TREATMENT OF XENOBIOTICS DURING ANAEROBIC DIGESTION AND ITS ENHANCEMENT UPON POST-OZONATION OF THE ANAEROBICALLY TREATED SLUDGE

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ABSTRACT

TREATMENT OF XENOBIOTICS DURING ANAEROBIC DIGESTION AND ITS ENHANCEMENT UPON POST-OZONATION OF THE ANAEROBICALLY TREATED SLUDGE

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Treatment of waste sludge has become an important issue in recent years around the world. However, the trend of waste sludge treatment has shifted from volume minimization and stabilization to reuse of the sludge and recover the energy potential of it. Therefore, anaerobic treatment of sludge is gaining popularity because of byproduct methane production and high percentage of VSS reduction. Pre-treatment of sludge before anaerobic digestion in order to increase methane production, and ozone pre-treatment in this context, is one such option. Domestic sludge also contains the recently recognized, so called, emerging compounds such as Endocrine Disrupting Compounds (EDCs). Therefore treatment of EDCs in sludge is another challenge in waste sludge treatment since direct discharge of such chemicals may harm the environment by causing gender shifts within the fauna. In this context two hormones (estrone and progesterone), three pharmaceuticals (acetaminophen, carbamazepine and diltiazem) and one plasticizer (benzyl-butyl phthalate) were routinely analyzed in sludge samples which were subjected to treatment during this study. Treatment of EDCs during anaerobic digestion and the effect of ozonation both on the performance of digestion and the treatability of EDCs were investigated in this study.

Four 2.5L anaerobic jars were used for anaerobic digestion connected to four 1L plastic graduated cylinders immersed in salt-water to collect the off gas. Anaerobic sludge culture of the reactor and the sludge feed to the reactors were obtained from Ankara Tatlar Wastewater Treatment Plant anaerobic digester and return activated sludge (RAS) line, respectively. One of the anaerobic digesters was used as control (no ozonation) and the others were fed with sludge samples ozonated at three different ozone doses 0.65, 1.33 and 2.65 mg ozone/g biomass. Sludge ages of the reactors were initially set to 25 days and the reactors were fed once every 2 days. The TSS, VSS, total gas volume, COD, pH, CH_4 percentage and EDCs were analyzed routinely. In the reactors, operated at 25 days, because of the observation of reduction of TSS, SRT was set to infinity; thus, sludge wastage was terminated.

Following the startup it was seen that at 2.65 mg ozone/g biomass dose TSS and VSS did not stay constant in the reactor and dropped sharply in the course of operation, indicating that system was not steady at this SRT. However, upon stoppage of sludge wastage from the reactors, thereby setting SRT to infinity, a steady culture could be maintained in the reactors. Both total gas production and CH_4 percentage increased with the increasing doses of ozone with respect to control reactor. For 2.65 mg/g ozonated reactor total gas volume doubled the amount produced in the control reactor.

All the EDCs within the scope of this study were analyzed in sludge using ultrasound-aided sequential sludge extraction method twice a week and the results showed that ozonation affected treatment of EDCs for up to 96%. The highest removal rate was obtained with natural hormones. Rates of treatment of pharmaceuticals were the second best.

Keywords: Anaerobic, ozone, endocrine disrupting chemicals, sludge digestion

ÖZ

SENTETİK ORGANİK KİRLETİCİLERİN HAVASIZ ÇÜRÜTME SÜRECİNDE ARITILMALARI VE SON OZONLAMANIN ARITIMA ETKİSİ

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Atık çamur arıtımının dünya çapında giderek daha da büyüyen bir önem arz ettiği genel anlamda bilinen bir gerçektir. Ancak, önceden yapılan arıtım teknikleri yani hacim azaltımı ve stabilizasyon tekniklerinden ziyade, çamur arıtımı yöntemlerinde yeni eğilim çamurun geri kullanımı ve enerji potansiyelinin ortaya çıkarılmasına doğru kaymıştır. Böylece, metan üretimi ve yüksek organic madde arıtımı yüzdesinden dolayı atık çamurların anaerobik arıtımı giderek popülerliğini artırmaktadır. Bunlara ek olarak, anaerobik çürütme öncesi yapılan ön-arıtım teknikleri de yaygınlaşmaya başlamaktadır. Güçlü bir kimyasal olan ozonun da bu proseslere bir örnek teşkil etmektedir. Bundan başka bilinen bir gerçek de çamurlarda düşük konsantrasyonlarda bulunmalarına rağmen ciddi rahatsızklara yol açan kimyasallar olan Endokrin Bozucu Maddelerin (EBM) atık çamurların arıtılmasında yeni sorunlara yol açtığıdır. Bu çalışma kapsamında, üç farmasötik (acteminophen, carbamazepine, diltiazem), iki doğal hormon (estrone, progesterone) ve bir plastiklerştirici (benzyl-butyl phthalate) analizleri LC (ESI) MS/MS kullanılarak yapılmıştır. Ayrıca, EBMlerin anaerobik çürütme sırasında arıtımları ve ozonlamanın hem bu maddelerin arıtımlarına hem de anaerobik çürütme performansına etkileri incelenmiştir.

Bu araştırmada, dört adet 2.5L'lik labaratuar ölçeğinde anaerobik çürütücü kullanılmıştır. Bu çürütücülerde Ankara Tatlar Atıksu Arıtma Tesisinin anaerobik çürütücüsünden alınan çamur numuneleri ve besleme çamuru olarak da aynı tesisisin atık çamur hattından alınan çamur kullanılmıştır. Bir reaktör kontrol grubu olarak kullanılmış ve ozonlanmamıştır. Diğer üç reaktörün besleme çamurları ise sırasıyla 0.65, 1.33 ve 2.65 mg ozon/g biyokütle dozu ile ozonlanarak beslenmiştir. Ilk once çamur yaşı 25 gün olarak ayarlanan reaktörlerde düzenli olarak toplam katı madde, uçucu katı madde, KOİ, toplam gaz üretimi, pH, metan yüzdesi ve hedef EBMlerin analizleri yapılmıştır. Reaktörlerde toplam katı madde miktarının ozonlama sonucunda devamlı olarak azalma eğilimi göstermesinden dolayı çamur yaşı sonsuz olarak ayarlanmış ve böylece reaktörlerden çamur atma işlemi durdurulmuştur.

Reaktör işletimi sonunda ön-arıtım olarak ozon kullanımının hem EBM arıtımı açısından hem de anaerobik reaktörlerin performansı açısından olumlu sonuçlar verdiği gözlemlenmiştir. Metan üretimi, ozonlanan reaktörlerde kontrol reaktörüne oranla artış gösterdiği gözlemlenmiştir. Özellikle 2.65 mg/g dozla ozonlanan reaktörde toplam gaz üretiminin kontrol reaktörüne gore iki katına çıktığı tespit edilmiştir.

EBM arıtımı açısından ise %96'ya kadar bir azalma tespit edilmiştir. En yüksek arıtım oranları doğal hormonlar olan estrone ve progesterondan elde edilmiştir. Farmasötükler de arıtım oranları ile ikinci sırada yer almıştır.

Anahtar Kelimeler: Anaerobik, ozon, endokrin bozucu maddeler, çamur çürütme

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CHAPTER 1

INTRODUCTION

The large amount of sludge produced during biological treatment of domestic effluents is a growing problem in wastewater treatment plant management. Disposal of waste activated sludge, WAS, has been reported to account for up to 65 % of total plant operation costs (Liu, 2003). In the EU countries, following the implementation of the Urban Waste Water Treatment Directive 91/271/EEC; the total amount of WAS to be handled was expected to exceed 10 million tons dry sludge per annum (Pe´rez-Elvira et al., 2006). The newly imposed stringent regulations and social and environmental concerns have since increased incentive to develop strategies to reduce excess sludge production (Wei et al., 2003).

Anaerobic digestion is now proven as the most energy efficient way of destroying and stabilizing waste activated sludge and the by-product methane is a form of fuel which helps cut down treatment costs. Furthermore it is also considered a form of green technology in combat against global warming, as methane is a renewable energy source. Efforts have since been deployed to increase gas yield in anaerobic digestion to increase the overall benefit. This included better conception of the microbiology involved, reactor design and substrate manipulations (Appels et al., 2008; Veeken et al.,2000; Hinken et al., 2008). Pre-treatment of the substrate sludge is considered a substrate manipulation with an aim to solubilize the particulate WAS material (Kianmehr et al., 2010; Elbeshbishy et al., 2011; Salsabil et al., 2010; Appels et al., 2008) so as to make it more available to the anaerobic microbial consortium.

Amongst the pre-treatment methodology, thermal treatment at elevated temperatures (Pérez-Elvira et al., 2008) or by freezing and thawing of WAS (Montusiewicz et al., 2010) have been well studied. For example around 160 % biogas enhancement,

relative to non-treated WAS, have been reported by Bougrier et al. (2008) upon pre-treatment of WAS at 190 ^{0}C . Ultrasonic pre-treatment has too been the subject of large number of scientific studies and reviews (Pilli et al.,2011; Bougrier et al.,2006; Pérez-Elvira et al., 2010). Ultrasonic disintegration has been reported to yield 100 % disintegration of the sludge solids but at high energy consumption (Dichtl et al.,1997)

Amongst other WAS pre-treatment techniques; chemical pre-treatment with acids and alkali.(Bordeleau and Droste, 2010; Kim et al., 2010); and with oxidants have special place in the literature. Oxidant treatment of WAS is well documented (Kianmehr et al., 2010; Salsabil et al. 2010, Carballa et al., 2009, Carlsson et al.,2012). Generally substrate pre-treatment strategies applied for WAS reduction are based on lysis-cryptic growth. The biomass growth on the lysate material is termed cryptic growth, to distinguish it from growth on the original organic substances (Mason et al., 1986).

Ozone is a strong oxidant and has extensively been used for wastewater and WAS treatment. During sludge treatment ozone rapidly decomposes into radicals and the produced radicals affect oxidation of both particulate and soluble organics (Cesbron et.al.,2003 and Salhi et.al., 2003). Among the oxidation processes, treatment using ozone is of special interest because no oxidant residues are remaining and no increase in salt concentration occurs (Weemaes et al., 2000 and Goel et al., 2003).

