(^{-1}))</td>
</tr>
<tr>
<td>F1</td>
<td>4.59</td>
<td>0.30</td>
<td>4.36</td>
<td>0.16</td>
<td>10.57</td>
</tr>
<tr>
<td>F2</td>
<td>6.89</td>
<td>0.26</td>
<td>6.64</td>
<td>0.18</td>
<td>8.16</td>
</tr>
<tr>
<td>F3 BF</td>
<td>6.30</td>
<td>0.04</td>
<td>7.55</td>
<td>0.10</td>
<td>4.85</td>
</tr>
<tr>
<td></td>
<td>(carbon &amp; sand)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>5.31</td>
<td>0.42</td>
<td>13.05</td>
<td>0.41</td>
<td>6.13</td>
</tr>
<tr>
<td>F5</td>
<td>5.48</td>
<td>0.99</td>
<td>7.91</td>
<td>0.85</td>
<td>5.86</td>
</tr>
<tr>
<td>F6</td>
<td>5.33</td>
<td>1.40</td>
<td>4.55</td>
<td>0.58</td>
<td>7.03</td>
</tr>
<tr>
<td>F7 BF</td>
<td>6.49</td>
<td>1.66</td>
<td>15.96</td>
<td>1.27</td>
<td>4.18</td>
</tr>
<tr>
<td></td>
<td>(carbon)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F8</td>
<td>4.31</td>
<td>0.16</td>
<td>10.99</td>
<td>0.73</td>
<td>3.63</td>
</tr>
</tbody>
</table>

Note: SD = Standard deviation

For the turbidity (Table 7A.9), the removal performance yielded by the photolysis process and filters with different media compositions varied. The poor removal of photolysis (-11%), F1 (29.6%) and F5 (31.4%), and the superior performance of GAC-sandwich filters was evidenced with the removal percentages of 65.9%-71.9% for F2, F3, F4 and 77.5%-79.3% for F6, F7, F8, respectively. The statistical analysis (Figure 7.6) using the ANOVA method showed that a significant removal difference was found for the F1, F2 and photolysis process from other column units at the 0.05 level. Here,
the greatest removal was achieved by the F6, F7 and F8 coupling with the photolysis pretreatment process though there was no significant difference from F2, F3 and F4 being observed. The removal of turbidity was primarily realised via sieving, straining and sedimentation (O’Melia and Crapps, 1964), which accounted for the poor turbidity reduction in the photolysis process due to the lack of retention lay in the reactor. Compared to the removal of 70% to 95% obtained for SSF in previous studies (Jenkins et al., 2011, Frank et al., 2014), the lower removal of SSF in this study was likely attributed to the low initial turbidity of feeding water (0.247-0.532 NTU), the same assumption was also mentioned by Frank et al. (2014) in that the greater removal of SSF could be achieved when the initial turbidity was high. Additionally, the negative turbidity removal of F1 and photolysis occasionally occurred, and this phenomenon was also observed by Sabogal-Paz et al. (2020), which might be ascribed to the occurrence of leaching of media or detachment of impurities accumulated on the reactor wall.
Figure 7.6 The average removal percentage of turbidity for photolysis and each filter unit from the single BF or photolysis-BF system. *: p<0.05, indicating the significant difference of turbidity removal for F1, F5 and photolysis.

The removal of total coliforms and *E. coli* in each filter was shown in Table 7A.10 and Table 7A.11. At the beginning of the operation stage the 100% removal of total coliforms and *E. coli* for all filters was determined. During the whole eight weeks of the operation time, the fluctuation in the removal of total coliforms and *E. coli* with the aging of the filters occurred, but greater than 90% removal was still reached except for the last week approaching the termination of operation. This result was within the recommended removal range from 90% to 100% achieved by the conventional SSF (Elliott et al., 2008). The poor removal of 56% for total coliforms and 40% for *E. coli* in
F3 was observed. Concurrently, the sharp rise of head loss in F3 (Figure 7.7) was also noted, and this is attributed to the clogging of the schmutzdecke which behaves as an additional porous media (Campos et al., 2006). Subsequently, the fluid could not penetrate through all portions of the media bed, and then the attached biofilm was influenced and shed off, causing the breakthrough of total coliforms and E.coli (Grace et al., 2016).

![Figure 7.7 The head loss of BF units throughout the operation stage.](image)

The BF units with photolysis pretreatment behaved stable on both microbial reduction and maintaining the head loss, it was because the photolysis process supplied an additional barrier to remove the bacterial indicators. In addition, UV irradiation was discovered effective to alleviate fouling issues of membrane filtration (Yu et al., 2016, Marconnet et al., 2011). Similarly, the pretreatment of UV in this study also provided
a control for microorganism concentration and mitigated increase of head loss (Figure 7.7) caused by the microbial activities, which was evidenced by the smaller amount of total protein determined in F5, F6, F7 and F8 than F1, F2, F3 and F4 presented in Table 7.2.

DOC and UV_{254} are usually indicative of the content of organic contaminants and the aromaticity of DOM, respectively, which are both crucial indicators in water treatment processes (Gao et al., 2020). Figure 7.8 displays the average absorbance of influents and effluents of BF and photolysis-BF systems at 254 nm and their average DOC concentrations through the operation period, in the meantime the error bars represents the standard deviation of these results. The variation of DOC resembles that of UV_{254}. For DOC, the average concentration of DOC occurring in the influent and effluent varied from 4.789 to 0.288 mg L^{-1}. The greatest reduction was observed for effluent of F8 which went through the system combining the photolysis and GASBF with 24 cm GAC layer. It was noticeable that the removal of DOC was not effectively achieved by the SSF compared with the GSBF, thus agreeing with the phenomenon that removal of DOC in conventional SSF was limited (Pompei et al., 2016). Moreover, the significant difference between SSF and GSBF was found using the ANOVA methods with p<0.01 and the superior performance of GSBF were due to the strong adsorption capacity of GAC particles induced by its inherent porous structure (Vilhunen et al., 2010). Additionally, the insignificant DOC change was found between before and after the photolysis process, agreeing with the observations reported by (Goslan et al., 2006a, Thomson et al., 2004). The neglectable DOC reduction was attributed to the fact that UVC light only results in the fragmentation of organic compounds, rather than the complete mineralisation and the products with smaller molecular size distribution.
were formed (Thomson et al., 2004). Nevertheless, a higher removal of DOC (30%) was achieved by UVC found in Parkinson et al. (2003)’s study. The discrepancy might be attributed to the resistance of the organics in this study to UV light or lower absorptivity coefficient for the examined water sample. More interesting here is that for GSBF units, the increase of GAC layer thickness and the installation of photolysis did not remarkably promote the reduction of UV$_{254}$ and DOC, a similar result was also obtained in a previous study (Li et al., 2018).

