THEROMDYNAMICS OF STEROID ESTROGEN SORPTION

TO ACTIVATED SLUDGE

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Kai Xu, son of Fengliang Xu and Zhaohong Fang, was born on the 23rd day of April in 1981 in Shanghai, China. After completed elementary and high school, he entered Beijing University of Technology in Beijing, China in August 1999 and was awarded a Bachelor of Science degree in Civil Engineering in June 2003. He served as a Project Engineer for Beijing Victor Star Co. Ltd. from September 2003 to November 2005. From August 2006, he came to Auburn University, Alabama and began work as a Graduate Research Assistant in pursuit of the degree of Master of Science.

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Sorption is an important mechanism for removal of 17α-ethinylestradiol (EE_2) in biological wastewater treatment. In an effort to deepen our understanding of this process, this work investigated the underlying thermodynamic parameters. Biomass was harvested from a membrane bioreactor (MBR) and a sequencing batch reactor (SBR), and sorption experiments were conducted over a range of temperatures. Sorption of EE_2 to activated sludge was spontaneous (ΔG values were between -16 and -11 KJ/mol), enthalpy-driven (ΔH values were -37 KJ/mol (MBR) and -48 KJ/mol (SBR)), and entropy-retarded (ΔS values were -74 (MBR) and -119 J/mol/K (SBR)). Although EE_2 is nonpolar, hydrophobic interactions were not dominant driving forces. The thermodynamic data also suggested that EE_2 sorption to biomass was primarily physisorption, but it also included low level chemisorption. The FT-IR results suggested that chemical reactions were not

significant enough to shift the detectable chemical bonding characteristics of the biomass functional groups.

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The author wishes to express his appreciation and respect to his home country and hope this work makes Her a better name.

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CHAPTER I INTRODUCTION

Pharmaceutical and personal care products (PPCPs), called emerging pollutants, in the environment have attracted increasing concerns by the government and scientists within the past decade (Daughton, 2004). Pharmaceuticals are medicinal drugs used in medical treatment on both human and veterinary to cure disease. Personal care products include skin care product (e.g. musk, body lotion), make up, and hygiene products. Most of the PPCPs contain synthetic bioactive compounds which are not easy to break down by the environment. Because of their indispensability of everyday usage and the difficulty in biodegradation, the metabolized residual compounds of PPCPs are continually discharged into the environment. Recent study from United States Geological Survey (USGS) showed that PPCP chemicals can be found in surface waters (Kolpin *et al.*, 2002). Additionally, the concentration of PPCPs in aquatic environments may exceed the non-effect concentration (Stuer-Lauridsen *et al.*, 2000; Santos *et al.*, 2007).

Endocrine disrupting chemicals (EDCs) are defined as a sort of natural, or synthetic compounds which imitate the natural hormones in the body. EDCs in the aquatic environment can affect the sexual character and reproduction behavior.

17α-Ethinylestradiol is an endocrine disruptor. It is widely used in contraceptives. In the aquatic environment, EE_2 causes developmental abnormalities in fish.

Overall

Because EE_2 is present in domestic wastewater, it must be removed during the wastewater treatment process. The overall goal of this research is to improve the understanding of how EE_2 is removed in the activated sludge process.

Organization of Thesis

This thesis utilizes journal paper format except for chapters I, II, and III. The organization of the thesis follows guidelines provided by *Guide to Preparation and Submission of Thesis and Dissertations* from Auburn University Graduate School.

This thesis is presented in the following order:

Chapter II presents a review of the current literatures from relevant studies to introduce some background and up-to-date progress.

Chapter III, IV and V describe the batch sorption experiments of EE_2 and the analysis of error involved in the experiment as well as the accuracy of result using different design strategies.

Chapter VI concludes the research work and recommends the future works.

CHAPTER II BACKGROUND

a. Pharmaceuticals and personal care products (PPCPs) in the environment

Pharmaceuticals and personal care products (PPCPs) refer to products used in human health and cosmetic reasons or used in enhancing the agricultural production and prohibiting the disease of livestock. Because of their wide application, a considerable amount of PPCPs are introduced into the environment, where they can negatively affect aquatic life. Since a large part of PPCPs discharged into the environment is from the domestic wastewater, wastewater treatment plants (WWTPs) become an important line of defense to keep those compounds from entering the environment.

i. Occurrence in rivers, lakes, and groundwater

Kolpin *et al.* (2002) used five separate analytic methods to determine the environmental extent of 95 kinds of PPCPs sampled from 139 stream sites across the nation. 80% of the 139 streams were found to be contaminated by the PPCPs. Additionally, 82 out of 95 different PPCPs were found at least once during the study. The concentration of one compound out of 82 PPCPs exceeded its maximum contaminant level (MCL) in a single site. Another compound exceeded its MCL in five sites. The occurance of PPCPs in aquatic environment and bottled water was reiterateed by the CNN News on three consecutive days in the middle March, 2008. At least one pharmaceutical was detected in the water supply of 24 major metropolitan areas, reported by Associated Press. Pharmaceuticals were found in nearly 20 states across the nation.

Boyd *et al.* (2003) found that the concentration of Naproxen, a pain killer, in Mississippi River, Lake Pontchartrain and Detroit River waters ranged from 22 to 107 ng/L. Clofibric acid in the Detroit River water was detected at concentration of 103ng/L. Ternes *et al.* (1999) detected the 17β-estradiol in the surface water at concentration of 0.2 to 2.6ng/L. The prevalence of PPCPs in the environment had also been demonstrated in Europe. The EU 5th Framework Poseidon project reported that the concentration of frequently detected PPCPs in surface waters was below 10ng/L. Herberer *et al.* (1998) investigated 30 individual Berlin surface waters and found the concentration of clofibric acid exceeded 1000ng/L and diclofenac concentration was above 100ng/L. Ayscough *et al.* (2000) detected up to 1 μg/L of a specific PPCP in UK, but most of the PPCPs concentrations were below 5-10ng/L. Farre *et al.* (2001) used Microtox bioluminescent test to determine the downstream PPCPs concentration in Spain and recorded 1.84μg/L lower than the toxicity threshold which was 19.1mg/L. The occurrence of pharmaceuticals was also prevalent in the water environment of Asia. These studies indicated that the PPCPs were prevalent in the surface water environment where people activities existed.

PPCPs also enter the ground and contaminate the groundwater. Heberer *et al.* (1997) detected diclofenac in groundwater samples. Sacher *et al.* (2001) reported 39 out of 105 samples contained pharmaceutical compounds whose concentrations above 10ng/L. More than one pharmaceutical compound was found in 24 of those 39 samples. Brownawell and Iden (2002) detected the concentration of PPCPs in the groundwater from monitor wells and found the concentration ranged from 2 to 200ng/L. Most of them were caffeine, paraxanthine, carbamazepine, and sulfamethoxazole.

ii. Effects on aquatic life

Since 1990s, the effects of PPCPs on the aquatic life have been studied. Even trace concentrations in surface water can affect the aquatic organisms (e.g. fish and amphibians). Ferrari *et al.* (2003) used *C. dubia* to test the acute and chronic toxicity of carbamazepine (CBZ) and Diclofenac. They found the acute criteria concentration which causes the 50% effect (EC_{50}) for CBZ was 77,700µg/L (48h) and the chronic criteria least observed effect concentration (LOEC) and no observed effect concentration (NOEC) was 100 µg/L (7 days) and 25 µg/L (7 days), respectively. For diclofenac, the EC_{50} was 22,704 µg/L (48h). LOEC and NOEC were 2000 µg/L (7 days) and 1,000 µg/L (7 days), respectively (Ferrari *et al.,* 2003). Halling-Sorensen *et al.* (1998) determined the toxicity of 17 α-ethinylestradiol, ibuprofen, bacitracin, and bromocyclen. The acute criteria concentrations were 105 µg/L (using *Daphnia*), 9060 µg/L (using *D. magna*, 48h), 30,000 µg/L (using *D. magna*, 48h), and 353 (using *D. Magna*), respectively. Herberer *et al.* (1997) reported the deleterious effect of a reproductive hormones which was at a level of <0.001 µg/L.

PPCPs in the aquatic environment can be ingest and accumulated by aquatic life. Gatermann *et al.* (1999) found the relatively high concentrations of musk ketone (4-acetyl-1-*tert*-butyl-3,5-dimethyl-2,6-dinitrobenzene, MK; maximum levels: mussels 2,200 ng/g lipid; winter flounder muscle 2,700 ng/g lipid; clams 17,700 ng/g lipid) and HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[g]-2-benzopyrane, 'galaxolide'; mussels 1,700 ng/g lipid; winter flounder 40 ng/g lipid; clams 3,000 ng/g lipid) inside the Halifax and Miramachi estuary. They also found relatively low level of

musk xylene (1-*tert*-butyl-3,5-dimetyl-2,4,6-trinitrobenzene, MX) and AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl-tetrahydro-napthalene, 'tonalide') to be contained inside Canadian aquatic fauna such as lobster, winter flounder, American eel, lake trout, clams and mussels (Gatermann *et al.*, 1999).