As was mentioned earlier sludge itself is a potential of pollutant. Moreover, it may also contain different forms of pollutants by way of sorption during contact with wastewaters. Such pollutants are called micro pollutants or emerging contaminants due to their trace quantities in sludge. Yet their effects on the biota may be significant due to interference with the hormonal systems even at these trace concentrations. Endocrine disrupting chemicals (EDCs) are considered under these type of pollutants. Although EDCs are newly recognized group of compounds, their number is growing dangerously. The properties of the selected EDCs for this study are given in Table 1.1. It is seen from this table that some of the EDCs are rather

high in concentration in sludge. Due to physicochemical properties of EDCs, these tend to adsorb and concentrate on sludge. However, there was no study that was analyzing the concentration of progesterone in sludge samples. Therefore, in this table progesterone concentrations found in sludge was given as N.A. (not applicable).

Table 1.1 Properties and usage of target EDCs

COMPOUND	INTENDED USE	EFFECT	CONCENTRATION FOUND IN SLUDGE (µg/g TS DW)	REFERENCE
Diltiazem	Hypertension, arrythmia, migraine	Endocrine disrupter	61-201	Final Report – Field Sampling Program CCME Project , 2010
Acetaminophen (or Paracetamol)	Analgesic	Endocrine disrupter	4.02-419	Final Report – Field Sampling Program CCME Project , 2010
Carbamazepine	Anticonvulsant, mood stabilizer	Endocrine disrupter	12-42	Final Report – Field Sampling Program CCME Project , 2010
BBP	Plasticizer	Endocrine disrupter	$100x10^{3}$	Final Report – Field Sampling Program CCME Project , 2010
Estrone	Natural hormone in female body	Endocrine disrupter	53-137	Final Report – Field Sampling Program CCME Project , 2010
Progesterone	Natural hormone in female body	Endocrine disrupter	N.A.	N.A.

CHAPTER 2

AIM OF THE STUDY

In this thesis study, recognizing the problems of waste sludge produced during wastewater treatment, treatability of endocrine disrupters during anaerobic sludge digestion and enhancement of the anaerobic digestion potential by ozone pre-treatment were investigated.

During this study, some target EDCs are analyzed using LC (ESI) MS/MS in anaerobic sludge. Ozone was applied to the anaerobic sludge and treatability of target EDCs and TSS and VSS reductions were tested. Second step of the study was to set-up a lab-scale anaerobic digester and investigate effects of pre-ozonation on anaerobic digestion performance. Treatability of target EDCs, COD, TSS, and VSS, total gas production, methane percentage and EDC analysis were done to follow the performance in anaerobic digestion. Therefore, at the end of the study, it should be possible to understand the fate and treatability of the chosen EDCs in anaerobic sludge treatment. Besides, the impact of ozone as a pre-treatment technique both on anaerobic digester performance (i.e. gas production) and EDC reduction would be clarified.

CHAPTER 3

LITERATURE REVIEW

3.1 Endocrine Disrupting Chemicals of Environmental Interest

20th century brought many different kinds of chemicals to our daily lives such as pharmaceuticals, household products and industrial products. However, no environmental risk assessment, ERA, studies were conducted with every chemical introduced to the market because it was impossible to follow the developments in chemical industry and rush ERas for each chemical. Therefore, up to 1970s there were no ERas, but from mid 70s to 80s EU started to list chemicals in use and after that time every new chemical must have risk assessment studies before the release to the market. Although their huge amount of production and extensive usage, drugs were not included in this list, because they were not considered suspect at such trace levels in the effluents.

On the other hand, it is a recently acknowledged that pharmaceuticals and personal care products (PPCPs) have some negative effects both on human health and environment although they ease our modern life styles. This situation has even moved a step forward so that PPCPs are nowadays considered to be endocrine disrupting compounds/chemicals (EDCs). OECD defines the problem of EDCs as "an exogenous substance or mixture that alters the function(s) of the endocrine systems and consequently causes adverse health effects in an intact organism, or its progeny or (sub) populations".

With this new concept of endocrine disruptiers, in 2001 EU started a new project called POSEIDON (Assessment of Technologies for the Removal of Pharmaceuticals and Personal Care Products in Sewage and Drinking Water Facilities to Improve the Indirect Potable Water Reuse) to gather information about the fate and behavior of those chemicals. The most important outcomes of this project can be summarized as follows:

- It is more common for polar compounds to stay in water and to be removed during biological treatment in WWTPs whereas hydrophobic compounds tend to adsorb onto biomass (Carballa et. al. , 2003).
- Advanced oxidation techniques, namely ozonation, seems to be a promising process for the removal of target EDCs and PPCPs (Carballa et. al. , 2003).
- Also, project highlighted the efficiency of conventional sludge treatment processes such as anaerobic digestion as well as the latest techniques like combination of conventional techniques with various sludge pre-treatment processes (Marta Carballa Acros, 2005).

3.2 Selection of EDCs

The reason behind the classification of EDCs is that there is huge amount of chemicals produced and released to the market and it is nearly impossible to conduct ERas in detail (Carballa et. al. , 2003). Therefore, a classification step is necessary for PPCPs.

3.2.1 Natural steroidal hormones

Natural estrogens are released to the environment by humans and animals through their urine and faeces in the form of inactive sulphates (Carballa et. al., 2007). In the scope of this thesis work, estrone and progesterone were selected under this group. The idea behind of this selection was that although the literature concentrations of these hormones are small, they still pose a huge potential as endocrine disrupters (Jobling et al, 1998; Santos et al, 2007; Williams et al, 2003).

3.2.3 Pharmaceuticals

For this category, carbamazepine, acetaminophen and diltiazem were selected because of their wide usage and detection all over the world (Kolpin et al., 2002; Metcalfe et al., 2004; Ternes, 1998; Ternes et al., 2001).

3.2.4 Plasticizers

Plasticizers are additives used to increase the fluidity or plasticity of the materials. They are another common group of compounds that show endocrine disrupting properties. Benzyl butyl phthalate (BBP) was chosen to represent this large group. It is a common plasticizer added especially to vinyl floor tiles, adhesives and synthetic leathers; which are essentials of the modern life.

3.3 Analytical methods for EDCs

As mentioned before, endocrine disrupting compounds started a new concern for both wastewater and waste activated sludge treatment. Therefore, it was necessary to establish high sensitivity analysis techniques. Because of the different chemical and psychochemical properties of EDCs, the analysis of these compounds poses a new challenge for analysis techniques. In addition to that, the properties of environmental samples such as the complexity of matrices and low concentrations of these compounds are other problems. Hence general and comprehensive techniques must be developed for the analysis.

The analytical determination of the above mentioned groups of EDCs, is dominated by chromatographic methods (GC and LC) coupled to sensitive and specific detection systems, such as MS, MS–MS or high-resolution MS (HRMS). All of these analytical methods require complicated and time-consuming sample preparation methods that are called extraction of compounds.

In order to choose the analytical method for the determination, properties of chemicals and analytical requirements must be known in detail. Usually, apolar and moderately polar compounds are analyzed using a GC and polar compounds are analyzed with LC. However, some compounds like natural hormones and steroids can be determined with both of apparatus.

3.3.1 Quantitative determination of steroids using LC

For the analysis of natural hormones, LC coupled with different detection systems, such as diode array detection (DAD) (M.J. López de Alda and D. Barceló, 2001), fluorescence (FL) (Snyder et. al., 1999) and MS (M. Lopez de Alda and D. Barceló, 2000; Baronti et. al. 2000; M. Lopez de Alda and D. Barceló, 2001; Ferguson et. al., 2001; Masunaga et. al., 2000; Johnson et. al., 2000; Laganà et. al., 2000 and Seifert et.al., 1999) have been used.

Estrogens and progestrogens are generally analyzed with various types of LCs with water and alcohol mixtures and usually organic solvents are used as mobile phases during analysis (M. Lopez de Alda and D. Barceló, 2000).

As mentioned before, using triple quadropole HPLC/MS/MS has many advantages over other types of LCs. One of these advantages is its high sensitivity and selectivity in determination of selected estrogens and progestrogens. Lower concentrations of these compounds in wastewaters can be determined in triple quadropole systems when it is compared with single HPLC/MS systems. Results obtained with GC/MS/MS systems are close or little higher than that of triple quadropoles (M.J. López de Alda and D. Barceló, 2001).

Although little better results are obtained with GC/MS/MS systems, they require a derivatization process which is a time and chemical consuming step. In spite of that, in LC systems no derivatization process is required and estrogens and progestrogenc can be analyzed in both conjugated and unconjugated forms (Huang and Sedlak, 2001; Z. Li et. al., 2004).

3.3.2 Quantitative determination of pharmaceuticals with LC

Developing technology in mass spectrometer systems along with the availability of high technology accessories of LC such as columns made outstanding improvements in the analysis of PPCPs in complex matrices.

In the studies of Camacho-Munoz (2009) and Galera and their colleagues (2010), using HPLC coupled with diode array UV absorbance and fluorescence in determination of pharmaceuticals is a low-cost technology, yet MS detection of these chemicals is the most developed technique for the trace analysis.

When PPCPs and EDCs were started to be an emerging issue for the treatment and reuse of wastewaters, single quadropole instruments were being used for the determination then they were followed by time-of-flight (TOF) instruments. After the development of triple quadropole analyzers, analysis of such chemicals were also developed thus lower detection limits for determination could be achieved (Hao et. al., 2007).

An ionization technique is necessary for the analysis in HPLC and Elctrospray ionization is the most widely used technique for te trace analysis of environmental samples. However the disadvantage of this technique is matrix effect and co-eluting of the components present in the matrix along with the target compounds. This situation causes significant decreases in sensitivity thus causes unreliable results. Mostly external standards are used in order to overcome this situation. Also, standard addition techniques can be used in alternative. However this procedure increases the duration period of the analysis (Hao et. al., 2007).

3.3.3 Quantitative determination of plasticizers with LC

Analytical methods of trace contaminants have been reviewed by Skinner (1992).
BBP may be analyzed by GC/MS and by HPLC. Usually the determination of BBP in GC is an exhausting process which includes severe extraction technique that is

followed by reverse osmosis. Therefore for environmental samples use of HPLC for the analysis of BBP is more common due to its less complexity for the preparation of samples (Pankow et al., 1988).