Figure 7. 8 The UV$_{254}$ (cm$^{-1}$) and DOC (mg L$^{-1}$) in influents and effluents of studied BF and photolysis-BF system.

Being partially in line with the removal trend of DOC, the modest UV$_{254}$ reduction was also observed to occur in SSF with the absence of the photolysis process, the reduction of UV$_{254}$ in the photolysis process was lower than that achieved by GSBF but significantly greater than F1 with t-test (p< 0.001). Here, specific UV$_{254}$ absorbance
(SUVA$_{254}$) was calculated using the absorbance at 254 nm dividing by the concentration of DOC (Vilhunen et al., 2010), which was usually used as a surrogate of the content of aromatic compounds in samples (Zheng et al., 2016). Furthermore, the rise of SUVA$_{254}$ was also indicative of the higher humified, aromatic and hydrophobic properties of ingredients and also implied the lower biodegradability (Yapsakli and Çeçen, 2010).

Table 7.3 The SUVA$_{254}$ of influents and effluents obtained for the studied treatment system.

<table>
<thead>
<tr>
<th>Filters</th>
<th>System configuration</th>
<th>SUVA$_{254}$ (L mg$^{-1}$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>SSF</td>
<td>0.025±0.004</td>
</tr>
<tr>
<td>F2</td>
<td>GSBF (8 cm GAC)</td>
<td>0.0149±0.0055</td>
</tr>
<tr>
<td>F3</td>
<td>GSBF (16 cm GAC)</td>
<td>0.0157±0.0068</td>
</tr>
<tr>
<td>F4</td>
<td>GSBF (24 cm GAC)</td>
<td>0.0161±0.0086</td>
</tr>
<tr>
<td>F5</td>
<td>Photolysis-SSF</td>
<td>0.0219±0.0055</td>
</tr>
<tr>
<td>F6</td>
<td>Photolysis-GSBF (8 cm GAC)</td>
<td>0.0121±0.0045</td>
</tr>
<tr>
<td>F7</td>
<td>Photolysis- GSBF (16 cm GAC)</td>
<td>0.0144±0.0054</td>
</tr>
<tr>
<td>F8</td>
<td>Photolysis- GSBF (24 cm GAC)</td>
<td>0.0141±0.0064</td>
</tr>
<tr>
<td>Influent 1</td>
<td></td>
<td>0.0221±0.0025</td>
</tr>
<tr>
<td>Influent 2</td>
<td></td>
<td>0.0163±0.0015</td>
</tr>
</tbody>
</table>

From Table 7.3, the remarkable reduction of SUVA$_{254}$ was observed in GSBF, having a SUVA$_{254}$ ranging from 0.0121 to 0.0161 L mg$^{-1}$ cm$^{-1}$ after feeding the influent 1 with a SUVA$_{254}$ of 0.0221 L mg$^{-1}$ cm$^{-1}$, during this course, the drop of SUVA$_{254}$ from 0.0221 to 0.0163 L mg$^{-1}$ in the photolysis process was also achieved, a similar observation was also reported by Vilhunen et al. (2010), demonstrating that the intermediates derived from photodegradation were more readily biodegradable. In the meantime, an
increase of SUVA$_{254}$ from 0.0221 to 0.025 or 0.0163 to 0.0219 L mg$^{-1}$ cm$^{-1}$ was found in the biodegradation- predominant BF (e.g. F1 and F5). It indicated that the hardly biodegradable molecules with high molecular weight were left.

Through the whole operation time of the treatment system, the low concentration of chlorophyll-a (< 0.05 µg L$^{-1}$) indicated a low biomass of phytoplankton.

The water quality parameters (e.g. pH, conductivity, ammonium, nitrate, nitrite, turbidity, *E.coli*, total coliforms, TOC) derived from each BF unit were listed in Figure 7A.16. Except for the removal of *E.coli* and total coliforms, other water quality parameters of each effluent are within the standard range for drinking water quality parameters set by the EU Directive or WHO (Table 7A. 15). Moreover, it was noticeable that photolysis-BF was yielding the treated water with better quality parameters, even for *E.coli* and total coliforms with the removal percentage ranging over 99%-100%.

7.3.5 Removal of free estrogens

The continuous treatment system containing BF units and photolysis-BF units was set up and operated with an effluent flow rate of 1 mL min$^{-1}$ (namely HRT 13.6 h). According to the DO concentration in influents and effluents, the operation of the treatment system was under aerobic conditions. After delivering the feeding water with an initial concentration of selected free estrogens (e.g. E1, E2, E3 and EE2) about 10 µg L$^{-1}$, their average removal percentage for BF units containing different media compositions with/without photolysis pretreatment was summarised in Figure 7.9, ranging over 20.95%–99.5% for BF units 1-8. The average estrogen removal percentage of 96.2%-99.5% in GSBF was still achieved through 8 weeks of the operation time, which was both observed with and without the photolysis pretreatment.
The superior removal was mainly attributed to the coexistence of the adsorption and biodegradation process (Ma et al., 2018). Compared to the result obtained from SSF, it was further assumed that adsorption acted as the dominant mechanism for the attenuation of estrogens.

Figure 7. 9 Average removal of estrogens E1, E2, E3 and EE2 achieved from the photolysis and BF units in studied treatment system.