Among PPCPs, endocrine disrupter chemicals (EDCs) had important adverse effects on the aquatic organisms (Jobling *et al.,* 1998; Lange *et al.,* 2001; Thorpe *et al.,* 2003; Parrott and Blunt, 2005). Lange *et al.* (2001) reported that exposed to 17α-ethinylestradiol (EE_2) at 4.0ng/L male fish cannot develop secondary sexual characteristics. However, such dosage had no effect on the female fish. Fenske *et al.* (2005) found that elevated vitellogenin (VTG) concentrations, gonadal feminization, and inhibited reproduction happened on the minnows that full life-cycle exposed to the aquatic environment contained 3ng/L EE_2. Segner *et al.* (2003) studied the estrogenic potency of EE_2 in surface waters compared natural steroidal estrogens. They found the estrogenic potency of EE_2 were 10~50-fold higher than that of estradiol-17β (E_2) and estrone (E_1). Pomati *et al.* (2007) investigated the effect of a mixture of 13 pharmaceuticals on growth and transcriptional regulation in zebrafish liver cells and reported the overexpression of oestrogen receptor beta and the oestrogen responsive protein GREB1 caused by the drugs. Kim *et al.* (2007) studied the ecological risks of four most abundantly used pharmaceuticals and six sulfonamide and estimated the hazard quotients derived from PECs and predicted no effect concentrations (PNECs) for sulfamethoxazole and acetaminophen were 6.3 and 1.8, respectively, which suggested potential environmental concerns. Steger-Hartmann *et al.* (1997) found an environmental

effect of enhanced mutation frequencies in eco-organisms when they studied the antineoplastic agent cyclophosphamide in sewage water at concentration from 7ng/L to 143ng/L. PPCPs have an adverse effect on the aquatic life at low concentrations.

iii. Discharge from wastewater treatment plants

PPCPs have been detected in effluent streams from wastewater treatment plants (WWTPs). Johnson *et al.* (2000) observed the removal of steroid oestrogen by activated sludge works in Italy and The Netherlands. Ethinyloestradiol (E_3) was found at concentration of 57ng/L in influent and 10ng/L in the effluent. Additionally, a range of <0.4~4.5ng/L of EE_2 and <0.5~54ng/L of E_1, <0.5~12ng/L of E_2, and <0.5~28ng/L of E_3 were found in the effluent of the activated sludge process (Johnson *et al.,* 2000). Baronti *et al.* (2000) monitored natural and synthetic estrogens at activated sludge sewage treatment plant in Italy and reported that the concentration of E_2 and EE_2 in the effluent of six activated sludge treatment plants were 1 and 0.45ng/L, respectively. The concentrations of E_1, E_2, E_3, and EE_2 downstream of the WWTPs were between 0.04 (EE_2) and 1.5ng/L (E_1) (Baronti *et al.* 2000). Another study from Denmark reported the effluent concentrations of steroid estrogens in from the WWTPs were 0.20±0.06% for E_1, 0.24±0.07% for E_2, and 0.29±0.07% for EE_2 (Andersen *et al.,* 2005). However, large variance of the removal rates existed between different WWTPs. Lishman *et al.* (2006) investigated the reductions of acidic drugs, triclosan, polycyclic musks, and estrogens by municipal wastewater treatment plants in Ontario, Canada. Ternes *et al.* (2001) reported up to 1.9µg/L of caffeine and up to 0.48µg/L of propyphenazone in the effluent of 14 WWTPs. Lacey *et al.* (2008) found 15 out of 20 pharmaceutical compounds in effluent of

WWTPs in Ireland using LC-MS method and identified salicylic acid and ibuprofen as the most abundant and 9.17 and 3.20μg/L, respectively. Feldmann *et al.* (2007) reported the occurrence of metamizole residues in a Germany WWTP effluent at concentration of 7μg/L. The majority (51~59%) of pharmaceuticals in the influent could be removed during secondary treatment with effluent concentration of 0.013 to 0.056μg/L (Thomas and Foster, 2005). Lee *et al.* (2004) in Canada reported the pharmaceutical removal efficiency by secondary treatment. For WWTPs in Canada, they found that it was 55~99% with effluent concentration at 0.01~17.3μg/L.

b. Steroidal compounds

i. Physical and chemical properties

Steroid is a general class of chemical compounds containing a 17-carbon 4-ring system and including the sterols and numerous hormones (as anabolic steroids or corticosteroids) and glycosides (Merriam-Webster Dictionary). Steroids can be from hormones, body constituents, and drugs. Thus, it has more than one meaning. The estrogen, a female hormone, is a steroid. Vitamin D, an important substance to maintain a healthy body, is another steroid. EE_2 is a steroid, too, that is used in medical prescription for contraceptives.

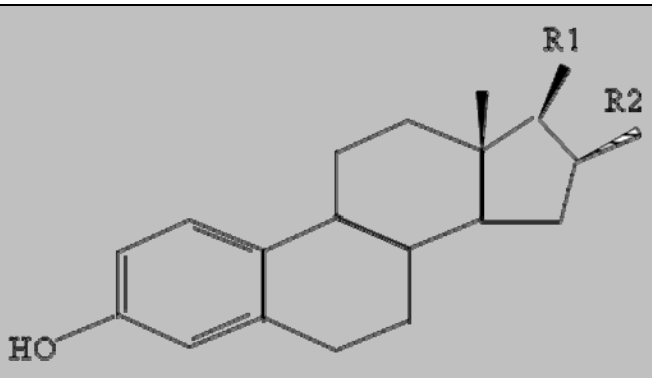
According to the function, steroids can be classified into animal steroids, plant steroids, and fungus steroids. Besides insect steroids in animal steroids, the vertebrate steroids contain steroid hormones and cholesterol. Sex steroids, corticosteroids, and anabolic steroids are three sub-classes of steroid hormones.

The word “steroids” often means the anabolic steroids which are synthetic hormones that are similar to the hormones inside the body. They can mimic the natural hormones, affect the binding between hormones and their receptors, affect the endocrine system in the body, and inhibit the function of self hormones.

Physiochemical properties of selected steroids are displayed in Table 2.1.

Table 2.1 Physical and chemical properties of Estriol, 17α-Ethinylestradiol, Estrone, and 17β-Estradiol

General Chemical Structure



Specific chemical structures and properties

	Estriol	17α-Ethinylestradiol	Estrone	17β-Estradiol
R1 group	-OH	-OH; -C≡OH	=O	-OH
R2 group	-OH	-	-	-
Molecular	$C_{18}H_{24}O_3$	$C_{20}H_{24}O_2$	$C_{18}H_{22}O_2$	$C_{18}H_{24}O_2$
Molecular weight	288.4	296.4	270.4	272.4
Water solubility (mg/L)	441	11.3	30	3.6
Melting Point (°C)	282	183	260.2	178.5
log K_{OW}	2.45	3.57	3.13	4.01
K_H	1.33E-12	7.94E-12	3.80E-7	3.64E-11
***Vapor Pressure* (mm Hg)**	1.97E-10	2.67E-9	1.42E-7	1.26E-8
***Atm OH Rate con* (cm^3/molec.-sec)**	1.29E-10	1.25E-10	1.26E-10	1.23E-10

Structure and Physiochemical properties retrieved from NLM. 2006. ChemIDplus Lite.

ii. Estimated discharge from anthropogenic sources

Incessantly, considerable amount of steroids discharge into the environment through human activity. Over 3.2 billion prescriptions were filled in 2003 which is 1 billion more than that in 1995 (National Association of Chain Drug Stores 2004-2005). This number would increase 10.1% in 2011 (Heffler *et al.,* 2005). Another data from Czech Statistical Office said that about 350,000 women between the ages of 15 and 54 years live in Prague, Czech. Between 25 and 100 μg/L estrogens as 17β-estroadiol, estriol, and estrone is secretes by each person. So the total amount of estrogen that input into environment can achieve 2.1 g/day (Morteani *et al.,* 2006). Turan (1995) reported an estrogen excretion rates in a range of 20~100μg per day by women depending on the phase of the menstrual cycle. They also showed that a 30 mg secretion of estrogen by the end of pregnancy. Lishman *et al.* (2006) studied the contribution of PPCPs and estrogens from anthropogenic source and estimated individual generation rates based on data (Ibuprofen 2.50, Gemfibrozil 0.20, Naproxen 2.27, Diclofenac 0.088, and Estrone 12.8mg per capital per day) from 12 municipal wastewater treatment plants along the Thames River, Canada. Such increasing input of estrogens to the environment needs scientists to study the behavior of those compounds and to develop a method to eliminate them.

c. The activated sludge processes

i. Overview

In the activated sludge process, microorganisms remove both organic and inorganic constituents from wastewater by chemical, physical, and biological reactions. A

conventional municipal activated sludge wastewater treatment plant (WWTP) schematic is shown in Figure 2.1.