The literature values for the determination of BBP were 1 µg/litre for samples of drinking-water (G. Halina, 1994) and 0.2 mg/kg dry weight for soil (Webber & Wang, 1995).

3.4 Fate and behavior of EDCs in sludge

In Stockholm Convention, 2001, various types of persistent compounds were officially recognized as endocrine disrupters (UNEP, 2001). After this convention, need for new remediation and treatment techniques was increased in order efficiently remove EDCs from various polluted environment such as water, wastewater sludge, sediments and soils (Duran and Esposito, 2000; Romantschuk et al., 2000).

Since the use of waste activated sludge has become widespread, the organic pollutants such as EDCs have increased the concern of the fate and treatability of these chemicals. Sludges taken from wastewater treatment plants have been used as value added products (biopesticides or other bio-control agents, microbial inoculants, industrial enzymes, bacterial bioplastics and other biopolymers) and the use of these products has been achieved with successful and encouraging results (Barnabé et al., 2005, Yezza et al., 2005). Production strategies were developed to enhance product yield as for example the increase of WAS biodegradability through pre-treatment (Barnabé et al., 2005).

Although there are mentioned benefits of reusing WAS as VAPs, WAS still contains various toxic compounds like heavy metals, micro pollutants and pathogens. When these untreated sludges are applied to soil, the hazardous compounds in WAS directly goes into urban water cycle and severely affect human health. Therefore, new regulations started to be applied in order to prevent these health problems (Yezza et al., 2005).

Recently, many studies investigating the effect of WAS treatment on EDCs removal were performed. In these studies encouraging results were obtained. Applying pre-treatment and bioconversion to WAS was seen to be a good technique in order to comply with the new regulation on the use of WAS as VAPs (Barnabé et al., 2005).

Since EDCs present in WAS eventually have an impact on public health, it is crucial to understand the treatability of those compounds. Thus, fate of these chemicals in sludge must be studied.

3.4.1 Pharmaceuticals

Pharmaceuticals mainly enter to the urban water cycle via wastewater treatment plants (Golet et al., 2002). Understanding the fate of pharmaceuticals was mainly based on the question whether or not these chemicals would create resistant bacterial pathogens (McArdell et al., 2003). Also, these pharmaceuticals themselves directly affect human health (Daughton and Ternes, 1999; Thiele-Bruhn, 2003).

Recently, a risk assessment investigating the effect of pharmaceuticals in Norwey was conducted. In this study, many agricultural soils amended with WAS whether or not they were infected with pharmaceutical compounds. Within the concept of this risk assessment, 1400 pharmaceuticals were analyzed. At the end of this study, drug concentration in soils amended with WAS were significantly lower than the concentration stated in regulations. As a conclusion, it was said that pharmaceuticals in WAS posed no significant risk to agricultural areas (Eriksen et al., 2009).

3.4.2 Steroids

Natural (17β-estradiol, estrone, estriol, progesterone) and synthetic hormones or steroid are discharged to the environment by humans and then they enter to WWTPs. After a partial treatment that enter to the receiving bodies mainly to water (Snyder et al., 2001). The other sources of natural hormones are majorly lactating cows (Kolodziej et al., 2004).

There were various studies investigating the fate of estrogens in WWTPs. Due to difficulties in determination of these compounds in sludge, only few studies revealed the concentrations of estrogenic compounds in such matrices (Gomes et al., 2004). Determined free estrogens in the effluents of WWTPs and receiving waters indicate that the transformation of estrogenic compounds happens between influent and effluent of WWTPs within few days (Shore et al., 1993; Desbrow et al., 1998; Ternes et al., 1999b, Ternes et al., 1999a; Korner et al., 2000; Layton et al., 2000; Hashimoto and Murakami, 2009). Mainly the degradation order was reported as: 17β-estradiol→estrone→estriol (Ternes et al., 1999a). however, determination of estrone in the effluent and receiving water bodies reveals the fact that estrogenic compounds does not fully degrade such that partial degradation also occurs (Barontri et al., 2000). The degradation rates usually reported to be 64–99.9% (Ternes et al., 1999b). Unfortunately, these studies did not report the distinguish between degradation and sludge adsorption (Schlusener and Bester, 2008). In the study of Anderson and colleagues (2003), it was reported that only 5% of estrogenic compounds was adsorbed onto sludge. Concentrations of estrone, which is one of the steroidal compounds within the concept of this study, found in sludge was given in Table 1.1. These low concentrations, combined with fast biodegradation rates in WWTP mass balance and laboratory studies suggest that steroids are unlikely to pose a risk to human health or the environment when land applying biosolids (Ternes et al., 1999b).

3.4.3 Plasticizers

When the literature was searched, there were many studies investigating the fate of various types of plasticizer both in WWTPs and waste sludge. Only some studies were measured BBP in sludge; however, none of them studied the fate of this compound neither in wastewater nor in sludge samples. The concentration of this compound in sludge was given in Table 1.1.

3.5 Anaerobic Treatment WAS

In treatment of wastewater sludge in order to decrease the biological activity and reduce the insoluble organic content, anaerobic digestion is a commonly used process. Also, methane production during digestion is a critical step for this type of process. However, usually methanogenesis is thought to be a rate-limiting step when the overall process is considered. Therefore, 20-30 days of SRTs are typical values for anaerobic sludge digestion (Pavlostathis and Gosset, 1986).

In order to decrease this time and increase the efficiency of anaerobic digestion, some different techniques have been applied to anaerobic digestion such as optimizing process conditions, applying thermophilic temperatures, pre-treating the input sludge or using a co-digestion with other substrates (Dohanyos et al., 2004).

The main aim of applying a treatment technique prior to anaerobic digestion is to increase the rate of hydrolysis step and consequently decreasing the stabilization duration and rise the degree of degradation. By this way, the amount of sludge to be disposed is diminished as well as the performance of anaerobic digestion is increased (Delgenes et al., 2000).

In order to accomplish this aim, different disintegration methods have been tried but real life applications still remain to be explored (Müller, 2000). Although mechanical disintegration techniques, among others, seem to be effective in solubilizing biomass, these kinds of processes are complicated and have higher cost (Weemaes and Verstraete, 1998). In sonication experiments, high rate of disintegration was achieved; however, similar to mechanical disintegration, this technique has also high cost (Dichtl et al., 1997). Although chemical and thermochemical pre-treatments reached high disintegration levels, these processes require specified material due to aggressive reaction conditions (Tanaka et al., 1997). Oxidation of sludge is another technique applied to disintegrate microbial cells. Ozonation is the most common used process among oxidative pre-treatments. Its highly preference depends on that

ozonation does not leave oxidant residues and does not increase salt concentration of the applied medium (Weemaes et al., 2000 and Goel et al., 2003).

Ozone is a very strong oxidizing agent, which reacts in two different ways: the direct and the indirect reaction, both reactions occurring simultaneously. The indirect reaction is based on the high reactivity of hydroxyl radicals, which do not react specifically, whereas the direct reaction rate with ozone depends more on the structure of the reactants. During sludge pre-treatment, the aim of ozone is to cause the hydrolysis and partial oxidation of the organic matter. A complete oxidation is avoided (Weemaes et al., 2000).

Depending on the efficiency of each sludge treatment technology, remaining PPCPs might be recycled with the liquid supernatant or disposed with the sludge (Carballa et al., in press). If the digested sludge is applied to soils, these contaminants could remain in the soil for a long time (months or even years) because of their sorption and slow rates of biodegradation (Wilson et al., 1997).

Effects of some PPCPs, e.g. contraceptives and diclofenac, in the aquatic environment are well-documented (Jobling et al., 2002 and Triebskorn et al., 2004), but not much is known about the behavior of these compounds in soils. Intake by plants (Wild et al., 1994), leaching into the groundwater (Kreuzinger et al., 2004) and negative impact on the terrestrial organism are not excluded (Jensen et al., 2001). Information dealing specifically with PPCPs behavior during sludge anaerobic digestion is very scarce and only the EU funded Poseidon project (http://poseidon.bafg.de) has reported some data (Carballa, 2005). Besides, it is known that the application of ozone at doses varying from 2 to 10 mg l−1 to biologically treated wastewater is sufficient for oxidizing many PPCPs by 90–99%, except X-ray contrast media, which showed little oxidation (Ternes et al., 2003and Huber et al., 2005).

The most important study conducted on the use of ozone pre-treatment for anaerobic digestion and investigation of its effect on EDCs and PPCPs degradation was

performed by Marta Carballa in 2005, as a PhD Thesis. In her study, Carballa et al. (2007), after ozonating WAS previously spiked with various PPCPs in a 10 L bubble column, and using an ozone dose of 20 mg O_3/g TSS; has fed this into a lab-scale anaerobic reactors. Total of eleven different PPCPs were spiked to the WAS samples that were being used for the feed. The PPCPs spiked included two musk, one antiepileptic, carbamazepine (CBZ); one tranquilizer, three estrogens, estrone (E1), 17b-estradiol (E2) and 17aethinylestradiol (EE2) and four other drug compounds. Although what was meant with 'concentration' is not clear, the spiked concentrations of the PPCPs were reported to lie in the range between 4 and 400 µg/ L.

Ozone treated WAS samples were fed into mesophilic (37 ^{o}C) and thermophilic (55 ^{o}C) anaerobic reactors. Controls received non-ozone treated WAS as feed. After 3 months of acclimatization, the feeding of reactors with ozone-treated WAS was commenced. From their data it can be deduced that, as compared to the non-ozonated control reactors, around 28 % increase in daily gas production in the mesophilic reactor and 17 % in the thermophilic reactor could be accounted for the ozone treatment. Percentage of methane in the off gas was ranging between 61.8 % in the mesophilic reactor and 65 % in the thermophilic. Regarding the PPCPs removal estrogens were removed close to 100 % in all the reactors. Removals of the two musks were close to 80 % in ozone treated mesophilic reactor which was much lower in the thermophilic reactors. Carbamazepine (CBZ) removal was very low in the mesophilic control reactors; whereas it was substantial in the ozone treated WAS fed reactor. Removals of the other PPCPs were on the medium range averaging between 20-60 % in all the reactors (Carballa, 2005).