Furthermore, the different thickness of GAC layer (e.g. 8 cm, 16 cm and 24 cm) in GSBF was evaluated using the ANOVA statistical analysis and the results showed that there was no significant difference on removing the targeted estrogens (p>0.9), being analogous to the study concerning the removal of paracetamol, caffeine and triclosan in GSBF with varying thickness of the GAC layer (10/20/30 cm) (Li et al., 2018). Based on the saturation adsorption capacity (Q) of GAC summarised in Table 5.4 of Chapter 5, the 0.217 mg g\(^{-1}\) for E1, 0.228 mg g\(^{-1}\) for E2, 0.145 mg g\(^{-1}\) for E3 and 0.160 mg g\(^{-1}\) for EE2, the loading of GAC in three GSBF was 18.1 g, 36.3 g, 54.5 g according
to the cross-sectional area of PVC column and density information in Table 7.1. Following the same operation conditions in this study, the adsorption capacity would be exhausted after eight to twenty-seven months theoretically. The specific time would be evaluated in the future, though the longer operation time was expected due to the layout of sandwich-type and installation of photolysis. By comparison, the remarkably lower removals of 60.88%, 44.5%, 34.78% and 20.95% for E1, E2, E3 and EE2 in SSF were respectively acquired, whose performance was not only significantly weaker than GSBF but also inferior to the SSF combined with photolysis process pronouncedly with p< 0.01. Prior to this study, the poor removal of E1, E3 and EE2, around 15% in conventional SSF, was observed by Kennedy et al. (2013). However, according to a study carried out by D’Alessio et al. (2015), the relatively better removal of 65% and 92% for E2 was respectively achieved in SSF as well. Similarly, the removal percentage over 90% for E2 was reached using the biologically active filter filling with adsorption-exhausted GAC particles or sand-anthracite dual media bed in Zhang et al. (2017)’s study. Additionally, the average removal percentages from -66.66% to 2.08% for E1, E2 and E3 were improved to 79.46% for E1, 34.58% for E2 and 11.66% for E3 via bioaugmentation with isolated estrogen degradation strains in SSF (Haig et al., 2016). The attenuation of estrogens derived from the SSF in this study was within the aforementioned range.

Regarding the photolysis pretreatment, its involvement provided the removal percentage of 71.4% for E1, 46.5% for E2, 35.2% for E3 and 44.4% for EE2, being consistent with the resistant characteristics to UVC irradiation described in Chapter 6. For the effluents entering F5, the removal of free estrogens ranged from 79.6% to 93.7% was finally obtained.
Throughout the operation, the dynamic concentration of targeted estrogens was profiled in Figure 7.10. There was no clear tendency of increase or decrease along with operation time being observed. Among the selected estrogens spiked in the feeding water of the treatment system, their concentrations occurring in GSBF with and without the photolysis process show inconsiderable discrepancy (0.21<p<0.99) within a range of 0.059–0.372 µg L⁻¹. On the contrary, the significantly different (p<0.01) concentrations between E1, E2, E3 and EE2 were found in effluents of SSF (F1) and there were 3.91 µg L⁻¹, 5.23 µg L⁻¹, 6.77 µg L⁻¹ and 8.54 µg L⁻¹ remaining, where the corresponding removals of E1, E2, E3 and EE2 were 60.88%, 44.47%, 34.78% and 20.95%, respectively. The recalcitrance of EE2 and E3 to biodegradation was confirmed and their persistence to biodegradation that took place in the AS process was reported in previous studies (Shi et al., 2004). For EE2, the presence of ethynyl group at C-17 group was assumed to lead to its lower biodegradability (Sornalingam et al., 2016). Apart from that, the removal performance in SSF was also depending on the strain types developing on the media (D'Alessio et al., 2015), showing various degradation capacities for different estrogens.
Figure 7. Concentration of free estrogens in influents and effluents through the eight weeks of operation time.
With the inclusion of photolysis as pretreatment for SSF (F5), the integration of photodegradation and biodegradation achieved much higher total removal presented in Figure 7.9. Here, it was also found that the biodegradable capacity of SSF for the studied estrogens, except for E1, was enhanced after the photolysis process. The drop of SUVA$_{254}$ after photolysis shown in Table 7.3 accounted for the reason as this declining parameter indicated the reduction of aromatic compounds in sample and then also demonstrated the increase of biodegradability (Zheng et al., 2016); therefore the greater biodegradation occurring in F5 than in F1 could be explained. Furthermore, the previous study Swift et al. (1981) also found that the contribution of photodegradation was especially important since this process preferentially attacked the same tertiary carbon atoms that tend to retard biodegradation. And it was also reported that photo-initiated intermediates were also likely to act as the primary substrates enhancing the biodegradation of the parent compounds (Lehto et al., 2003).

The analogous scenarios showing the promotion of UV photolysis for the post-biodegradation of pyridine was also reported previously (Zhang et al., 2013). In the meantime, the decreased biodegradation of E1 in Filter 5 was presumed to be induced by the conversion that occurred between E2 and E1 in the treatment system. This hypothesis was supported by Yu et al. (2007) who found the occurrence of transformation from E2 to E1 was generally observed for many E2 degrading bacteria but no further E1 degradation was achieved. Moreover, this conversion from E2 to E1 was also detected in the photolysis process (Lin and Reinhard, 2005a). Thus, the accumulation of E1 in these processes was reflected via the weakening biodegradation in F5. Overall, the installation of UVC pretreatment allowed a significant improvement for SSF.
7.3.6 Estrogenic potency variation

In addition to profiling the concentration of targeted estrogens, it is also important to measure the variation of estrogenic potency for evaluating the performance of the system. The relative estrogenic activity (EA/E\textsubscript{A0}) calculated via dividing the In of effluent by that of influents was shown in Figure 7.11. The negligible suppression in estrogenic activity of effluents from SSF, photolysis and photolysis-SSF was evidenced with EA/E\textsubscript{A0} of 1.242, 0.750 and 0.755, respectively. In previous cases, the negative removal of estrogenic activity in nonaugmented SSF was reported by Haig et al. (2016). It was highlighted that the biodegradation products might possess higher estrogenic potency than their parent compounds (McNaught and Wilkinson, 1997). With regard to the photolysis process, the estrogenic activity merely decreased 25% though the average photochemical degradation of E1, E2, E3 and EE2 reached to 71.4%, 46.5%, 35.2% and 44.5%, respectively. So, it was assumed that the limited reduction of estrogenic potency was not only attributed to the low degradation of E2, E3 and EE2 but also because of the formation of by-products having estrogenic potency. For example, the lumiestrone which has estrogenicity arisen from the direct photolysis of E1 in Whidbey et al. (2012)’s work. In addition, dehydro-estradiol derived from the photodegradation of E2 was also found to present high estrogenic potency (Li et al., 2016). These studies gave support to what was observed in this study, while the verification for the specific by-products contributing to the estrogenic activity after the photodegradation and biodegradation was further demanded in the future.
Figure 7.11 The comparison between the relative estrogenic activity, DOC and UV254.

In the investigated systems, GSBF did achieve a significantly higher reduction in the overall estrogenic activity than SSF and photolysis-SSF did with p<0.05. It was observed that the trend of EA/E\textsubscript{A_0} in effluents in different filter units was in accordance with the parameters of UV\textsubscript{254} and the concentration of DOC (Figure 7.2), which was further proved via the Pearson correlation analysis for the key water quality parameters and estrogenic activity in Table 7.4.
Table 7. 4 The correlation between the main water quality parameters and estrogenic activity (n=80).