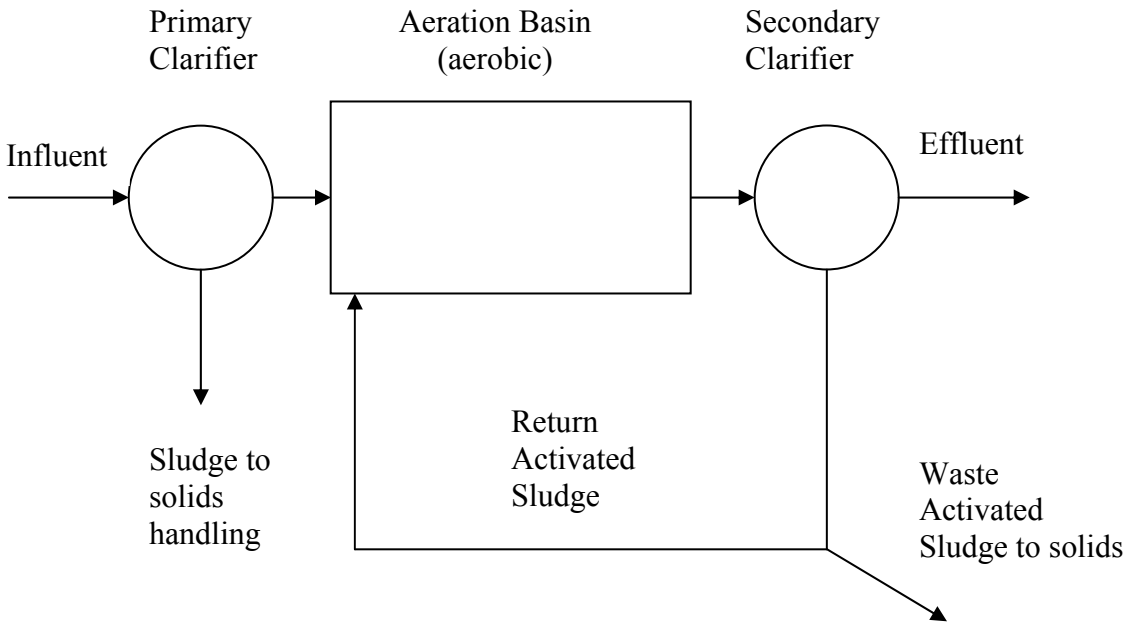


Figure 2.1 Conventional activated sludge wastewater treatment plant schematic (Yi and Harper, 2007)

Influent wastewater is fed to a primary clarifier for removal of settleable particulate matter. The primary effluent is then fed to an aeration basin where particulate and dissolved organics, nutrients, and metals are removed by a flocculent biomass. The wastewater is then routed to a secondary clarifier for biomass recycle and for solids separation to produce a clarified secondary effluent.

ii. Modifications to the activated sludge process

For typical municipal wastewater, the activated sludge process removes some but not all N and P by incorporation through growth into biomass. Because many receiving waters are subject to eutrophication, activated sludge processes that enhance N and P removal have been developed (Biological Nutrient Removal (BNR) Processes). Enhanced Biological Phosphorus Removal (EBPR) is one of these BNR processes.

iii. Removal of steroidal compounds in the activated sludge processes

Numerous reports have explored the removal of various classes of PPCPs at full scale, generally attempting to evaluate whether municipal WWTPs are acting as persistent point sources for PPCP discharge to the environment. Ternes (1998) showed that the removal efficiencies ranged from 10 to 90% in wastewater treatment plants in Germany, and Ternes *et al.* (1999) showed that removal efficiencies for polar PPCPs varied from 12 to 90% for WWTPs in Brazil. Gomez *et al.* (2007) conducted a one-year monitoring study at a sewage treatment plant in Spain, and they found that the removal efficiencies for 14 organic micropollutants varied from 20% (carbamazepine) to 99% (acetaminophen). Joss *et al.* 2006 showed that only 4 out of 35 compounds are 90% removed using state-of-the-art biological treatment systems, and 17 out of 35 are

removed at less than 50% efficiency. These studies are in addition to others that present high removal efficiencies. Oppenheimer and Stephenson[5] found that removal efficiencies for frequently detected PPCPs were generally high (>80%), and another study by Jones *et al.,* 2005 found that ibuprofen, paracetamol, salbutamol and mefenamic acid were removed at approx. 90% within a large sewage treatment plant in England. Overall, these efforts have shown that the removal efficiencies vary greatly.

That conclusion that PPCP removal in full-scale systems varies considerably is further supported by Lishman *et al.* (2006) who investigated the presence of selected acidic drugs, triclosan, polycyclic musks, and selected estrogens in WWTP influent and effluent at sites in Canada. They found that three analytes were never detected during the survey (clofibric acid, fenoprofen, fenofibrate) and two analytes were always removed at high efficiency for all treatment configurations (ibuprofen, naproxen, triclosan). Two analytes were removed at a low efficiencies (gemfibrozil, diclofenac), but better removals were observed for treatment configurations with higher solids retention times. Five polycyclic musks were surveyed; general conclusions could not be reached because of the small data set and because of numerous nonquantifiable results, but removal efficiencies generally were variable. E_2 and E_1 were both removed at high efficiency for all treatment systems. Even where conventional WWTPs are concerned, removal efficiencies for different PPCPs can vary significantly. Diclofenac removal efficiency is negative suggesting that diclofenac may be deconjugated during the treatment process. Generally, these full-scale studies have not collected the type and amount of data necessary to organize mass balances for specific PPCPs, so that a clear articulation of the relative roles

of sorption and biodegradation in the full-scale process is generally unavailable. Some studies have complemented full-scale studies with batch experiments, so that the potential for sorption and/or biodegradation at full-scale can be assessed.

Removal efficiencies can vary as a function of the type of compound. Carballa *et al.* (2004) surveyed two cosmetic ingredients (galaxolide, tonalide), eight pharmaceuticals (carbamazepine, diazepam, diclofenac, ibuprofen, naproxen, roxithromycin, sulfamethoxazole and iopromide) and three hormones (estrone, 17β-estradiol and 17α-ethinylestradiol) at municipal WWTPs in Spain. They found that the overall removal efficiencies ranged between 70–90% for the fragrances, 40–65% for the anti-inflammatories, approximately 65% for 17β-estradiol and 60% for sulfamethoxazole. However, the concentration of estrone increased along the treatment due to the partial oxidation of 17β-estradiol in the aeration tank. Nakada *et al.* (2006) measured a host of compounds, including six acidic analgesics or anti-inflammatories (aspirin, ibuprofen, naproxen, ketoprofen, fenoprofen, mefenamic acid), two phenolic antiseptics (thymol, triclosan), four amide pharmaceuticals (propyphenazone, crotamiton, carbamazepine, diethyltoluamide), three phenolic endocrine disrupting chemicals (nonylphenol, octylphenol, bisphenol A), and three natural estrogens (17β-estradiol, estrone, estriol) in 24-h composite samples of influents and secondary effluents from municipal WWTPs in Tokyo. They found that aspirin, ibuprofen, and thymol were removed efficiently during secondary treatment (>90% efficiency). They also found that amide-type pharmaceuticals, ketoprofen, and naproxen showed poor removal (<50% efficiency), probably because of their lower hydrophobicity (log K_{OW}<3). This study was also the

first to report the presence of crotamiton (a topical treatment for *scabies*), and to show that it is persistent during secondary treatment (Nakada *et al.,* 2006). Overall, these results reinforce the conclusion that removal efficiencies vary for the various PPCPs and suggest that chemical characteristics also may play an important role in determining the fate of each compound in biological wastewater treatment.

Removal efficiencies also can vary as a function of the sludge retention time (SRT). Oppenheimer and Stephenson[5] studied the removal of 20 PPCPs in full-scale and pilot scale WWTPs in the U.S, and they organized their data using a BIN assignment system, which assigned each detected compound into a category related to the frequency of detection (i.e. infrequent, variable, and frequent) and into another category related to the removal efficiencies (excellent removal, moderate removal, poor removal). They found that half of the PPCPs were frequently detected and were removed at less than 80% efficiency at a sludge retention time (SRT) of 5 days of less. Caffeine and ibuprofen were among 9 compounds that were both frequently detected and removed well for all the systems in the study. Galaxolide and musk ketone were also frequently detected but removed at 80% only when the SRT exceeded 25 days.

Membrane bioreactor systems (MBRs) have been evaluated as a possibly better technology for removing PPCPs. MBRs use a suspended growth bioreactor, like in conventional activated sludge, but replaces gravity sedimentation with micro- or ultra-filtration. The MBR is an attractive treatment configuration because it eliminates the need for secondary clarification, which in turn allows the overall treatment process to be sited on a much smaller footprint. Kim *et al.* (2007) found that the MBR system was efficient

for hormones (e.g., estriol, testosterone, androstenedione) and certain pharmaceuticals (e.g., acetaminophen, ibuprofen, and caffeine) with approximately 99% removal, but MBR treatment did not decrease the concentration of erythromycin, trimethoprim, naproxen, diclofenac, and carbamazepine. Oppenheimer and Stephenson (2006) used a limited data set to suggest that MBR provided no additional PPCP removal, when compared to similarly operated conventional systems. Kimura *et al.* (2005) found that MBRs exhibited much better removal regarding ketoprofen and naproxen, but with respect to the other compounds, comparable removal was observed between the MBRs and conventional systems. These data suggest that MBRs likely offer no inherent advantage over conventional systems for removing PPCPs, but because MBRs are operated at long solids retention times and at high mixed liquor suspended solids (MLSS) concentrations, those operational factors are likely the cause of any measured differences in PPCP removal efficiencies.

Finally, there remains a need to continue to conduct full-scale studies, with the goal of organizing accurate mass balance and fate data. To accomplish this, rigorous wastewater sampling methods must be employed. For example, these full scale studies collected data using time-weighted composite sampling using automatic samplers, equipped with sample storage in cooled compartments. This strategy allowed the reports to collect data that is likely to represent a reasonable estimate of the PPCP concentrations of interest, as well as the inherent variability; but this approach is not infallible. Many of the PPCPs of interest are biodegradable, and may be transformed while the samples remain stored in the collection container. Still other compounds are highly hydrophobic

and sorb strongly to biomass solids and colloidal materials that are also present in the original sample. In these cases, it is possible to underestimate the concentrations of interest, either because the solids are not properly re-suspended before sample analysis, or because of inadequate extraction techniques. Finally, time-weighted sampling collects a given wastewater volume at given time intervals, even if the wastewater flow is low. This means that time-weighted sampling may cause low-flow PPCP concentrations to be over-represented in the composite sample. For these reasons, future sampling campaigns should consider the use of flow-weighted sampling in combination with frequent grab sampling to minimize the error associated with sample collection. Each collected sample should also be mixed vigorously to resuspend settled material, and PPCP analysis should be carried out on both the filtered and unfiltered samples. Improvements in sample collection methodology will strengthen the reliability of the data, which in turn will no doubt be the basis for future treatment plant optimization and regulatory action.

d. Sorption of ethinylestradiol in activated sludge

In general, the partitioning of organic compounds from water onto activated sludge biomass is referred to as adsorption, although it may be more appropriate to refer to this as sorption because there may be some uncertainty as to whether the compound is on the surface (adsorption) or partitioning into another phase (absorption). When sorption is of interest, it is important to establish a relationship between what is on the surface and what is in the aqueous phase, a relationship generally referred to as a sorption isotherm. The term isotherm comes from the idea that the equilibrium is reached at a constant temperature to distinguish this type of partitioning from condensation. These

relationships are determined experimentally and then the data is used to determine a partitioning coefficient, which is a measure for the affinity of a given compound for the activated sludge biomass.