In view of the scanned literature the objective of this study was set around low cost ozone treatment of WAS generated during sewage treatment, in order to improve anaerobic digestion by increasing biogas production and reducing the amount of biomass produced. Another aim was to remove EDCs sorbed by the sludge simultaneously during digestion. To the best of knowledge of the present author, and as already reviewed, ozone pre-treatment of sludge prior to anaerobic digestion has not been too successful in terms of cost and efficiency increase to warrant a full scale

application. Close analysis of the experimental protocols so far employed in the literature suggest that ozone have been directly or partially applied to the anaerobic sludge causing rapid depletion of ozone by the ample amount of soluble reductants present in the liquor and leaving little available ozone for sludge disintegration. Therefore, in this study, waste sludge from aerobic process was ozonated prior to feeding to the anaerobic digester to affect superior disintegration of sludge solids and obtain higher gas yields. Moreover, obtaining lower or no biomass production during the process and effective EDCs removal at the same time were aimed.

CHAPTER 4

MATERIALS AND METHODS

4.1 LC/MS/MS Instrument

4.1.1 Description

In the scope of this thesis work, an Agilent 6410A type quadropole MS detector consisting of autosampler, degasser and binary pump equipped with electrospray ionization (ESI) attached to an Agilent 1200 HPLC was used for the analysis.

4.1.2 Operation principles

An Agilent 1200 type HPLC with 6410A type quadruple MS detector consisting of autosampler and electrospray ionization was used for analysis. The triple quadropole mass spectrometer consists of an ion source, followed by ion optics that transfer the ions to the first quadropole positioned to the right of it. A diagram of the spectrometer is shown in Figure 4.1.

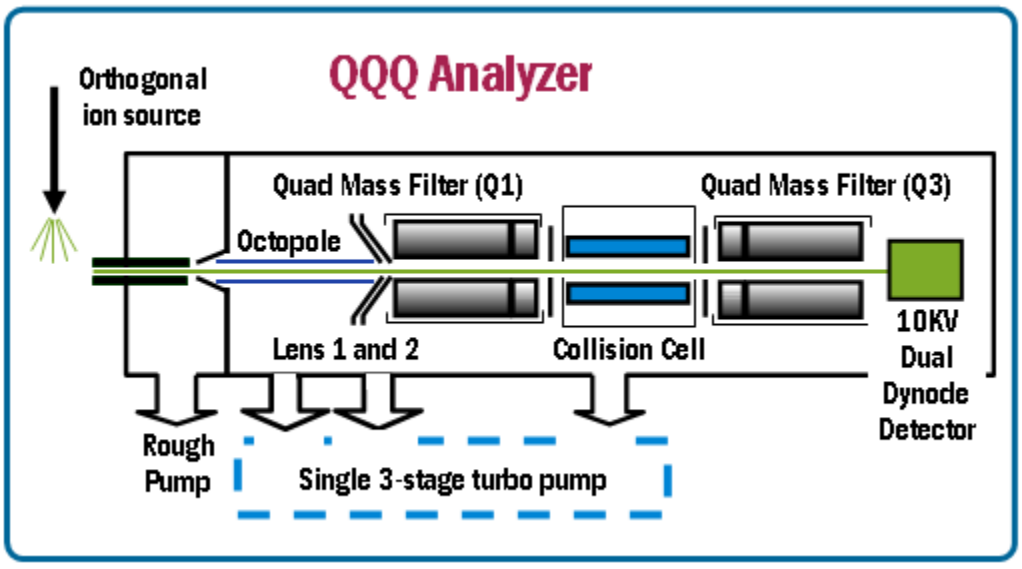

Figure 4.1 Diagram of triple quadropole mass spectrometer (Agilent Guide, 2009)

The quadropole consists of four parallel hyperbolic rods through which selected ions are filtered before reaching a collision cell where they are fragmented. The collision cell is typically called the second quadropole, but in this case, geometrically it is actually a hexapole filled with nitrogen, the same gas used in the ion source. The fragment ions formed in the collision cell are then sent to the third quadropole for a second filtering stage to enable a user to isolate and examine one precursor and one product ion.

Representing the quadropole mass analyzers as moving belts, a collision cell can be placed between the belts to fragment the ions. The first belt can be fixed to select which precursor ion travels to the collision cell. Different types of collision cells can be used. The cell can be another quadropole, a hexapole (six rods like the one used in the Agilent 6410 Triple Quad LC/MS), an octopole (eight rods), or even a transverse wave guide.

Whichever geometry is used, a collision gas is required—an inert, non-reactive gas such as nitrogen or argon. Nitrogen is used here. Since there was a need of constant and ultra-pure supply of nitrogen for the apparatus, a nitrogen generator was purchased and integrated to the system. Therefore, bitter surprises caused by unexpected and sudden consumption of pure nitrogen gas were prevented. In addition, the voltages applied to the collision cell must be different from those applied to the quadropoles to enhance the movement of all of the product ions toward the third quadropole.

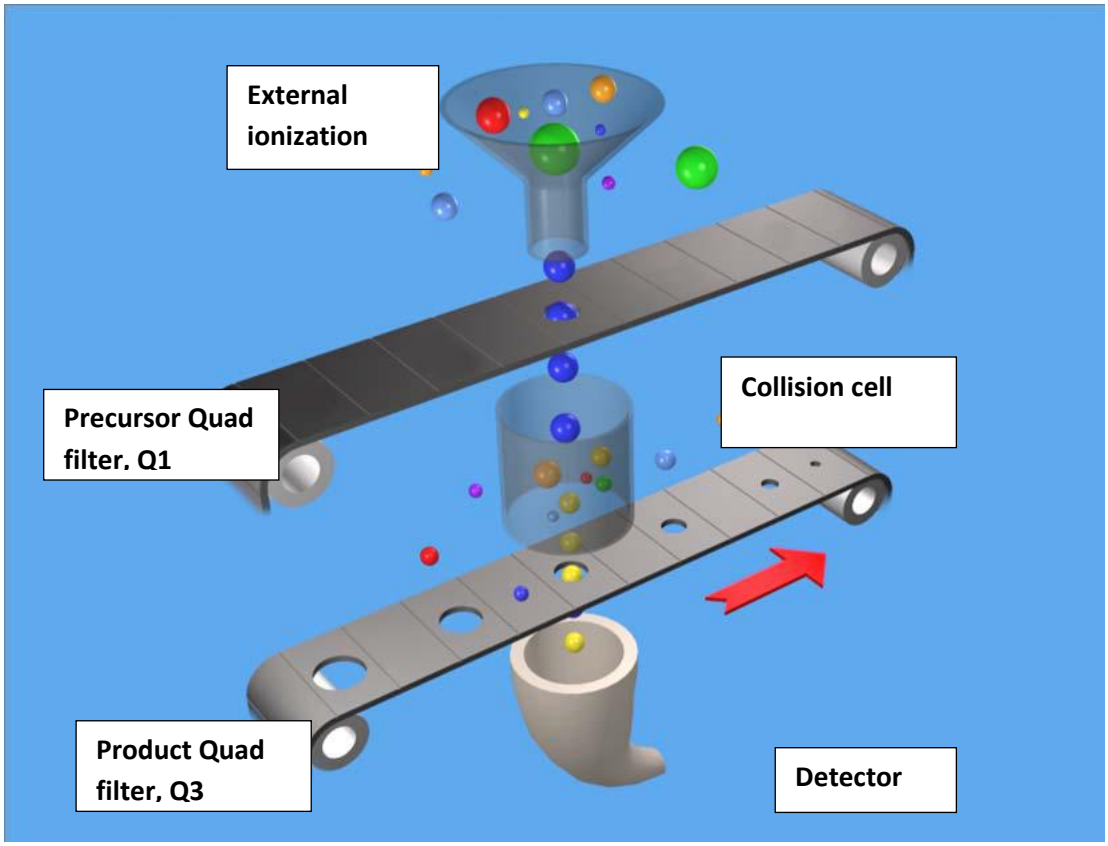

Figure 4.2 Conceptual model of a triple quadrupole mass spectrometer (Agilent Guide, 2009)

4.1.3 Optimization of the Instrument

Agilent 1200 type HPLC and 6410A type quadruple MS detector with autosampler and equipped with Electrospray Ionization was used for analyzes. Gradient elution was used to get sufficient separation of analytes.

At the beginning of the study, fragmantor voltage (FV), product ions and collision energies (CE) were optimized. Column was disconnected from the HPLC-ESI-MS/MS to decrease the analysis time. Two options, positive and negative ions, were tested during the optimization. EDCs tested were found in the positive region during optimization owing to the one H- ion absorbed. Parameters kept constant in the optimization of ES-MS/MS system are nebulizer pressure 50 psi, emv 400 V, drying gas (N2) temperature and volume 350 °C, 11.0 L/min, injection volume 30 µL, flow rate 0.2 mL/min, drew speed 200 µL/min.

In order to separate the analytes from each other, gradient elution was used. 0.1% formic acid + 5.0 mM ammonium format in ultra-pure H_2O for Mobile Phase A and 10% of 0.1% formic acid + 5.0 mM ammonium format in CH_3OH for Mobile Phase B were used. During the analysis, the nebulizer pressure was 50 psi, emv 400 V, drying gas (N_2) temperature and volume were 350 C, 11.0 L min^{-1}, injection volume was 20 mL, flow rate was 0.5 mL min1 and the sample suction flow rate applied was 200 mL min1. Separation conditions for HPLC are given in Table 4.1.