<table>
<thead>
<tr>
<th></th>
<th>turbidity</th>
<th>conductivity</th>
<th>TDS</th>
<th>pH</th>
<th>DO</th>
<th>UV$_{254}$</th>
<th>DOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>conductivity</td>
<td>0.37873</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDS</td>
<td>0.35065</td>
<td>0.99456*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.56203</td>
<td>-0.71686*</td>
<td>-0.6922*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO</td>
<td>0.77207*</td>
<td>0.6621*</td>
<td>0.67061*</td>
<td>-0.75783*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV$_{254}$</td>
<td>0.90628*</td>
<td>0.22321</td>
<td>0.19344</td>
<td>-0.51062</td>
<td>0.76149*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>0.97838*</td>
<td>0.29874</td>
<td>0.27162</td>
<td>-0.55394</td>
<td>0.7832*</td>
<td>0.96756*</td>
<td></td>
</tr>
<tr>
<td>EA/EA$_0$</td>
<td>0.84777*</td>
<td>0.08215</td>
<td>0.05584</td>
<td>-0.42635</td>
<td>0.68308*</td>
<td>0.98*</td>
<td>0.93439*</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level.
The correlation coefficient 0.986 and 0.934 accounted for the significant correlation of EA/E_{A0} with UV_{254} or DOC, as both of these two parameters can represent the total concentration of estrogen residues and the intermediates derived from biodegradation or photolysis in this study (Tang et al., 2014, Oh et al., 2006). So, based on the estrogen removal results in this study, the estrogenic activity partially induced by the by-products produced in SSF biodegradation or photolysis was confirmed.

Overall, the combined system of photolysis pretreatment coupling with GSBF unit not only performed well on maintaining the stable operation performance for guaranteeing the water quality, but also achieved constant effective removal of estrogens and estrogenic activity.

7.4 Conclusions

In this chapter, the photolysis-BF hybrid system was set up on a bench scale and its applicability was evaluated in terms of the operation performance, capability of removing targeted free estrogens (E1, E2, E3 and EE2) and reduction of estrogenic activity. In the meantime, the BF units disconnected with the photoreactor were operated for investigating the effects of the photolysis process. Additionally, the BF units consisting of SSF, 8 cm GSBF, 16 cm GSBF and 24 cm GSBF were also operated simultaneously to study the impacts of GAC layer thickness.

Over eight weeks of operation time, no significant difference was found for pH, conductivity, TDS, ammonium, nitrite, nitrate, phosphate and DO between different BF units. Concerning the turbidity, DOC and UV_{254}, the GSBF with or without photolysis process both presented greater removal than SSF units. Coupling with the photolysis process, it was observed that the microbial reduction between 92% and 100% was constantly achieved by SSF and GSBF. The slower increase of head loss was also
realised with the inclusion of photolysis as the excessive growth of microorganisms was prevented under irradiation of UVC irradiation.

The targeted estrogens were greatly removed by GSBF, the average reduction ranged from 96.2% to 99.5%. Moreover, it was found that the thickness of the GAC layer in GABF units did not show a significant difference on estrogen removal at the 0.05 level.

The impacts of photolysis here were found to promote the biodegradation of E2, E3 and EE2 in SSF, increasing their removal to 79.6%-93.7%.

In addition, the limited reduction of estrogenic activity was observed for SSF, photolysis, and photolysis-SSF. By comparison, all GSBF units with and without photolysis pretreatment exhibited superior removal on estrogenic activity and the value of $\frac{EA}{EA_0} < 0.05$ was obtained.

Overall, the hybrid photolysis-GSBF system was evidently capable of mitigating both estrogen concentration and estrogenic activity, and the installation of UVC reactor could effectively prevent the clogging issue via inhibiting excessive accumulation of microorganisms. These characteristics make it a promising and sustainable technique for alleviating the estrogen contamination in water.
Chapter 8 Conclusion and future work

In this thesis, the investigation on the attenuation of estrogens and their estrogenic activity using the GSBF units coupled with photolysis pretreatment was carried out. Regarding this goal, the estrogen measurement method using LC-MS coupled with SPE pre-concentration procedure was established first, in which the optimisation of SPE and LC-MS method was conducted in succession. For better understanding the mechanisms of the photolysis-GSBF system, the adsorption capacity of GAC for the targeted estrogens was studied regarding the properties of carbon adsorbent and influence of water matrix. Then the photolysis of estrogens under UVC irradiation was also investigated individually, except for studying the impact derived from the flow mode; and similarly the influence of natural water matrix on the degradation was also assessed. Finally, the photolysis-BF hybrid system was setup after characterising the sand and GAC particles, the performance of BF units, which had various media composition, was evaluated in terms of general operation performance, the removal of targeted estrogens and reduction of estrogenic activity. In addition, the inclusion of photolysis in the hybrid system was also analysed simultaneously. The key findings of the research were summarised in the following sections. In addition, the future work was also recommended in this chapter based on the findings of the thesis.

8.1 Conclusion

8.1.1 Development of LC-MS and SPE method

Due to the inherent weak ionisation of free estrogens and limitation of analytical instrument, a novel LC-MS method based on derivatisation with DMIS was established to achieve simultaneous detection of free and conjugated estrogens at trace level.
Introduction of DMIS label to the C-3 position of free estrogens E1, E2, E3, EE2 and E2-17 significantly enhanced their mass spectrometric signal under ESI conditions without compromising the detection of other conjugated estrogens such as E1-3S, E1-3G, E2-3S, E2-3G and E3-3S. Moreover, the derivatives affixing DMIS label were found capable of providing specific structural characterisation for the confirmation of the selected compounds using MS/MS, especially for isomers E2-17G and E2-3G, alleviating the susceptibility of determination in the presence of interferences. In addition, the chromatographic separation of targeted compounds was achieved using Hypersil GOLD C18 capillary column.

Following a feasible SPE procedure, the enrichment of analytes from samples was carried out using Oasis HLB sorbent, after loading sample with a flow rate of 5 mL min⁻¹, impurities were cleaned with Milli-Q water followed by elution using 8 mL methanol. The results of method validation showed that the satisfactory relative recovery ranging 76.4 % to 95.4 % was obtained using the surface water as the matrix. Finally, the MQLs for all chosen estrogens ranging from 7.0 to 132.3 pg L⁻¹ in surface water was achieved, which was within the range usually obtained using more advanced instruments. And the applicability of this method was also proved via profiling the concentration of estrogens in lake water from the Regent’s Park, the average concentration of 0.05, 0.11, 0.19, 0.21 and 0.32 ng L⁻¹ was determined for E2-3S, E1-3S, E3, E2 and EE1.