Partitioning coefficients (K_d) have been determined in a number of studies to investigate PPCP sorption to activated sludge. Ternes *et al.* (1999) conducted a series of batch tests with primary and secondary sludge slurries to determine partitioning coefficients for a number of target PPCPs. They found that the K_d values of pharmaceuticals ranged from <1 to 500 L kg^{-1}, while that of the polycyclic musk fragrances AHTN and HHCB proved to be much higher and up to 5300 and 4900 L kg^{-1}, respectively. They also found significant differences between the K_d values obtained between primary sludge and secondary sludge; for acidic pharmaceuticals and musk fragrances, the K_d values were higher when measured with primary sludge; the opposite was true with neutral pharmaceuticals, iopromide, and ethinyl estradiol.

The sorption equilibrium partitioning coefficients determined for steroid estrogens with activated sludge show some limited variability, but they are generally in good agreement. Clara *et al.* (2005) found that the log (K_d) for steroid estrogens were 2.84 (2.64–2.97) and 2.84 (2.71–3.00) for E2 and EE2, respectively. In the work by Ternes *et al.* (1999) the log (K_d) for EE_2 was determined to be 2.54 (2.49–2.58). Yi *et al.* (2006) found that the log (K_d) for EE_2 was 2.7 for membrane bioreactor sludge and 2.3 when the sludge was taken from a sequencing batch reactor; since the MBR particle sizes were significantly smaller than the SBR particles, this result suggested that particle size may explain some of the variability that is reported for steroid estrogen partitioning

coefficients. Andersen *et al.* (2005) determined distribution coefficients (K_d) with activated sludge biomass for the steroid estrogens , estrone (E_1), 17β-estradiol (E_2) and 17α-ethinylestradiol (EE_2) in batch experiments, and they determined log K_d values for steroid estrogens of 2.6, 2.7, 2.8 respectively. When Andersen *et al.* (2005) corrected their *log* (K_d) values to account for the organic carbon content of the sludge, they found that the *log* (K_d) values were 3.16, 3.24, 3.32 respectively. These values were remarkably consistent with the sorption partitioning coefficients determined where soil is used as the sorbate. Taken together, these partitioning coefficients enable practitioners to model PPCP sorption in activated sludge processes, and numerically evaluate the importance of sorption as a removal mechanism.

Sorption is not always an important removal mechanism. Ternes *et al.* (2000) found that, for compounds with the K_d values less than 500 L/Kg, only 20% of the target compound mass was associated with the sludge solids, which showed that the majority of the mass of the target compounds remained in solution. This result supported the idea that sorption is not an important removal mechanism for many pharmaceutical compounds. Yu *et al.* (2006) conducted aerobic batch biodegradation (using activated sludge as microbial inocula) experiments to evaluate the biodegradation behavior of 18 target PPCPs at initial concentrations of 50, 10, and 1 μg/L. The target compounds included a number of antiseptics, barbiturates, and anticonvulsants. Their sterile control studies showed no loss of target PPCPs during the entire incubation period, and sorption to the biomass was found to be negligible for all testing conditions (Yu *et al.,* 2006). Urase and Kikuta (2005) conducted a series batch experiment to examine the removal of three

steroid estrogens (i.e. 17β-estradiol), two endocrine disruptors (i.e. bisphenol A), and 10 pharmaceutical substances by activated sludge. Many of the target PPCPs in this study were hydrophilic, had lower water–sludge partition coefficients than the steroid estrogens, and remained in the aqueous phase, with only a small fraction partitioning to the activated sludge.

When sorption is important there is a sorption/desorption cycle that should be investigated experimentally. In some cases, desorption fails to restore the full capacity of the sorbent, and when this happens, some of the sorption sites remain occupied. This is referred to as sorption hysteresis, and this has been reported for many organic compounds where either soil or sludge acts as the sorbent (Huang *et al.,* 2003; Kim *et al.,* 2005; Conrad *et al.,* 2006). Hysteresis has thus far received little attention where PPCP sorption to sludge is concerned. Recently, Kim *et al.* (2005) showed sorption hysteresis in the case of tetracycline sorption/desorption with activated sludge, but this is probably because tetracycline forms strong complexes with Ca (II) and other divalent cations known to be important for floc stability (Sobeck and Higgins 2002; Schwarzenbach *et al.,* 2003). PPCP sorption hysteresis is a basic and relevant process that has not received great attention to date.

One cause of sorption hysteresis may be related to particle characteristics (e.g. size), and there is a need to study the possible fundamental connections. Yi and Harper (2007) hypothesized that sorption hysteresis is more pronounced as the biomass particle size distribution shifts toward larger sizes. The rationale for this was that smaller flocs are denser and less permeable than larger flocs (Snidaro *et al.,* 1997; Chu *et al.,* 2005)

therefore allowing for much less intraparticle entrapment of PPCPs. In general, activated sludge particles in conventional processes are typically 80-300 um in diameter (Metcalf and Eddy, 2003), and this structure typically consists of smaller microcolonies (approx 8-15 um) connected by exocellular polymeric and inorganic material, and with a few large flow channels that facilitate transport (Snidaro *et al.,* 1997; Chu *et al.,* 2005). Smaller activated sludge particles can be found in bioreactors like membrane bioreactors (MBR) (Andersen *et al.,* 2005; Ng and Hermanowicz, 2005), and smaller particles have less internal polymer, a higher number of cells per unit volume (Snidaro *et al.,* 1997) and they do not have the large flow channels that facilitate transport.

Yi *et al.* (2007) investigated this hypothesis by operating two laboratory-scale bioreactor systems, a MBR and conventional bioreactor (CBR), both operated in continuous flow mode. The experimental strategy was to harvest biomass from the bioreactors for use in a series of sorption/desorption batch tests. The data retrieved from the batch tests was used to determine sorption and desorption isotherms, from which the partitioning coefficients (K_d and K_{ds} respectively) and sorption hysteresis (HI) index values were calculated (Yi and Harper Jr., 2007). Sorption hysteresis index (HI) was calculated as follows:

$$HI = \frac{K_{ds} - K_d}{K_d}\Bigg|_{T,Cr} \qquad (1)$$

The subscript T (23°C) and C_r (C_r level is 0.5) refer to specific conditions of constant temperature and residual solution phase concentration ratio, respectively. The partitioning coefficient determined from the sorption experiments is K_d, and the partitioning

coefficient determined from the desorption experiments is K_{ds} . Samples were also collected for biomass particle size analysis.

A typical sorption/desorption result is shown following figure;

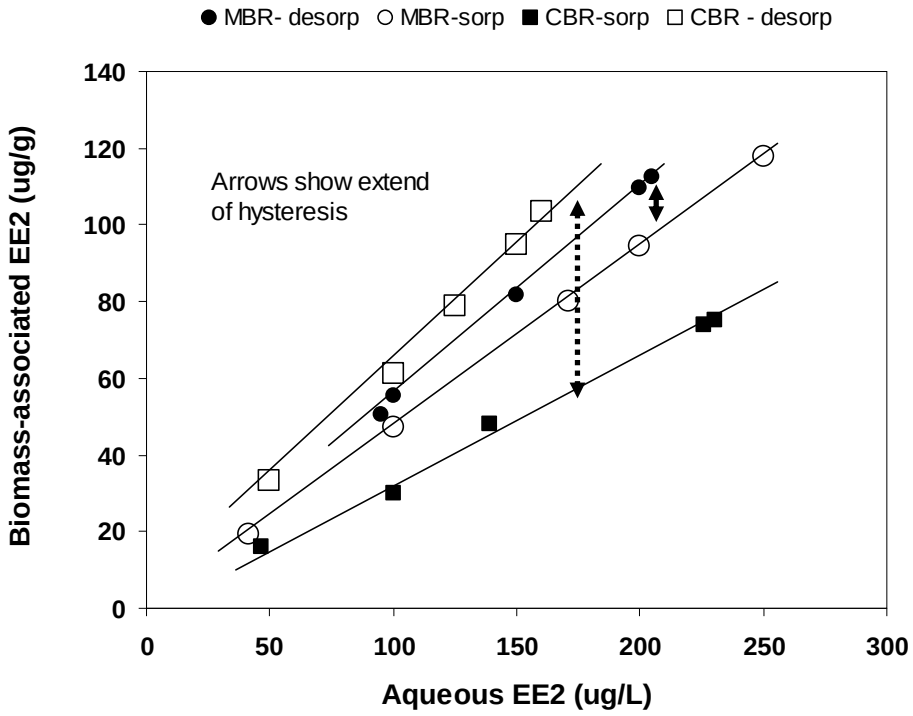


Figure 2.2 Sorption/desorption of EE_2 onto/from activated sludge. (Yi et al., 2007)

for the two different biomass floc suspensions. The suspension taken from the MBR had a mean particle size of 10 μm, while that of the CBR had a mean particle size of 120 μm. In this example, the sorption/desorption experiment yielded K_d and K_{ds} values of 0.47 L/g and 0.56 L/g for the MBR biomass, and 0.32 L/g and 0.61 L/g for the CBR biomass respectively. Using these values, the hysteresis index values for the MBR and CBR were 0.19 and 0.89 respectively. Results such as these suggest that the particle size influenced the hysteresis index for EE_2 sorption. Yi and Harper Jr. (2007) found that as the mean particle size increased from 10 um to 230 um, the HI increased nonlinearly from approximately to 0.2 to 0.9. This result showed that the biomass particle size can have a dramatic effect on the entrapment of EE_2 within activated sludge floc, which in turn may affect the ultimate fate of EE_2.