Table 4.1 HPLC seperation conditionss for selected EDCs

Parameter	HPLC
HPLC Column	Agilent, Zorbax, SB-C8 (100 _ 2.1 mm _ 3.5 mm)
Mobile Phase Program	i) 0–0.3 min, 90% of 0.1% Formic Acid + 5.0 mM Ammonium, Format in ultra pure H_2O (Mobile Phase A), 10% of 0.1% Formic Acid + 5.0 mM Ammonium, Format in CH_3OH (Mobile Phase B) ii) 0.3–1.0 min, 90–5.0% of Mobile Phase A, 10–95% of Mobile Phase B iii) 1–5 min, 5% of Mobile Phase A, 95% of Mobile Phase B iv) 5–5.1 min, 5–90% of Mobile Phase A, 95–10% of Mobile Phase B v) 5.1–10 min,90% of Mobile Phase A, 10% of Mobile Phase B
Sample Flow Rate, mL min_1	0.50
Loop Volume, mL	50.0

4.1.4 Calibration

Calibration of the LC (ESI) MS/MS is the most important step before routine analysis of sludge samples. As mentioned before six EDCs were selected within the context of this study and all those compounds were calibrated before each analysis to overcome any sensitivity changes in LC (ESI) MS/MS. For all the calibration sets each compound was prepared from 100 ppm stock solution at 0.05-100 ppb range in 25% methanol-water mix. After the analysis in LC, with the help of its software calibration curves were drawn. These calibration curves are given in Appendix part, Figure A.1-6. These calibration curves were drawn using at least 4 points which represent signals of corresponding concentration. In the given calibration curves, open dots show the points not used for the drawing of calibration. Only points represented by full dots were used for curves. No internal standard was used.

In addition to that, in order to prevent any mistakes caused by sensitivity changes in the apparatus, a new calibration set was sent to LC with each sample to be analyzed, and the EDC concentrations in these samples were calculated according to new calibration curves. However in the appendices only one example to calibration curves is given.

4.1.5 Ultrasound aided sequential extraction

The other crucial step for EDC analysis in sludge samples is the extracting selected compounds from these samples and preparing them to be analyzed in LC. When the literature was scanned, it was seen that the most widely used method for sludge extraction is ultrasound aided sequential extraction.

To apply this method 0.5 g sludge sample dried in 105°C was taken and put in 100 ml of methanol, after 30 min of sonication in ultrasound bath the aliquot was centrifuged at 2500 rpm for 10 min to prevent suspend solids interference. Then the aliquot was collected in a flask. The sonication step was repeated 3 times. Therefore, 300 ml of aliquot was collected in total, and then this aliquot was filtered using glass

fiber filter to prevent further matrix effect due to suspended solids. After the filtration step the aliquot was completely dried under N_2 flow, and then the compounds were taken into 3 ml of 25% methanol-water mixture. Method was also explained schematically in Figure 4.3.

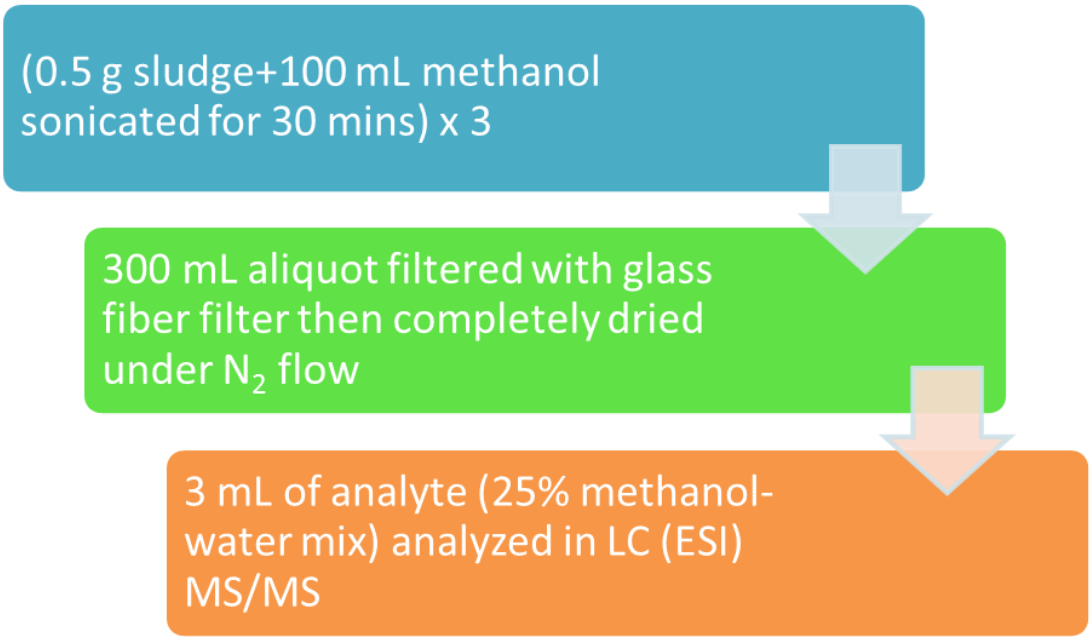

Figure 4.3 Schematic description of ultrasound aided sequential sludge extraction method

For the recovery studies, the method was applied in the same way but after dehumidification, and also after washing the sludge to get rid of any target EDCs that can interfere with the recovery results, then sludge was injected with selected EDCs at 20 ppb concentration. Comparative chromatograms of carbamazepine of standard injection and sludge are given in Figures A.7-8. After the analysis, recovery of this analysis procedure was calculated according to the Equation 4.1; where 'concentration' represents the corresponding EDC concentration as obtained by LC analysis in the aliquots; '0.5 g' represents the sludge sample amount used in extraction and ; '3mL' stands for the aliquot volume obtained at the end of sludge extraction process.

$$Recovery\ (\%) = \frac{concentration}{05.g \times 3\ ml} \times 100$$ Equation 4.1

4.2 Post-Ozone Treatment process

4.2.1 Ozone generator description

In the experiments, ozone was supplied by OSC-Modular 4HC, WEDECO ITT INDUSTRIES (2007) ozone generator. Operating pressure of the generator is 5 bars and the gas flow rate is 10-140 L/hr with a rated capacity of 4 g/hr.

During the ozonation experiments the gas flow rate from the ozone generator was kept at 80 L/hr; the pressure of the generator was set to 3.5 bars.

4.2.2 Ozone experimental procedure

At the start of experimental phase of this study, anaerobic sludge samples taken from Ankara Tatlar Wastewater Treatment Plant anaerobic digester and belt filter were ozonated according to optimum ozone dose. The purpose of this experiment was to see the effect of post ozonation both on sludge digestion and treatment of EDCs. Therefore, 300 ml taken from digester and belt filter sludge samples were ozonated for durations which are 20, 30 and 45 min. After ozonation change in TSS and VSS were investigated. However the TSS and VSS reductions were below than expected and this consequence was related to a single ozone dose application.

Hence, it was then decided to ozonate samples taken from the return activated sludge, RAS, line and operate a lab-scale anaerobic digester to examine the effect of ozone on anaerobic digestion process efficiency and EDC treatability. In order to accomplish this, four 2.5L anaerobic jars attached to gas collection tubes which were submerged at the free ends into salty water to capture gas produced by the system. All the reactors were initially operated at SRT of 25 days. One of the reactors was

used as control and received non-ozonated RAS feed, as obtained from Ankara Tatlar WWTP. One other reactor was fed with ozonated RAS at the dose of 2.65 mg ozone /g biomass. The other two reactors were operated at infinite SRT and no sludge was wasted from these two reactors. They were fed with ozonated RAS samples at doses of 1.33 and 0.65 mg ozone/g biomass.

For the reactor operation, reactor sludge was initially filled with 20 ppb EDC spiked sludge. During the experiments sludge feed to the reactors were not spiked. Therefore initial EDC content gradually diluted out. For the routine experiments, 100 mL of non-spiked RAS sample, which was kept in the fridge, was ozonated daily at the given doses before feeding into the reactors. Before feeding to the reactors, ozonated sludge samples were flushed under N_2 flow for 10 minutes in order to eliminate any residual oxygen from ozonation in order to preserve anaerobic conditions in the reactors. After that, samples were fed to the reactors. For the reactors operated at 25 d of SRT 100 mL of sludge was wasted daily. TSS and VSS of the reactor were determined using wasted sludge. The drawn sludge sample from the reactors were dried and kept in the fridge for EDC analysis. For the infinite-SRT operated reactors, a similar volume to the feed sludge were drawn from the aliquots of the settled reactors in order to keep the liquid volume constant. Since there was no sludge wastage from these reactors, only small amounts of sludge samples, enough to carry out TSS, VSS and EDCs analysis, were taken as required. For all the reactors total gas volume was measured daily with the help of graduated cylinders. Additionally, gas compositions of the reactors were analyzed using a GC once every two days. The pH and sCOD of the reactors were routinely measured.

Before the routine analysis, anaerobic digesters were operated for 30 days with daily feed of RAS at 25 SRT. By this way, all the digesters were brought to steady-state conditions prior to actual experimentation.

4.3 Chemical analysis

4.3.1 Ozone analysis

The ozone amount imparted into the liquid was determined by measuring ozone concentration in the liquid spectrophotometrically according to the Standard Method 8021 (DPD chlorine reagent) (APHA, 1998) and consulting a calibration curve. The amount of ozone imparted into the liquid by using the ozone generator was linearly proportional with the duration of ozonation, as shown in Figure 4.4. As can be seen from this figure 0.122 mg O_3/min-L is imparted.

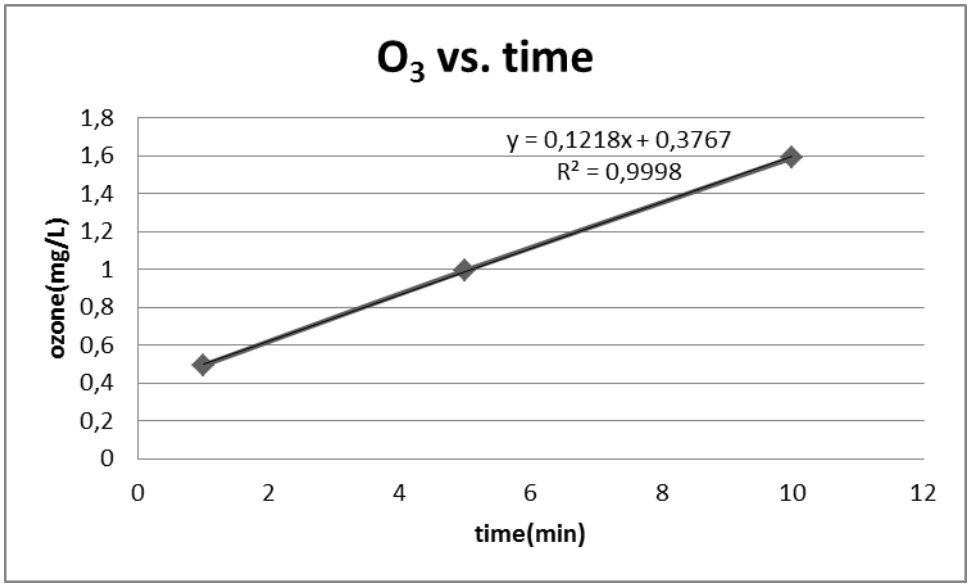

Figure 4.4 Calibration of ozone imparted to distilled water

4.3.2 COD

For routine COD analysis of the reactors Hach high range TNT plus COD kits were used. For this analysis, 2 mL of analyte was taken from the sample and if necessary it was diluted. Then these were put in a COD digester. After digestion period of the kits, they were analyzed using DR 2800™.