8.1.2 Adsorption mechanisms of estrogens onto GAC

The batch adsorption experiments were conducted for studying the simultaneous adsorption behaviour of estrogens E1, E2, E3 and EE2 onto two kinds of commercial
GAC adsorbents for the first time. Good removal (>90%) of free estrogens via the adsorption onto GAC was observed.

In comparison with the pseudo-first-order kinetic model, the time depending experimental data was found to fit well with the pseudo-second-order kinetic model with correlation coefficient $R^2 > 0.99$ when the estrogen spike concentration was 10 µg L$^{-1}$. In addition, the conclusion that the surface diffusion was the main rate-limiting step in the adsorption process was obtained via analysing the intra-particle diffusion model.

With the presence of NOM, the Freundlich adsorption constants of AC-i dropped by 9.4%- 31.7% for the synthetic humic solution and 89.8%-91.5% for natural water matrix. For adsorbent A-ii, the Freundlich adsorption constants dropped by 45.2%-52.1% for the synthetic humic solution and 94.5%-96.4% for natural water matrix, demonstrating the adsorption capacity was subjected to the NOM in water due to the competition for the adsorption sites. Therefore, the pretreatment such as UV photolysis was suggested to minimize the influence of NOM for preventing the pronounced reduction of adsorption capacity.

8.1.3 The photodegradation of estrogens under UVC irradiation

The photodegradation of E1, E2, E3 and EE2 under the UV-C irradiation was well-described by the pseudo-first-order kinetics with correlation factors $R^2 > 0.981$, depending on the obtained photolysis rate constants and $t_{1/2}$, the estrogen recalcitrance to the employed UV-light followed the order of E3>EE2>E2>E1. The greatest molar absorption coefficient of 402.4 M$^{-1}$ cm$^{-1}$ and quantum field of 0.065 mol E$^{-1}$ at $\lambda=253.7$ for E1 accounted for E1 being more prone to photodegradation than the other three estrogens under the identical exposure conditions.
The $t_{1/2}$ derived in the stationary operation mode for E1, E2, E3 and EE2 were 39.8 min, 161.2 min, 301.4 min and 173.3 min, respectively. By comparison, the significantly less $t_{1/2}$ values for photolysis of estrogens (29.7 min, 78.8, 177.7 and 100.5 min for E1, E2, E3 and EE2) using the up-flow mode were noticed, this promotion was presumably ascribed to the higher content of dissolved oxygen in flow mode, which enhanced the oxidation of estrogens.

The positive impact of surface water matrix on the photodegradation of estrogens was observed according to the acquired shorter $t_{1/2}$ ranging from 23.1 to 126.8 min, even though the statistically significant difference was only attained for E1. This may indicate that the most common constituents such as DOM, nitrate and chloride ion worked together under the UV-C irradiation to achieve the overall positive impact on the photolysis performance.

8.1.4 The removal of estrogens in photolysis-BF system

The removal of estrogens and estrogenic activity in photolysis-GSBF was investigated for the first time. Over the eight weeks of operation time, the BF coupling with the photolysis process constantly yielded the effluents with great water quality parameters and slower increase of headloss was also observed in the filters with the inclusion of photolysis.

Good average removals ranging from 96.2% to 99.5% of estrogens were achieved by the GSBF system. The 8 cm, 16 cm and 24 cm thickness of GAC layer in the GSBF did not show significant difference on estrogen removal. Here, photolysis pretreatment was found to promote the biodegradation of E2, E3 and EE2 in SSF, increasing their removal to 79.6%-93.7%, being better than the removal of estrogens reported by previous studies (Haig et al., 2016).
Cocurrently, the limited reduction of estrogenic activity was observed for SSF, photolysis, and photolysis-SSF. By comparison, all GSBF filters with and without photolysis pretreatment exhibited superior removal on estrogenic activity, the value of $EA/EA_0 < 0.05$ was obtained.

Overall, the hybrid photolysis-GSBF system was evident, capable of mitigating both estrogen concentration and estrogenic activity, and the installation of UVC reactor could effectively prevent the clogging issue via inhibiting excessive accumulation of microorganism.

8.1.5 Overall conclusions

Based on the investigations on the removal of estrogens with adsorption, photolysis and hybrid photolysis-biofiltration systems, it was concluded that adsorption capacity provided by GAC not only allowed the good removal for the four chosen estrogens (>90%) in batch experiments, but also played a crucial role in the elimination of estrogens and reduction of estrogenicity in GSBF and the photolysis-GSBF system. By comparison, the single process of photolysis under the UVC irradiation or biodegradation in SSF contributed less to the decrease of estrogen concentrations, for more photo sensitive and biodegradable E1, around 60% average removal was achieved, while E2, E3 and EE2 were poorly removed (less than 40% averagely). The combination of photolysis pretreatment with SSF unit promoted the degradation of all assayed estrogens and especially EE2, but showed limitations concerning the mitigation of estrogenicity as the same as the single photolysis and SSF unit. However, photolysis was a feasible pretreatment process as a result of controlling the overgrowth of microorganisms and inhibiting the increase of headloss, also producing the effluent with stable removal of *E.coli* and total coliforms (greater than 99%) over
the period of operation. Additionally, both photolysis installed as pretreatment and biodegradation primarily occurred in biofilm formed on the surface of the sand layer, being helpful to alleviate the negative impacts of natural water matrix on the GAC particles, which could prolong the lifespan of adsorbents. Thus, due to the excellent performance for removing estrogens and capacity of producing the effluents with good water qualities, the system of photolysis-GSBF in the series is a comprising water treatment technique for practical life.

8.2 Future work

Being subject to factors such as timescale, instruments and materials, there existed some limitations for the experimental investigation designed in this thesis. These limitations are summarised and listed together with the recommendations for future investigation below.

The estrogen determination method developed in this thesis was corresponded to the natural lake water. However, the presence of estrogens in other more complicated matrix is also prevalent, such as wastewater, dairy products, sentiments and sludge (Capriotti et al., 2015, Kuster et al., 2004, Baronti et al., 2000). Confronting the interferences presented in these matrices, the parameters of LC-MS and SPE method can be optimised further and be made more versatile for various types of matrix. Additionally, the LC-MS analytical method without derivatisation process are also expected not only for the higher throughput but also for the easier confirmation of intermediates produced in the studied process in comparison with the derivatisation method.
In the adsorption experiment described in Chapter 5, the influence of NOM was evaluated using the synthetic humic acid solution and natural surface water. However, the more specific influences in terms of the size distribution of NOM on the estrogen adsorption capacity of carbon materials are still unclear. The molecular weight distribution of NOM could be profiled using the high performance liquid chromatography system installed with a size exclusion chromatography column (Li et al., 2003). In the future, the influence of NOM size distribution on the adsorption of estrogens can be investigated accordingly.