CHAPTER III RESEARCH OBJECTIVES

Removing PPCPs from wastewater continues to represent an important priority in the water quality community. The primary reason for this is because discharging these chemicals into water bodies may cause genetic and developmental abnormalities in microorganisms, aquatic life, and possibly even humans. Water and wastewater characterization studies have clearly shown that there are a wide range of PPCPs present, and that in many cases a significant fraction of these chemicals pass through the biological wastewater treatment process (Ternes 1998; Ternes *et al.,* 1999; Joss *et al.,* 2006). Recent applied research has demonstrated that PPCP removal efficiencies depend on a number of factors including the chemical characteristics of the compound(s) (e.g. log K_{OW}), the operating solids retention time (SRT) of the treatment plant, and the biomass particle characteristics (Carballa *et al.,* 2004; Yi and Harper Jr., 2007; Oppenhiemer and Stephenson, 2006). Although these results are largely observational, they can be used to design hypothesis-driven experiments capable of uncovering the underlying removal mechanisms that are still generally unclear for most PPCPs.

17α-ethinylestradiol (EE_2) is a synthetic steroidal PPCP that has attracted considerable attention in the literature, and because of this, the mechanisms involved with removing EE_2 compounds have become clearer. EE_2 is nonpolar and hydrophobic, so that it adsorbs strongly onto activated sludge particles. Recent research has shown that sorption is an important removal mechanism in biological wastewater treatment systems

(Cirja *et al.,* 2007). Equilibrium partitioning coefficients (K_d) have been determined by previous sorption studies. The log K_d values have been reported over the range of 2.3 to 3.0 (Clara *et al.,* 2004; Ternes *et al.,* 2004; Yi *et al.*, 2006), values that enable practitioners to empirically estimate removal due to sorption only. The current need is to deepen the understanding of EE_2 sorption to activated sludge, a goal that can be accomplished by clarifying the basic and relevant thermodynamic parameters. Most of the previous work was not conducted in a way that allowed for the determination of the activation energy or the enthalpic and entropic characteristics, and this knowledge gap means, #1) it is difficult to clearly understand the role of chemical reactions (chemisorption) versus the role of physical reactions (physisorption) in the EE_2 sorption process, #2) the driving forces for EE_2 sorption remain unclear, #3) possible functional groups involved with EE_2 attachment are unknown and , #4) the variations in measured log K_d values can't be explained in meaningful way (e.g. if EE_2 sorption is entropy-driven, it can be inhibited as the temperature of the medium is decreased). The goals of the current work are to #1) determine the activation energy (E_a) and the changes in enthalpy (ΔH) and entropy (ΔS) that are associated with EE_2 sorption to activated sludge biomass, #2) use thermodynamic parameters to determine whether EE_2 sorption is physisorption, chemisorption, or a combination of the two, and #3) use FT-IR to attempt to uncover the biomass associated functional groups possibly involved with EE_2 sorption.

Ren *et al.* (2007) recently produced important information related to the thermodynamics of EE_2 sorption to activated sludge biomass. They conducted sorption

equilibrium and kinetic experiments at two temperatures (4 and 20°C), and they found that the ΔH was approximately -4.6kcal/mole. This result suggests that EE_2 sorption to sludge is an exothermic process involving physisorption (i.e. Van de waals forces), however, the approach used by the authors did not permit the determination of ΔS and E_a values because this requires sorption experiments incubated at least four different temperatures. The accuracy of ΔH estimates may also be improved by conducting sorption experiments at more temperature values. One of the temperature values used (4ºC) probably only applies during the winter at wastewater treatment systems found in cold climates (Metcalf and Eddy, 2003). The current approach retrieved more thermodynamic information by conducting sorption experiments at 6 different temperature values over the temperature range more typically found in activated sludge aeration basins.

CHAPTER IV MATERIALS AND METHODS

a. Experimental overview.

Two laboratory-scale bioreactor systems were operated, a membrane bioreactor (MBR) and sequencing bioreactor (SBR). Since MBRs have smaller and more hydrophobic particles than SBRs (Yi *et al.,* 2006; Yi and Harper Jr., 2007), it is possible to explore the effect of particle characteristics on the thermodynamic properties of EE_2 by utilizing biomass from both of these two bioreactors. Both bioreactors were originally seeded with mixed liquor from the City of Auburn Southside Wastewater Treatment Facility. The experimental strategy was to harvest biomass from the bioreactors for use in a series of sorption batch tests. The sorption experiments were carried out over a range of temperatures. Kinetic data was retrieved to determine the activation energy, and equilibrium data was collected to determine changes in enthalpy and entropy. These parameters were then used to investigate the whether EE_2 sorption is controlled by physisorption, chemisorption, or a combination of both.

b. Bioreactor operation.

The membrane bioreactor had a working volume of 60 L and was equipped with one, vertically-mounted membrane module (pore size, 0.08um; physical size, 0.55m in total length; surface area, 0.5 m^2; courtesy of Vivendi/US Filter), completely submerged in a plexiglass vessel. The module had a diameter of 8 cm. The height of the vessel was 91 cm, with 16 cm of freeboard, and a 76 cm water depth. The module was placed to

allow a 10 cm clearance both from the vessel bottom and the water surface. The module was mounted in the middle of the vessel, and held in place by a plexiglass U-shaped support apparatus. The influent flow rate recommended by the vendor was kept at $40ml/\min\cdot m^2$ and $30l/d$ using masterflex pumps. The HRT of the reactor was 2d, and the SRT was 20d. The DO in the reactor was maintained at $2mg/l$ with airflow rate at $10l/\min$. The pH was kept between 6.8 and 7.3 using 0.1M HCL and 0.1M NaOH and inspected by the auto-pH meter (alpha pH 200 1/8-DIN pH/ORP Controller, EUTECH Instrument Pte Ltd, Singapore) and pH electrode (Thermo Orion Glass pH electrode, Orion Research, INC. Beverly, MA).

The SBR had a working volume of 4 liters. Aerobic conditions were maintained by bubbling ambient air through a porous diffuser. Aerobic conditions were verified by measuring dissolved oxygen (YSI Model 57 Oxygen Meter with YSI Model 5793 Standard Membranes, YSI Incorporated, Yellow Springs, Ohio) and redox potential (Eutech Instruments, Model 200, Singapore). The pH was controlled with an auto-pH meter (WDP Series Dual Input pH/ORP Controller, Walchem Corporation, Holliston, MA) and pH electrode (WEL-PHF-NN electrode, Walchem Corporation, Holliston, MA) with a protective housing (Model 102606, Walchem Corporation, Holliston, MA). The pH of each reactor was maintained in the range of 6.8 to 7.3 by the addition of 0.1 M HCL solution or 0.1 M NaOH solution. There was no pH control during the settling or effluent withdrawal phases. The temperature was ambient (approx. 24ºC). The HRT was 12 hours and the SRT was 20 days.

c. Synthetic wastewater.

The synthetic wastewater used for the MBR and the SBR was the same. The organic substrate (acetic acid) and inorganic nutrients were added in separate feed streams. The composition of the synthetic feed was (on mg COD /L basis: acetate (360), casamino acids (20), yeast extract (< 1)). The inorganic salts content was (as mg/L total influent concentration): KCl (210), $MgCl_2$-$6H_20$ (394), $MgSO_4$-$7H_20$ (26), $CaCl_2$ (80), H_3BO_3 (0.11, $ZnSO_4$-$7H_20$ (0.0.50), KI (0.027), $CuSO_4$-$5H_20$ (0.11), $Co(NO3)_2$-$6H_20$ (0.135), $NaMoO_4$-$2H_20$ (0.056), $MnSO_4$-H_20 (0.62), and $FeSO_4$-$7H_20$ (0.55). The influent P concentration was supplied as NaH_2PO_4-$2H_2O$ and was always 8.0 mg P/L. The influent N was supplied as NH_4Cl and was always 40.3 mg N/L.

d. Determination of *ΔH* and *ΔS*: Protocol for equilibrium sorption experiments.

The EE_2 equilibrium sorption experiments were conducted using 200 ml biomass samples taken from the SBR and MBR. Sorption of EE_2 onto the biomass was determined by adding EE_2 into glass bottles at different concentrations ranging between 100 and 5000 μg/L. These concentrations are higher than those measured in real wastewater, but this does not present a problem in this study because the thermodynamic parameters associated with sorption are not concentration dependant (Annamalai and Puri, 2002). To prevent biodegradation, sodium azide was added at 0.2 % w/w (200 mg/L); this concentration inhibited biodegradation but did not cause cell lysis (as confirmed with soluble carbohydrate measurements, Figure 4.1.).