4.3.3 TSS and VSS

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) of the reactors were analyzed according to Standard Methods 2540B and 2540E, respectively.

4.3.4 Gas Analysis

Gas samples were taken from reactors with sterile Hamilton syringes and analyzed in Thermo Trace GC Ultra gas chromatography once in a week. The result chromatograms were evaluated using ChromQuest 5.0. Calibration of GC was done with a gas mixture containing 40% H_2, 20% CH_4, 20% N_2 and 20% CO_2.

4.3.5 Target EDCs

The selected compounds, estrone (>99%), diltiazem (>99%), progesterone (>99%) were purchased from Sigma, Benzyl Butyl Phthalate (>98%) was purchased from Aldrich, carbamazepine (>99%) and acetaminophen (>99%) was from Sigma-Aldrich. These chemical were chosen according to their availability, common usage; hence high amounts of discharge. In addition to these, each of these compounds represents an important group of EDCs and these group and their properties were given in Table 4.2. This table reveals the fact that all the endocrine disrupters listed in this table are high in sludge concentration.

Table 4.2 characterics of target EDCs used in this study

Compounds	**CAS**	**MW g/mol**	**Log Kow**	**Melting Point ^{o}C**
Acetaminophen	103-90-2	151.2	0.46	196
Estrone	53-16-7	270.4	3.13	256
BBP	85-68-7	312.4	4.73	-35
Progesterone	57-83-0	314.5	3.87	131

Diltiazem	42399-41-7	414.5	2.80	231
Carbamezapine	298-46-4	236.5	2.67	191

In order to analyze the EDCs in sludge samples and to see the effect of anaerobic digestion coupled with ozone pre-treatment on EDC removal, it was decided to spike the anaerobic digester initially with the target EDCs at a concentration of 20 ppb each. The initial RAS sludge to start the anaerobic test reactors were kept in the anaerobic rectors for 30 days at 25 days SRT, prior to experimentation. At the end of this period sludge present in the reactors were transferred into conical flasks, where they were spiked with target EDCs to give 20 ppb in the liquid phase. Sludge was shaken on an orbital shaker at 70 rpm for 10 minutes. Any residual EDC at the end of this period was analyzed in the liquid. When no remaining EDC was observed in the water phase the sludge mixtures were again placed in the reactors. After reaching approximately 20 ppb concentration of each target EDC in sludge, feeding the reactors with pre-ozonated sludge feed (no EDC) were commenced.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 EDC Analysis

5.1.1 Parameter and Chemical Optimization

In HPLC (ESI) MS/MS, when performing chemical optimization there must be at least two product ions. As example, carbamazepine is given from now on and the optimization results of the rest of the EDCs are given in appendix part. As it is seen in Table 4.2 the molecular weight of the carbamazepine is 237 g/mole. The product ions of this chemical was determined as 194 and 192 g/mole and the chromatographic images can be seen from Figure 5.1.

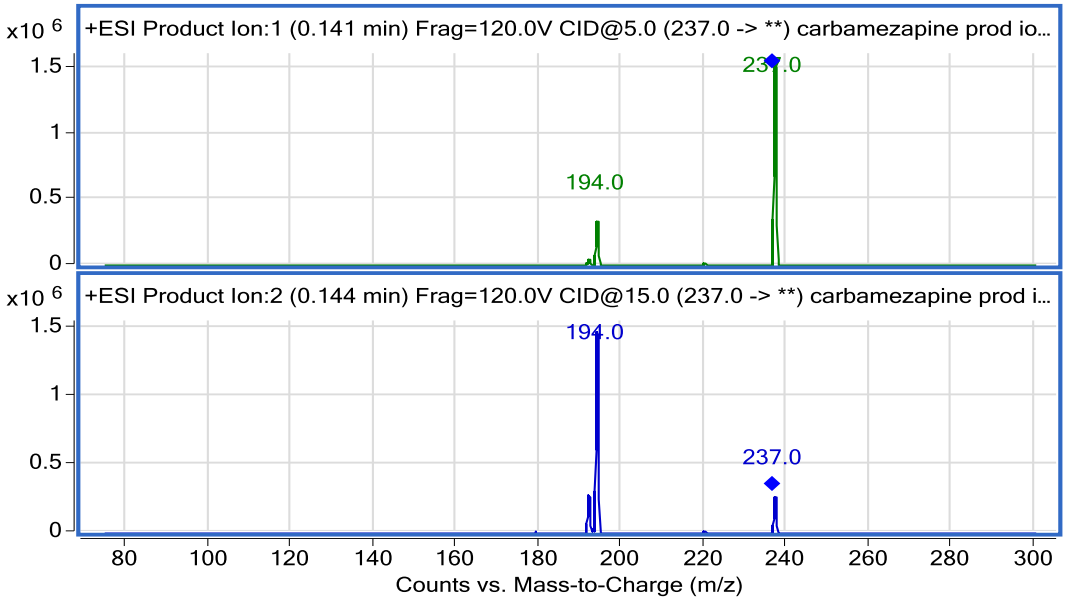

Figure 5.1 Product ions of Carbamazepine

After the definition of product ions, it must be decided on which product ion will be quantitative or qualitative. The ion with higher signal becomes the quantitative and the other becomes qualitative ion. Quantitative ion defines the concentration of the compound whereas the qualitative ion indicates the presence of the compound with analyzed quantitative ion.

After the definition of quantitative and qualitative ions, mobile phase optimization was performed. Mobile phase is one of the most important differences between GC and HPLC. In HPLC, analytes are carried in a liquid phase called mobile phase differently in GC those are carried with gas flow. Therefore, it is a must to optimize the mobile phase ingredients and flow rate. In order to achieve this aim, first, mobile phase was optimized with different types of buffers at different concentrations and in different solvents. The optimization chart is given in Table-3.2.

Table 5.1 Different types of mobile phases used during the optimization

Number	**Mobile Phase-A**	**Mobile Phase-B**
1	Distilled water + 0,1% F.A*.	Methanol+ 0,1% F.A.
2	Distilled water + 26 mM A.F*.	Methanol+ 26 mM A.F.
3	Distilled water + 0,1% F.A. + 5mM A.F.	Methanol+0,1% F.A. + 5 mM A.F.

*: F.A.: Formic Acid, A.F.: Ammonium Formate

Table 5.1 reveals that in HPLC two mobile phases are used: Mobile Phase A and B. The only difference between these two mobile phases is that Mobile Phase A is prepared in distilled water and the other is prepared in high purity methanol. After the analysis of the three scenarios of mobile phases given in Table 5.1, the results showed that the best signals were acquired with Mobile Phases number 3. The signals of this comparison were given in the Appendix section, in Figures A.9-11. as it was seen from these figures that there was no major difference between these mobile phases. Therefore, it was seen appropriate to apply different fragmantor

voltages to decide the mobile phase. According to Figures A.12-14, highest signals for carbamazepine were acquired with Mobile Phase-3. Also, best fragmantor voltage for carbamazepine was established as 140 V. All these steps were applied exactly to other target EDCs.

After this point, optimization of target compounds were carried out with at first the given parameters in Table 5.2, and then optimization was completed changing these parameters. Finally, optimum analysis parameters for target EDCs were shown in Table 5.3. as it can be seen from this table, both quantifier (Quant) and qualifier (qual) ions were found. Quantifier ions were used to calculate or quantify the concentration of EDCs while qualifier ions verify the presence of quantifier ion. Therefore, the optimization of HPLC (ESI) MS/MS period was completed.

Table 5.2 Default parameters used in analysis

Parameter	**Value**
Flow	0.4 ml/min
Mobile Phase A	95%
Mobile Phase B	5%
Nebulizer	50
EMV	400

Table 5.3 Optimization results for each compound

Compound	**MW**	**Q1**	**Q3 (Quant)**	**Q3 (Qual)**	**FV**	**CE**	**Polarity**
Diltiazem	414.5	415	177.9	309.9	130	24-30	Positive
Progesterone	314.5	315	109	97	120	30-23	Positive
BBP	312..4	313	91	148.9	70	20-9	Positive
Estrone	270.4	271	253	159	110	9-20	Positive

Carbamazepine	236.5	237	194	192	120	18-22	Positive
Acetaminophen	151.1	152	110	93.1	90	14-22	Positive

5.1.2 Recovery Tests

The second step before starting the routine analysis and anaerobic reactor set-up; optimization of the selected sludge extraction method described in Chapter 2 must be performed. First optimization study was to find out the correct solvent for the extraction method. In order to achieve this aim two solvents were selected: dichloromethane and methanol. Sludge samples were extracted according to the method described using both methanol and DCM separately. In Figure 5.2, Total Ion Chromatograms (TIC) of these experiments were shown. As it can be seen from this figure, signals acquired with methanol were better than that of DCM's. Therefore, after this point methanol was used for extractions as solvent.