The limited photolysis removal of free estrogens, especially E2, E3 and EE2 with exposure to UV-C light, was observed in both Chapters 6 and 7. To increase the removal of estrogens, a different light source can be examined. For example, Wen et al. (2018) found VUV/UV exhibited a greater removal performance for contaminants without the increase of cost. Therefore, in the future the irradiation experiment can be carried out and the production of reactive oxygen species should be monitored experimentally for explaining the photodegradation better. Furthermore, the transformation of estrogens reflected by the LC-MS and estrogenic activity essay are also expected to verify the patterns and effectivity of degradation.

The biological activity in SSF and GSBF is complex and the degradation of estrogens might be subject to the abiotic effect, glucuronidase effect, sulfatase effect and the general enzyme effect besides general microbial activity as described by (Ben et al., 2017). However, in this study, the removal path ways of estrogens were analysed from merely adsorption and biodegradation. For better understanding the degradation mechanisms, the control tests are expected to proceed via inhibiting corresponding effects individually and the decomposition pathways of targeted estrogens can be indicated. In addition, the piezometer for measuring headloss in this study was
connected to the bottom of media bed and the total headloss of each filter was
determined only. In future work, the connection ports of headloss should be set at
different depth of media bed for demonstrating the headloss contribution from different
media layers in detail, being helpful to the maintenance of the filtration system.
Similarly, the sampling points for effluents of the filtration system can be set at different
depth of media bed for accounting for the contribution of different media layers to
estrogen removal.

In Chapter 7, the photolysis pretreatment followed by SSF was found to promote the
biodegradation of E2, E3 and EE2, and this promotion was analysed from the
chemistry aspect that more biodegradable intermediates were formed in the photolysis
process. However, the microbial community may also be influenced by the installation
of the photolysis pretreatment. Previous studies (Chang et al., 2021, Xia et al., 2011)
investigated the microbial community alteration with direct exposure to UV light, the
impact of UV as pretreatment on the microbial community has not been studied yet.

At present, the studies carried out here are all on the bench scale. In the future, the
studied treatment should be scaled up and treat more complicated water matrix for
proving the practical applicability.
Reference


with household bleach for the removal of endocrine disrupting compounds. 

*Journal of Environmental Science and Health, Part A, 48, 753-759.*


LEE, C. & YOON, J. 2008. UV direct photolysis of 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) in aqueous solution: Kinetics and


benzene, toluene and chlorobenzene removal from contaminated groundwater by combined UV/H2O2 photo-oxidation and aeration. Water research, 120, 245-255.


MATEJICEK, D., HOUSEROVA, P. & KUBAN, V. 2007. Combined isolation and purification procedures prior to the high-performance liquid chromatographic-


SABOGAL-PAZ, L. P., CAMPOS, L. C., BOGUSH, A. & CANALES, M. 2020. Household slow sand filters in intermittent and continuous flows to treat water
containing low mineral ion concentrations and Bisphenol A. *Sci Total Environ*, 702, 135078.


SANCHES, S., PENETRA, A., RODRIGUES, A., CARDOSO, V. V., FERREIRA, E., BENOLIEL, M. J., BARRETO CRESPO, M. T., CRESPO, J. G. & PEREIRA, V.


SILVA, T. L. D., SÁNCHEZ-ROMÁN, R. M., QUELUZ, J. G. T. & PLETSCH, T. A. 2020. Chapter One - Treatment options for the direct reuse of reclaimed water in


the combination of microwave-assisted extraction and LC-MS/MS. *Talanta*, 85, 1825-34.


detected new intermediary product and investigating the kinetics at various environmental conditions. *Chemosphere*, 72, 389-99.


ZHANG, K. 2006. *Occurrence, microbial, and photochemical degradation of endocrine disrupting estrogens in surface water*, University of Massachusetts Lowell.


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</tr>
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A (i)

Estrone_10ppm_161004144536 #64  RT: 0.84  AV: 1  NL: 1.75E4
F: ITMS + c ESI Full ms [100.00-300.00]

B (i)

E1 NEW_16110416837 #3  RT: 0.01  AV: 1  NL: 6.84E5
T: ITMS + c ESI Full ms [150.00-500.00]
Figure 4A. 1. Mass spectra (i) at derived from tune method and chromatograms (ii) of representative free estrogen—E1 with and without derivatization reaction: A) E1 at 10 mg L$^{-1}$ without derivatization reaction; B) E1 at 0.1 mg L$^{-1}$ after derivatization reaction
ITMS + c ESI Full ms [115.00-600.00]

- 139.24
- 199.14
- 239.38
- 309.45
- 317.06
- 326.70
- 353.52
- 381.53
- 415.00
- 431.45
- 453.35
- 484.31
- 510.91
- 550.92
- 580.80
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Table 4A. 1 Calibration curves and instrumental limits of detection and quantification for estrogen standard diluents.