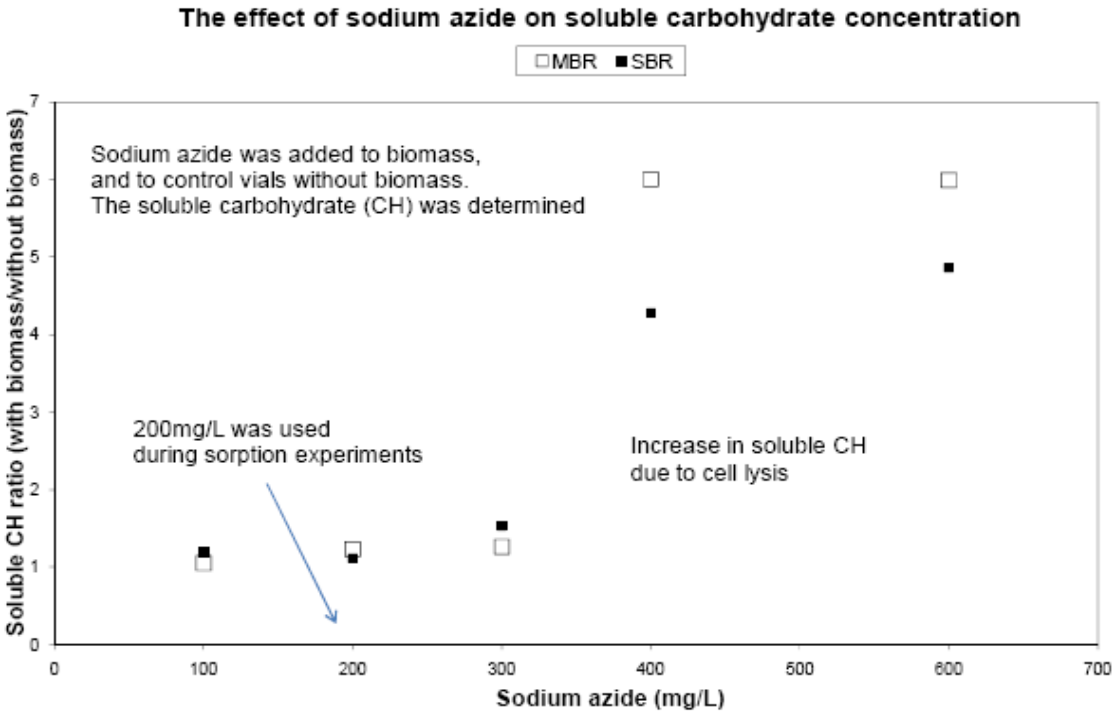


Figure 4.1 The effect of sodium azide on soluble carbohydrate concentration

Carbohydrate measurements were conducted as described in Jenkins *et al.* (1993). Samples were mixed on an orbital shaker at 200rpm, and additional control bottles were included without biomass to make sure that EE_2 was not being lost to the glassware. Samples were taken after 5h; preliminary kinetic tests indicated that equilibrium was reached in this time. The samples were centrifuged at 1500g for 5min, filtered (0.2-μm Teflon filter), and then analyzed for EE_2. Biomass-bound EE_2 concentrations were determined by mass balance. All sorption tests were done in triplicate. The equilibrium sorption experiments were conducted at 6 different temperatures (18, 22, 24, 26, 28 and 30°C). The lowest temperature used for SBR equilibrium experiments was 16°C (not 18°C).

The solid phase concentrations (q_s) of EE_2 were calculated by the following formula:

$$q_s = \frac{C_0 - C_e}{m} V ,$$

where C_0 and C_e (mg/L) are the initial and final concentration of EE_2, m is the dry weight of biomass in solution and V is the volume of the solution (L).

Data from the sorption experiments (q_s vs. C_e) were fitted by linear regression. The partition coefficients (K_d) of each temperature were derived from the slope of each line. By plotting lnK_d vs. $1/T$ the changes of enthalpy and entropy could be obtained from the slope and y-intercept using the following equation:

$$\ln K_d = -\left(\frac{\Delta H}{R}\right)\left(\frac{1}{T}\right) + \frac{\Delta S}{R} ,$$

R is the gas constant (8.314 J/mol·K), and T is the temperature in Kelvin. ΔG was

calculated at each temperature (=ΔH -$T\Delta S$).

e. Determination of Ea: Protocol for short-term kinetic sorption experiments.

The short-term batch experiments were conducted as described above, except that samples were collected over a short time frame (0 to 25 min) and the experiments were conducted at 7 temperatures (18, 20, 22, 24, 26, 28 and 30°C). The raw data from each kinetic test was used to determine a first order rate constant k. The sorption activation energy (E_a) was calculated using the Arrhenius equation: $k = Ae^{-\frac{E_a}{RT}}$, where k is the rate constant, A was frequency factor, R is the gas constant (8.314 J/mol·K), T is the temperature in Kelvin. The Arrhenius equation was linearized using the natural log function: $\ln k = -\frac{E_a}{RT} + \ln A$. The activation energy was determined from the slope ($-\frac{E_a}{R}$) by plotting $\ln k$ vs. $\frac{1}{T}$.

f. Analytical methods.

EE_2 was detected by HPLC (Hewlett-Packard, HP 1100). The system consisted of a degasser (G1322A), a Quaternary pump (G1311A), an ALS auto-sampler (G1313A), a Colcomp column oven (G1316A) and Variable Wavelength UV-VIS Detector (G1314A). A Hypersil ODS C18, (125x46 mm, 5μm) column was used. HPLC operating conditions were as follows; UV detector wavelength, 197nm and mobile phase, acetonitrile and water (40:60) with solvent delivered at a constant flow rate of 1mL/min. The total runtime of the HPLC analysis was 10min. Each batch of samples included spiked surrogate samples, and recovery always exceeded 90%. Total suspended solids (TSS) and volatile suspended solids (VSS) analysis was done according to Standard Methods

(APHA, 1992). Typical TSS concentrations were approximately 500 and 800 mg/L for the SBR and MBR respectively, and the VSS/TSS ratios for both bioreactors were typically 0.8. Particle size distribution and total specific surface area were determined on 15 ml samples from the MBR and SBR sludges utilizing a Horiba LA-920 laser scattering particle size distribution analyzer (Delta Analytical Instruments, North Huntington, PA). The measurement range for this instrument is 0.02 to 2000 microns. Typically the SBR biomass had a 120 um mean particle size, while the MBR biomass had a 50 um mean particle size.

g. FT-IR measurements.

The FT-IR spectrum of activated sludge with and without EE_2 sorbed on it was derived from the FTIR Spectrophotometer (Nicolet Avatar 360 FT-IR E.S.P). Samples were dried in the oven at 60°C and stored in the desiccators. 200 milligrams of KBr and 10 milligrams of sample were mixed thoroughly and pressed to pellet. Nitrogen gas was introduced into the chamber to exclude air, especially CO_2 and water vapor which would cause noise in the output. The range of FT-IR spectra was in the range of 400~4000cm^{-1}.

CHAPTER V RESULTS

Equilibrium Sorption Isotherms. Figure 5.1 shows the family of MBR sorption isotherms.

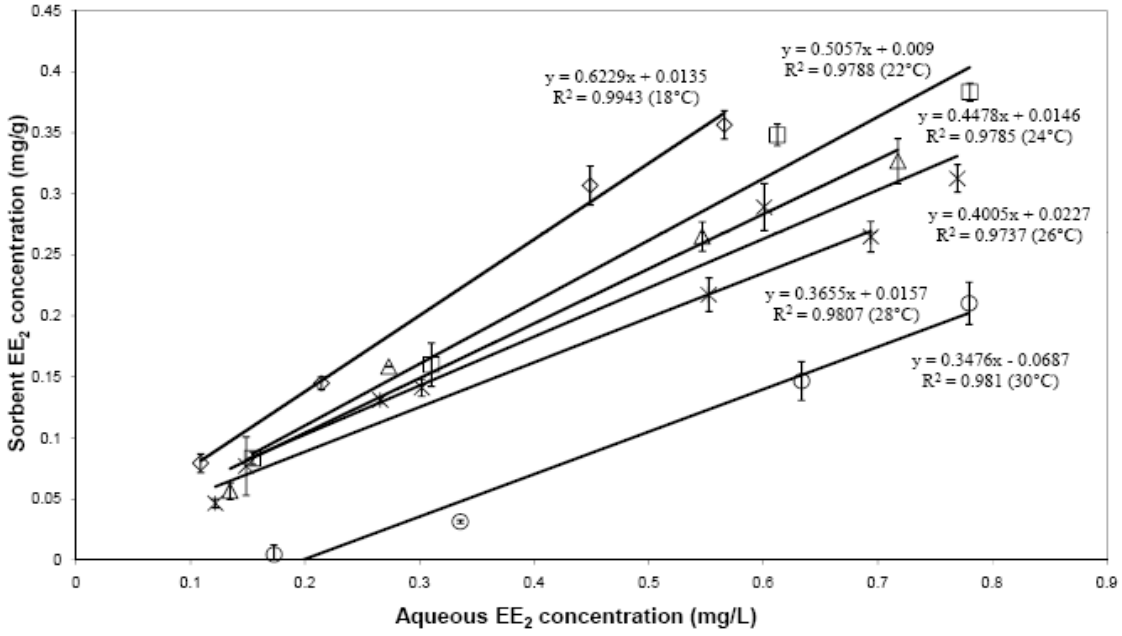


Figure 5.1 EE_2 sorption isotherms determined with MBR sludge

All of the sorption profiles were best approximated by a linear regression, consistent with the Freundlich (n =1) model. This means that, at a given temperature, the attractiveness of the sorbent (biomass) for the sorbate (EE_2) remained the same over the range of concentrations tested. The linear regressions also produced a small y-intercept value, which is important because the "true" isotherms must pass through the origin. The presence of a y-intercept is due to the experimental error inherent in the isotherm determination. The isotherm slope represents the partitioning coefficient, and the results showed that the partitioning coefficient decreased as the temperature was increased; it was 0.63 L/g at 18ºC and decreased to 0.35 L/g at 30ºC. These values are equivalent to a logK_d of 2.8 (at 18ºC) and 2.5 (at 30ºC) respectively, and they agree well with what has been measured previously (Clara *et al.,* 2004; Ternes *et al.,* 2004; Yi *et al.,* 2006). The SBR sorption experiments showed that the measured partitioning coefficients also decreased as the temperature was increased (Figure 5.2).