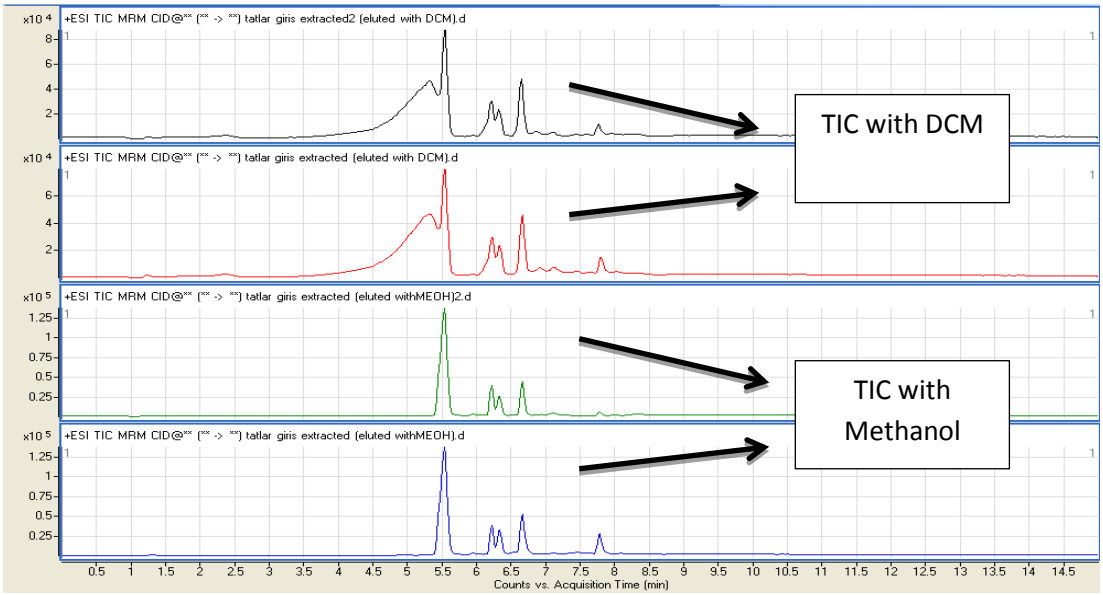

Figure 5.2 Optimization results of solvents

Secondly, the duration of sludge extraction method was optimized. In order to accomplish this step, three different methods was applied: second sludge samples were extracted 3 times with 100 mL of methanol for 30 and 45 minutes and the last sludge sample was extracted 6 times with 50 mL of methanol for 30 minutes. The

results given in Figure 5.3 revealed that the method with 3 times of duplication and 30 minutes of duration was the best among other tested methods.

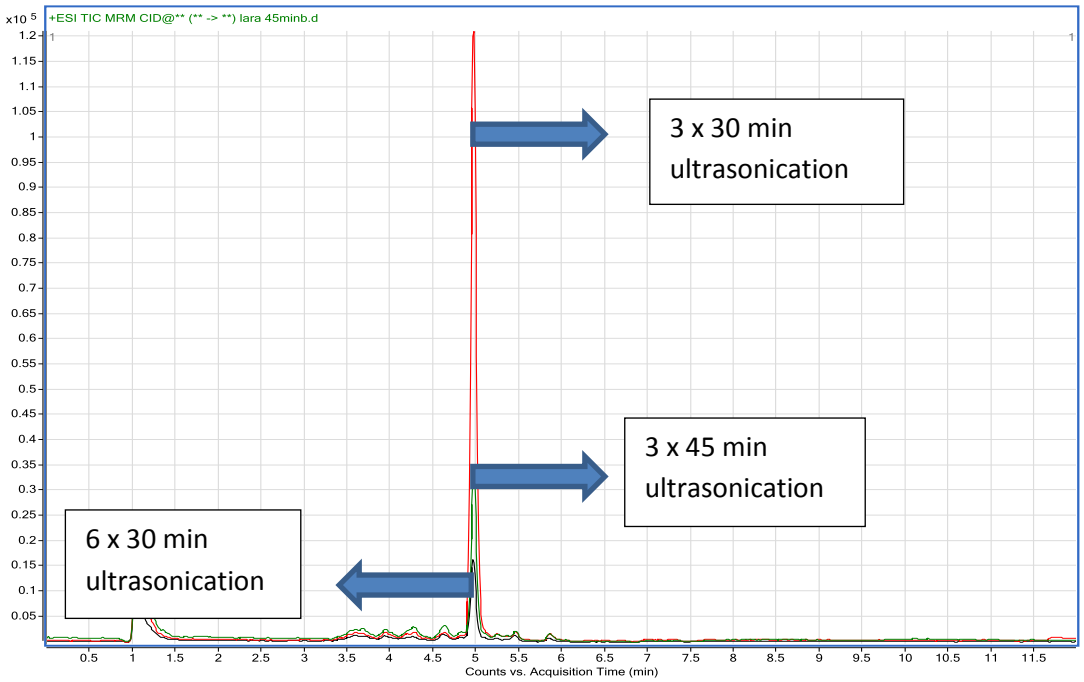

Figure 5.3 Comparison of TICs of different sludge extraction methods

Lastly, recovery percentages must be calculated before the routine analysis and recoveries were calculated using the Equation 4.1. The results of this study are given in Table 5.4. These results indicate that when the sludge extraction recovery percentages in literature are compared, the current method is applicable.

Table 5.4 Recovery percentages of sludge extraction method

Componds	**Recovery (%)**
Diltiazem	93 ± 0.04
Progesterone	96 ± 0.03
BBP	92 ± 0.05
Estrone	91 ± 0.06
Carbamazepine	94 ± 0.04
Acetaminophen	96 ± 0.02

5.2 Anaerobic Sludge Ozonation

5.2.1 Sludge Lysis

Ozone, being a highly oxidizing chemical, when introduced into a medium it immediately undergoes reaction with the available organics present in the medium. In the case when ozone is applied to sludge samples, it is expected that cell walls will be destroyed releasing soluble cellular contents. The Figures 5.4 and Figure 5.5 show soluble COD release into the supernatants and the corresponding TSS disintegration, respectively, at differing ozone dozes administered on RAS suspended at 2.3 g/L concentration, in tap water. As can be seen from both figures the COD release and TSS disintegration both proceeded at an increasing rate until 2 mg ozone/g biomass dose. From this point on disintegration effect of ozone was much less conspicuous.

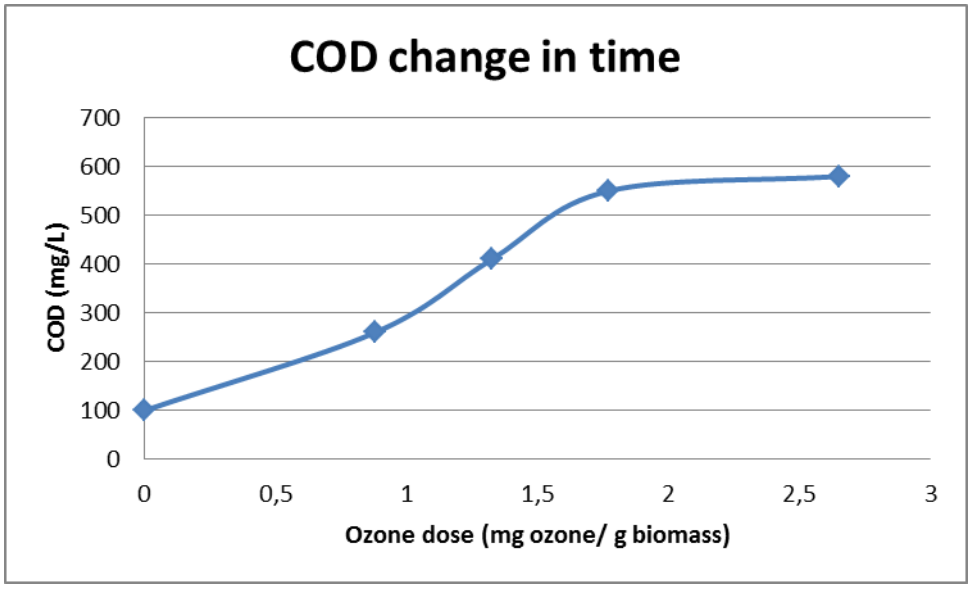

Figure 5.4 The soluble COD release upon ozonation of sludge samples at different ozone doses

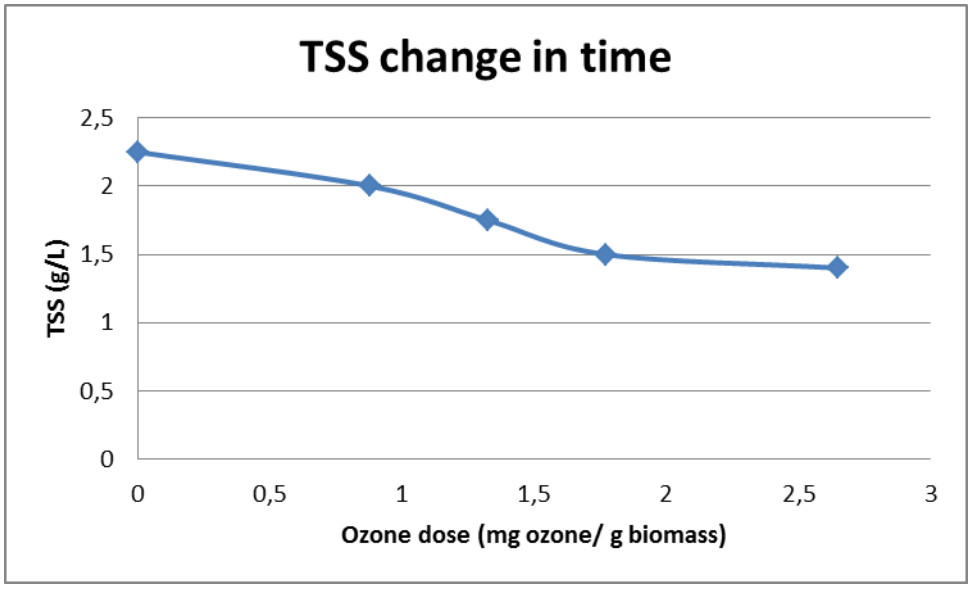

Figure 5.5 Total Suspended Solids destruction versus ozone dose

5.2.2 Ozone Dose Requirements

Prior to digester experiments Waste Activated Sludge (WAS) samples taken from Ankara Central Wastewater Treatment Plant RAS line were ozonated using ozone generator at durations of 2, 3 and 4 minutes. From Figure 5.5, it can be seen that after 2 mg ozone/g biomass dose was no significant change in TSS; as in the case of COD release in Figure 5.4. However the highest disintegration by ozone was achieved at 2.65 mg ozone/g biomass, from Figure 5.5. This ozone dose was set as reference for the further studies. Figure 5.6 shows VSS change with cumulative ozone dose applied. Six mg/L was observed as the optimum concentration to affect highest disintegration at the optimum expense of ozone. When this figure was divided with 2.3 g/L VSS present in the medium 2.65 mg ozone/g biomass dose is reached.