<table>
<thead>
<tr>
<th>Estrogens</th>
<th>Standard diluents</th>
<th>Linear range (µg L(^{-1}))</th>
<th>Linear slope</th>
<th>R(^2)</th>
<th>IDL (ng L(^{-1}))</th>
<th>IQL (ng L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>0.01-1000</td>
<td>181.1 ± 0.92</td>
<td>0.9992</td>
<td>4.6</td>
<td>15.2</td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>0.01-1000</td>
<td>172.1 ± 0.42</td>
<td>0.9993</td>
<td>5.8</td>
<td>19.2</td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>0.05-1000</td>
<td>72.9 ± 1.36</td>
<td>0.9993</td>
<td>21.4</td>
<td>71.4</td>
<td></td>
</tr>
<tr>
<td>EE2</td>
<td>0.01-1000</td>
<td>157.2 ± 0.35</td>
<td>0.9996</td>
<td>4.7</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>E2-17G</td>
<td>0.02-1000</td>
<td>96.3 ± 1.68</td>
<td>0.9977</td>
<td>14.0</td>
<td>46.7</td>
<td></td>
</tr>
<tr>
<td>E1-3S</td>
<td>0.005-1000</td>
<td>321.2 ± 1.59</td>
<td>0.9998</td>
<td>2.5</td>
<td>8.5</td>
<td></td>
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<tr>
<td>E1-3G</td>
<td>0.05-1000</td>
<td>29.6 ± 0.31</td>
<td>0.9993</td>
<td>27.8</td>
<td>92.7</td>
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<tr>
<td>E2-3S</td>
<td>0.005-1000</td>
<td>460.7 ± 1.85</td>
<td>0.9991</td>
<td>1.8</td>
<td>5.9</td>
<td></td>
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<tr>
<td>E2-3G</td>
<td>0.05-1000</td>
<td>54.7 ± 0.42</td>
<td>0.9996</td>
<td>29.0</td>
<td>96.6</td>
<td></td>
</tr>
<tr>
<td>E3-3S</td>
<td>0.05-1000</td>
<td>76.9 ± 1.01</td>
<td>0.9995</td>
<td>16.8</td>
<td>55.8</td>
<td></td>
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</table>
Figure 5A. 1 Characterisation of GAC via adsorption of nitrogen. A or B) GAC purchased from Chemviron Carbon or DARCO®: (i) adsorption and desorption isotherms using nitrogen gas at 77 K; (ii) distribution of pore size; B) GAC purchased from DARCO®.
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Figure 5A. 2. (A) Effect of contact time on adsorption of estrogens onto Chemviron Carbon GAC: i) E1; ii) E2; iii) E3 and iv) EE2.
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Table 5A. 1 Single point distribution coefficients ($k_d$) and their specific surface area normalised species ($k_d'$) at various equilibrium concentrations in pure water.

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>$k_{d,1%}$</th>
<th>$k_{d,1%}'$</th>
<th>$k_{d,50%}$</th>
<th>$k_{d,50%}'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>s</td>
<td>(L g$^{-1}$)</td>
<td>(L m$^{-2}$)</td>
<td>(L g$^{-1}$)</td>
<td>(L m$^{-2}$)</td>
</tr>
<tr>
<td>E1</td>
<td>80.1</td>
<td>0.144</td>
<td>0.122</td>
<td>0.037</td>
</tr>
<tr>
<td>E2</td>
<td>97.2</td>
<td>0.174</td>
<td>20.78</td>
<td>0.04</td>
</tr>
<tr>
<td>E3</td>
<td>57.1</td>
<td>0.103</td>
<td>13.12</td>
<td>0.024</td>
</tr>
<tr>
<td>EE2</td>
<td>67.8</td>
<td>23.85</td>
<td>17.73</td>
<td>0.032</td>
</tr>
<tr>
<td>AC-i</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-ii</td>
<td>110.7</td>
<td>0.172</td>
<td>0.131</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>177.3</td>
<td>0.276</td>
<td>30.20</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>63.3</td>
<td>0.098</td>
<td>42.02</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>83.9</td>
<td>0.131</td>
<td>24.76</td>
<td>0.039</td>
</tr>
</tbody>
</table>

The $k_{d,1}$ and $k_{d,50}$ ($q_e/C_e$) values are designated as the distribution coefficient within adsorbent-solution system at which the equilibrium concentrations of four free estrogens were equal to 1% and 50% of their initial concentration in accordance with the Freundlich model, and $k_{d,1}'$ and $k_{d,50}'$ are their homologous normalised species by the specific surface area.
Table 5A. 2 Diameter of free estrogens and humic acid

<table>
<thead>
<tr>
<th>Compounds name</th>
<th>Diameter (Å)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>E1</td>
<td>8.2</td>
<td>Rossner et al. (2009)</td>
</tr>
<tr>
<td>E2</td>
<td>8.3</td>
<td>Rossner et al. (2009)</td>
</tr>
<tr>
<td>E3</td>
<td>8.3</td>
<td>Rossner et al. (2009)</td>
</tr>
<tr>
<td>EE2</td>
<td>8.4</td>
<td>Rossner et al. (2009)</td>
</tr>
<tr>
<td>Humic acid</td>
<td>12-139</td>
<td>Tagliavini et al. (2017)</td>
</tr>
</tbody>
</table>

Table 5A. 3 Characteristics of humic acid solution and natural surface water.

<table>
<thead>
<tr>
<th>Water matrix</th>
<th>Adsorbance (cm$^{-1}$)</th>
<th>DOC (mg L$^{-1}$)</th>
<th>SUVA$_{254}$ (L mg$^{-1}$ m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humic acid solution (30 mg L$^{-1}$)</td>
<td>0.861</td>
<td>12.78</td>
<td>6.7</td>
</tr>
<tr>
<td>Natural surface water</td>
<td>0.143</td>
<td>6.75</td>
<td>2.1</td>
</tr>
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</table>
Table 5A. 4 Single point distribution coefficients (kd) and their percent reduction values (RAC-i or RAC-ii) at various equilibrium concentrations in humic solution and natural surface water.

<table>
<thead>
<tr>
<th>Adsorbents</th>
<th>Humic acid solution</th>
<th>Natural surface water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k_{d,1}% * (L g^{-1})</td>
<td>k_{d,50}% * (L g^{-1})</td>
</tr>
<tr>
<td></td>
<td>E1</td>
<td>E2</td>
</tr>
<tr>
<td>AC-i</td>
<td>61.30</td>
<td>52.71</td>
</tr>
<tr>
<td>RAC-i</td>
<td>0.765</td>
<td>0.543</td>
</tr>
<tr>
<td>AC-ii</td>
<td>48.18</td>
<td>62.54</td>
</tr>
<tr>
<td>RAC-ii</td>
<td>0.435</td>
<td>0.353</td>
</tr>
</tbody>
</table>

* The k_{d,1} and k_{d,50} (q_{e}/C_{e}) values are designated as the distribution coefficient within adsorbent-solution system at which the equilibrium concentrations of four free estrogens were equal to 1% and 50% of their initial concentration in accordance with the Freundlich model.
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Table 7A. 1 The pH values determined for influents and effluents of biofiltration and photolysis-biofiltration hybrid system over the period of operation (after maturation of biofilters).