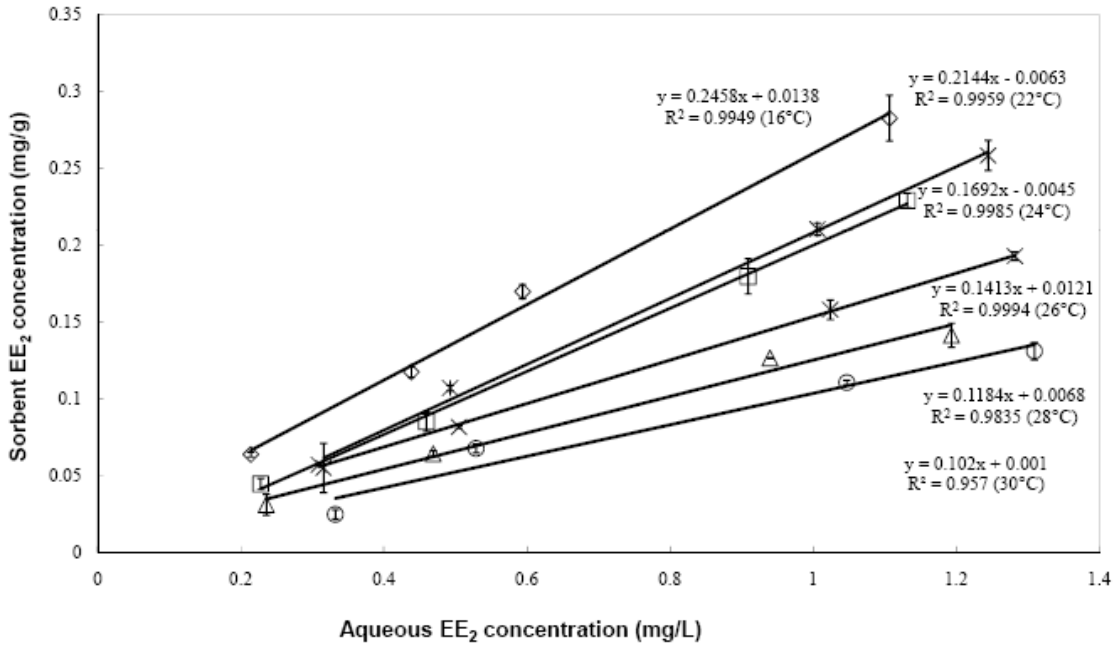


Figure 5.2 EE_2 sorption isotherms determined with SBR sludge

The partitioning coefficient was 0.21 L/g ($\log K_d = 2.3$) at 18ºC and 0.1 L/g ($\log K_d = 2$) at 30ºC. At each temperature, the SBR K_d value was lower than the associated MBR K_d value, a result previously reported by Yi *et al.* (2006). These results demonstrate the effect of temperature on K_d values over a wide temperature range and for 6 different temperature values, thus allowing for rigorous determination of the enthalpy and entropy changes governing EE_2 sorption to activated sludge.

The partitioning coefficient data also allows for determination of the Gibbs free energy change associated with sorption to bioreactor sludge (Table 1).

Table 5.1 Gibbs free energy involved in the sorption

Gibbs Free Energy Determination

	Partitioning Coefficient (K_d) L/g		Gibbs Free Energy (ΔG) KJ/mol	
Temperature °C	MBR	SBR	MBR	SBR
16	---	0.25	---	-13.2
18	0.62	---	-15.6	---
22	0.51	0.21	-15.3	-13.2
24	0.45	0.17	-15.1	-12.7
26	0.40	0.14	-15.0	-12.3
28	0.37	0.12	-14.8	-12.0
30	0.35	0.1	-14.7	-11.7

The MBR and SBR ΔG values were between -16 and -11 KJ/mol. These negative ΔG values show that sorption is spontaneous. The sorption characteristics were further investigated using FT-IR to detect changes in the chemical bonding characteristics of biomass functional groups. Figure 5.3 shows a typical biomass FT-IR profile both before and after exposure to EE_2.

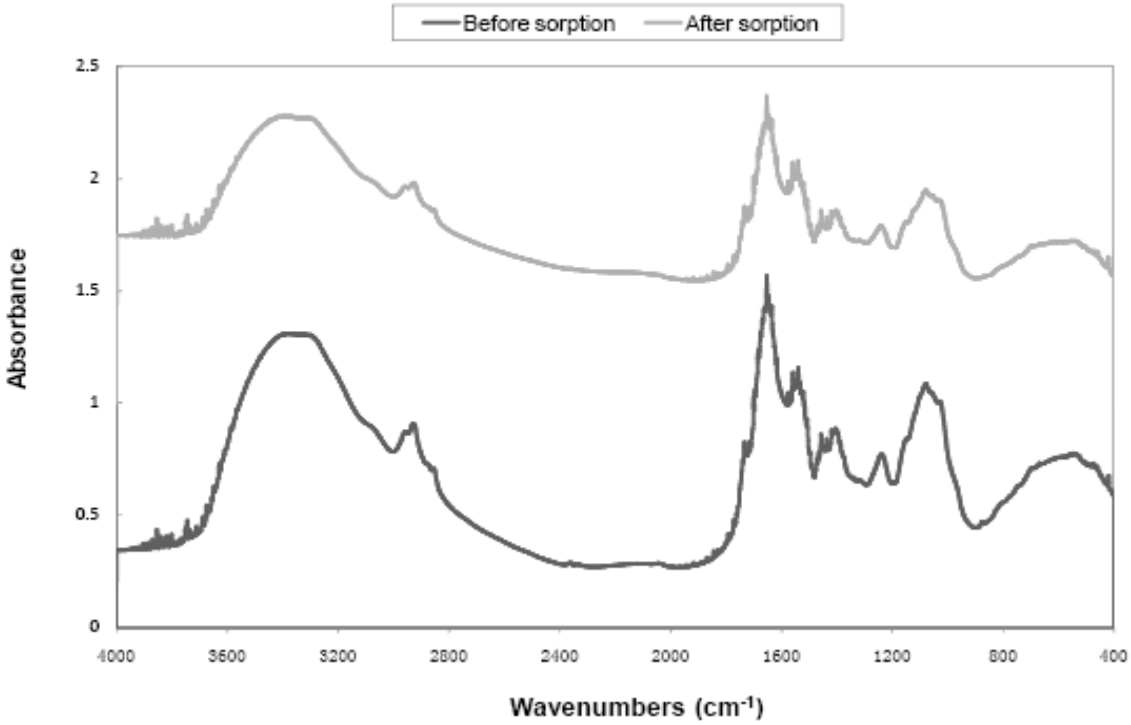


Figure 5.3 The effect of EE_2 sorption FT-IR spectra

Infrared spectroscopy works because chemical bonds have specific frequencies at which they vibrate. FT-IR is therefore capable of detecting changes in the chemical bonding characteristics of activated sludge functional groups (Choi and Yun, 2006). Figure 5.3 shows that the location of the absorbance peaks was not affected by EE_2 sorption. It appears then that the character or quantity of the biomass-associated chemical bonds did not change after exposure to EE_2, and this means that chemical bonding reactions are not significant enough to be detected by FT-IR. This is consistent with the notion that physisorption is dominant, and that the interactions are primary weak molecular interactions and not strong covalent or ionic bonding. It appears that biomass-associated functional groups do not play a significant role in EE_2 sorption.

The MBR and SBR $\ln K_d$ values were plotted against inverse temperature, as shown in Figure 5.4.

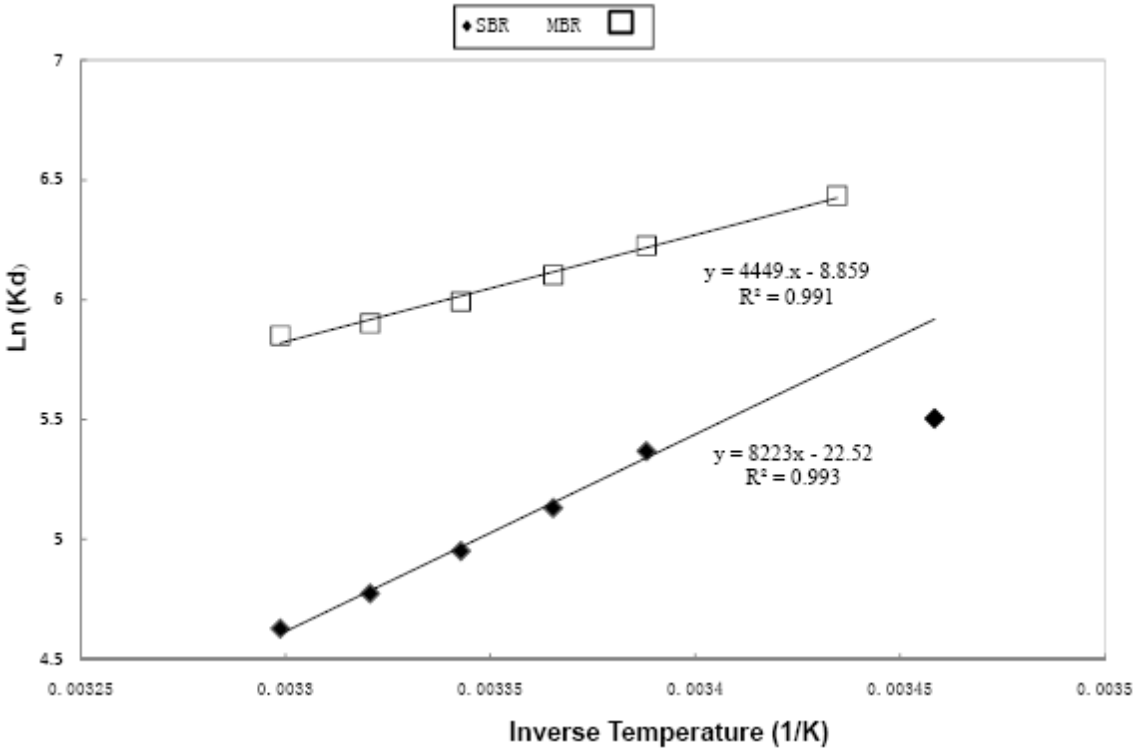


Figure 5.4 Enthalpy and entropy change determination for EE_2 sorption to bioreactor sludge.