<table>
<thead>
<tr>
<th>Week</th>
<th>Influent 1</th>
<th>Influent 2</th>
<th>Effluent of biofiltration only</th>
<th>Effluent of hybrid system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F1</td>
<td>F2</td>
</tr>
<tr>
<td>1</td>
<td>8.27</td>
<td>8.29</td>
<td>8.31</td>
<td>8.26</td>
</tr>
<tr>
<td>2</td>
<td>8.28</td>
<td>8.36</td>
<td>8.29</td>
<td>8.32</td>
</tr>
<tr>
<td>3</td>
<td>8.33</td>
<td>8.43</td>
<td>8.42</td>
<td>8.3</td>
</tr>
<tr>
<td>4</td>
<td>8.34</td>
<td>8.49</td>
<td>8.44</td>
<td>8.28</td>
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<tr>
<td>5</td>
<td>8.23</td>
<td>8.16</td>
<td>8.34</td>
<td>8.24</td>
</tr>
<tr>
<td>6</td>
<td>8.08</td>
<td>8.27</td>
<td>8.28</td>
<td>8.28</td>
</tr>
<tr>
<td>7</td>
<td>8.31</td>
<td>8.43</td>
<td>8.37</td>
<td>8.36</td>
</tr>
<tr>
<td>8</td>
<td>8.02</td>
<td>7.91</td>
<td>8.16</td>
<td>8.16</td>
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</tbody>
</table>
Table 7A. The value of conductivity (µs cm⁻¹) determined for influents and effluents of biofiltration and photolysis-biofiltration hybrid system over the period of operation (after maturation of biofilters).

<table>
<thead>
<tr>
<th>Week</th>
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<th>Influent 2</th>
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Table 7A. 3 The value of TDS (mg L⁻¹) determined for influents and effluents of biofiltration and photolysis-biofiltration hybrid system over the period of operation (after maturation of biofilters).

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Table 7A. The ammonium concentration (mg L\(^{-1}\)) in influents and effluents in assayed treatment system with estrogen spike after finishing maturation.

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Table 7A. 5 The nitrite concentration (mg L-1) in influents and effluents in assayed treatment system with estrogen spike after finishing maturation.

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</table>
Table 7A. 6 The nitrate concentration (mg L⁻¹) in influents and effluents in assayed treatment system with estrogen spike after finishing maturation.

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<tr>
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<td>6.674</td>
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</table>
Table 7A. The phosphate concentration (mg L⁻¹) in influents and effluents in assayed treatment system with estrogen spike after finishing maturation.

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<tr>
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<th>Effluent of biofiltration only</th>
<th>Effluent of hybrid system</th>
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Table 7A. 8 The DO (mg L⁻¹) in influents and effluents in assayed treatment system with estrogen spike after finishing maturation.

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Table 7A. The turbidity (NTU) of influents and effluents in assayed treatment system with estrogen spike after finishing maturation.

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Table 7A. The total coliforms removal of effluents from the biofiltration and photolysis-biofiltration hybrid system over the period of operation (after maturation of biofilters)

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<tr>
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<td>6</td>
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Table 7A. 11 The E.coli removal determined for effluents of biofiltration and photolysis-biofiltration hybrid system over the period of operation (after maturation of biofilters).

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Table 7A. The headloss (cm) determined for each biofilters over the period of operation (after maturation of biofilters).

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Table 7A. 13 The DOC (mg L⁻¹) of influents and effluents in assayed treatment system with estrogen spike after finishing maturation.

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Table 7A. 14 The UV254 (cm\(^{-1}\)) of influents and effluents in assayed treatment system with estrogen spike after finishing maturation.

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<td>0.0667</td>
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</table>
Table 7A. The standard for drinking water quality parameters set by the EU Directive or WHO.

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<td>0.5 mg L⁻¹ *</td>
</tr>
<tr>
<td>nitrate</td>
<td>50 mg L⁻¹ &amp;</td>
</tr>
<tr>
<td>Nitrite</td>
<td>3 mg L⁻¹ &amp;</td>
</tr>
<tr>
<td>Turbidity</td>
<td>1 NTU *</td>
</tr>
<tr>
<td>E.coli</td>
<td>0/100 mL *</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>0/100 mL *</td>
</tr>
<tr>
<td>TOC</td>
<td>No abnormal change *</td>
</tr>
</tbody>
</table>

*:Drinking Water Directive 98/83/EC; &: (Organization, 1993)

Table 7A. The average water quality parameters of effluent of each BF unit corresponding to the drinking water quality parameter standard.

<table>
<thead>
<tr>
<th>Drinking water quality parameters</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.33 ± 0.09</td>
<td>8.28 ± 0.06</td>
<td>8.36 ± 0.08</td>
<td>8.36 ± 0.07</td>
<td>8.39 ± 0.10</td>
<td>8.36 ± 0.10</td>
<td>8.33 ± 0.08</td>
<td>8.40 ± 0.08</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻¹)</td>
<td>1009 ± 38</td>
<td>1016 ± 38</td>
<td>1011 ± 4</td>
<td>1014 ± 50</td>
<td>1013 ± 41</td>
<td>1016 ± 38</td>
<td>1015 ± 39</td>
<td>1012 ± 44</td>
</tr>
<tr>
<td>Ammonium (mg L⁻¹)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Nitrate (mg L⁻¹)</td>
<td>3.7 ± 4</td>
<td>5.1 ± 4.5</td>
<td>4.3 ± 4.5</td>
<td>5.2 ± 3.2</td>
<td>2.1 ± 3.2</td>
<td>3.6 ± 4.8</td>
<td>0.4 ± 0.2</td>
<td>3.5 ± 3.7</td>
</tr>
<tr>
<td>Nitrite (mg L⁻¹)</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>0.22 ± 0.08</td>
<td>0.12 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.27 ± 0.12</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>E.coli removal (%)</td>
<td>96 ± 0.03</td>
<td>99 ± 0.02</td>
<td>90 ± 0.20</td>
<td>98 ± 0.03</td>
<td>99 ± 0.01</td>
<td>100 ± 0.00</td>
<td>99 ± 0.03</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td>Total coliforms removal (%)</td>
<td>98 ± 0.03</td>
<td>97 ± 0.03</td>
<td>93 ± 0.15</td>
<td>98 ± 0.02</td>
<td>99 ± 0.01</td>
<td>100 ± 0.00</td>
<td>99 ± 0.01</td>
<td>100 ± 0.01</td>
</tr>
<tr>
<td>TOC (mg L⁻¹)</td>
<td>3.19 ± 0.92</td>
<td>0.97 ± 0.46</td>
<td>0.81 ± 0.48</td>
<td>0.94 ± 0.77</td>
<td>2.81 ± 0.87</td>
<td>0.81 ± 0.49</td>
<td>0.70 ± 0.40</td>
<td>0.77 ± 0.60</td>
</tr>
</tbody>
</table>