The MBR and SBR enthalpy changes were -37 KJ/mol and -48 KJ/mol respectively, which shows that in both cases EE_2 sorption to bioreactor sludge was exothermic and that the relative magnitude of the ΔH was similar for both bioreactors. Ren *et al.* (2007) also found that EE_2 sorption was exothermic. Enthalpy change data is useful for distinguishing physisorption and chemisorption. Physisorption is typically associated with heats of adsorption in the 5-20 KJ/mol range, while chemisorption is typically associated with much larger ΔH values (i.e. 100-400 KJ/mol) (Weber Jr. and DiGiano, 1996). The current result lays between these two ranges, which suggests that physisorption processes are being supplemented with a few chemical reactions. The MBR and SBR entropy changes were -74 and -119 J/mol/K, which means that EE_2 sorption to bioreactor sludge is entropy-retarded. This finding means that the changes associated molecular ordering actually impede the sorption process. The observed free energy changes (shown in Table 1) are favorable because the energy release (i.e. ΔH) overcomes the unfavorable entropic change. EE_2 is relatively hydrophobic (Log K_{OW} = 3.9 (Holthaus *et al.,* 2002)) which suggests that hydrophobic interactions may cause sorption to be entropy-driven; the current results are in conflict with this notion. Hydrophobic interactions are hypothesized to result in greater overall system entropy because of the decrease in the ordering of water molecules surrounding the hydrophobic compounds of interest (in this case, the sludge floc and EE_2). The current results show that the ordering of water molecules does not drive the overall entropy change associated with EE_2 sorption to bioreactor sludge.

Each kinetic test produced a first order rate constant k, and the ln$k$ was plotted versus the temperature inverse (Figure 5.5).

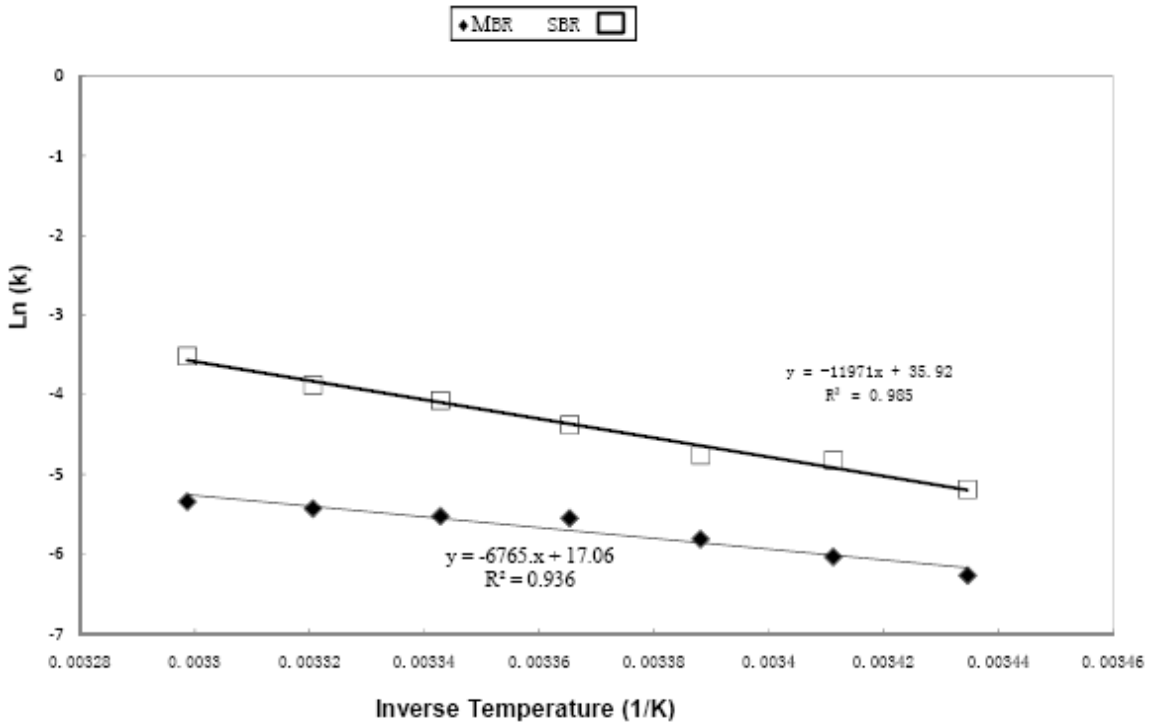


Figure 5.5 Activation energy determination for EE_2 sorption to bioreactor sludge.

The SBR rate constants were greater than the MBR rate constants, perhaps because the SBR particles are larger and more porous, allowing for more intraparticle mass transfer. The slopes of the ln*k* versus inverse temperature relationships yield MBR and SBR E_a values of 56 and 100 KJ/mol respectively, values that land in the lower end of the chemisorption range (40-800 KJ/mol, (Bekci *et al.,* 2006))). Physisorption is typically associated with E_a values up to ~ 40 kJ/mol (Bekci *et al.,* 2006)). This result suggests that EE_2 sorption to bioreactor biomass may include a few chemical reactions. The aforementioned enthalpy change estimates also suggest that both physical and chemical sorption is present. The E_a determination suggests the presence of low-level chemical sorption, and the aforementioned FT-IR result reflects that the fact that chemisorption is not prominent.

In general, profiles like those shown in Figure 6 are sometimes not approximated well by linear regressions. For the current data, this can be seen for the MBR regression. There are two possible reasons. The first possibility is experimental error, but this can also happen when the rate-controlling mechanism changes from (for example) diffusion-control at lower temperature to activation energy-control at higher temperature. In cases like this, the dimensionless thiele modulus (Φ) helps reveal the relevant process limitation: $\Phi = L(k/Đ)^{0.5}$, where L is the characteristic length along the diffusion path (a quantity that is related to the biomass size), k is the first order rate of reaction, and Đ is the effective molecular diffusivity of the EE_2 through the medium. For diffusion controlled reactions, $\Phi > 1$. It is possible to further investigate this mechanism shift experimentally by directly measure the diffusion coefficient of EE_2 using a diffusion cell,

and then calculating the Φ at each temperature to identify diffusion (or reaction) - limitations. This approach is recommended in further research. The available data suggests that Φ values are probably much greater than 1, because the effective molecular diffusivity of the EE_2 in water is very small (e.g., between 10^{-16} - 10^{-18} cm^2/s (Weber Jr. and DiGiano, 1996; Bromberg and Magner, 1999)). These values need to be accurately measured in a relevant wastewater medium and along with biomass particle size information, used to calculate Φ values.

This research impacts the study of EE_2 sorption to activated sludge in three practical ways. First, the mechanistic work shown here explains the reversibility of EE_2 sorption. Physisorption is usually reversible, while chemisorption is typically irreversible. Yi and Harper (2007) found that EE_2 sorption to bioreactor biomass is only partially reversible, and the current results clarify this previous finding. EE_2 sorption involves physical sorption *in combination* with a relatively low level of chemical reactions. The presence of chemisorption is probably why EE_2 sorption to bioreactor biomass is not completely reversible. Second, this study showed that EE_2 sorption to activated sludge biomass is spontaneous ($\Delta G<0$); this result applies directly to full scale wastewater treatment plants. EE_2 concentrations detected in raw wastewater are often in the low ug/L or ng/L concentration range, much lower than what was used in this study (100-5000 ug/L). This fact does not impact the applicability of the current findings because the thermodynamic character (i.e. ΔS, ΔH, E_a) of EE_2 sorption is not concentration dependant (Annamalai and Puri 2002). Lastly, the current results inform modeling efforts. Physisorption is normally diffusion-limited while chemisorption is typically reaction-rate

limited. Detailed kinetic models must account for both of these processes. Also, since EE_2 sorption to MBR and SBR sludge is mechanistically similar, it is possible to develop thermodynamically-based kinetic models that apply to both MBR and SBR systems.

CHAPTER VI CONCLUSIONS

The conclusions from this study are as follows:

- EE_2 sorption to bioreactor biomass is spontaneous, enthalpy-driven and entropy-retarded. The MBR and SBR ΔG values were between -16 and -11 KJ/mol. The ΔH determined using MBR and SBR biomass were -37KJ/mol and -48KJ/mol respectively. The ΔS values determined using MBR and SBR biomass were -74 and -119 J/mol/K. Hydrophobic interactions are not important driving forces;
- The ΔH and E_a values suggest that physical sorption is being augmented with a relatively low level of chemical reactions. The FT-IR results suggest that the chemical reactions are not significant enough to shift the detectable chemical bonding characteristics of the biomass functional groups;
- For a given temperature, SBR K_d values were always lower than MBR K_d values, a result that confirms previous findings.

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