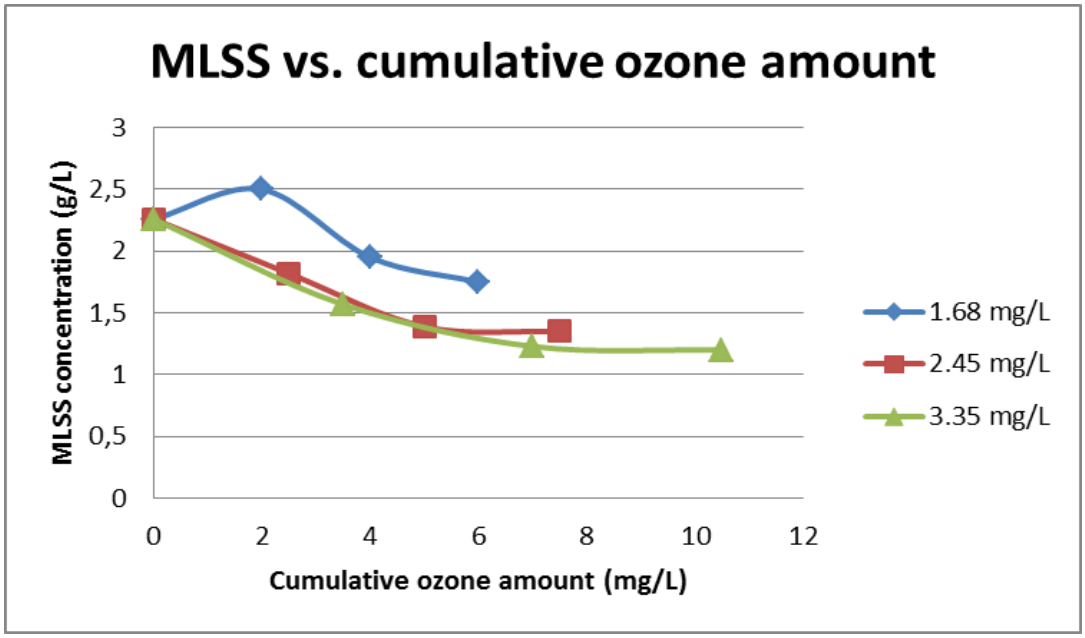

Figure 5.6 Reduction of MLSS with ozone applied to the sludge sample

5.2.3 Ozonation of Digester and Belt Filter Sludge Samples

Sludge samples were taken from Ankara Central Wastewater Treatment Plant at the points of *anaerobic digester* and belt filter effluent. The samples were ozonated at three different durations: 20, 30 and 45 minutes. TSS reduction and treatability of target EDCs were investigated. The Figures 5.6 – 5.7 summarizes target EDCs removal attained at these ozone concentrations. The TSS removals achieved at these ozone doses are expressed in Table 5.5.

As can be seen from Table 5.5, as opposed to RAS ozonation results presented in Figures 5.5 and 5.6 very little, merely around 8 %, reduction or disintegration of sludge could be achieved at the expense of such high ozone doses. This is somewhat in accord with the results of Carballa et. al., 2007. Moreover, removal of EDCs in sludge samples were also on the low side, as can be seen in Figures 5.7 and 5.8. This extremely lower disintegration of the sludge, as opposed to RAS ozonation presented in Figure 5.6 as the attributed to the nature of the sludge sample. RAS Samples were taken from the aeration tank outlet, whereas digester samples were taken from the anaerobic digester and belt filter effluent of Ankara Tatlar WWTP. Clearly the

reducing medium carried along with the anaerobic sludge samples heavily consumed ozone making very little of it available to sludge destruction and EDCs removal.

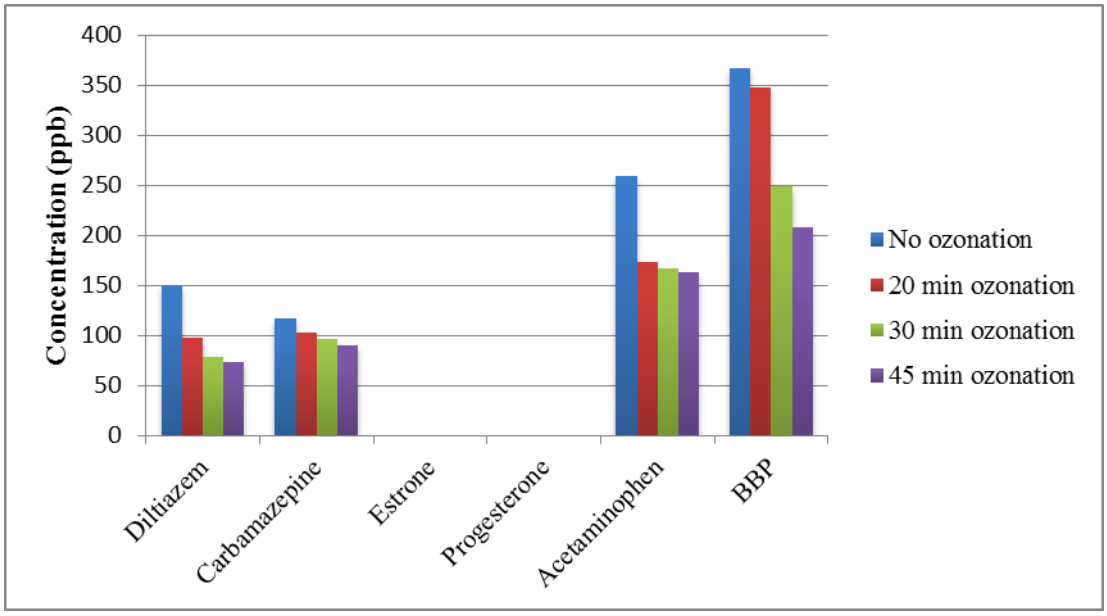

Figure 5.7 Treatability of target EDCs in digester sludge sample

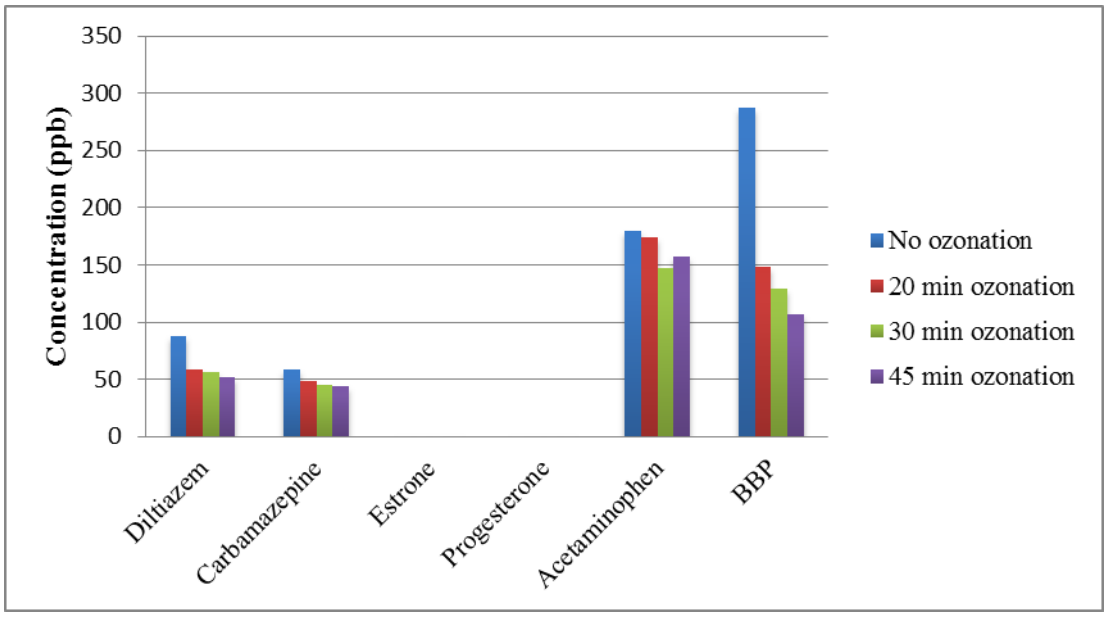

Figure 5.8 Treatability of target EDCs in belt filter effluent sludge sample

Table 5.5 TSS reduction percentages at different ozonation durations

Ozonation duration	**20 min**	**30 min**	**45 min**
Digester Sludge	8,9	1,1	8,6
Belt Filter Sludge	8,4	8,6	8,2

Therefore further studies were conducted with sludge samples obtained from RAS line, and not the digesters.

5.3 Ozonation experiments by using RAS sludge samples.

The composition of the common RAS feed applied to the reactors are given in Table 5.6. As can be deduced from this table while total COD remained constant the soluble fraction changed with the degree of ozonation.

Table 5.6 Composition of the RAS feed to the reactors

Parameter	**Concentration (mg/L)**
TSS	4010
VSS	1200
SCOD	778
SCOD 0.66	1440
SCOD 1,33	2100
SCOD 2.65	3400

Although batch ozone application reactors should give an idea about effectiveness of ozonation on sludge digestion and EDCs removal, yet effect on continuous digesters, such as in the real case, remains to be explored. Therefore ozonated RAS samples were fed to continuous anaerobic digesters.

Total gas volume was measured daily and percentage of methane in the biogas and analysis of EDCs content of sludge were routinely carried out once per week bases. Control reactor, whose TSS and VSS concentrations were steady around 9 g/L and 7.5 g/L respectively; was being fed at 100 mL/day with RAS feed having 3.87 g/L

TSS and 2.4 g/L as VSS. The control reactor was being operated at 25 days SRT, with routine discharge of excess sludge. The TSS and VSS concentrations in the control reactor are presented in Figure 5.9.

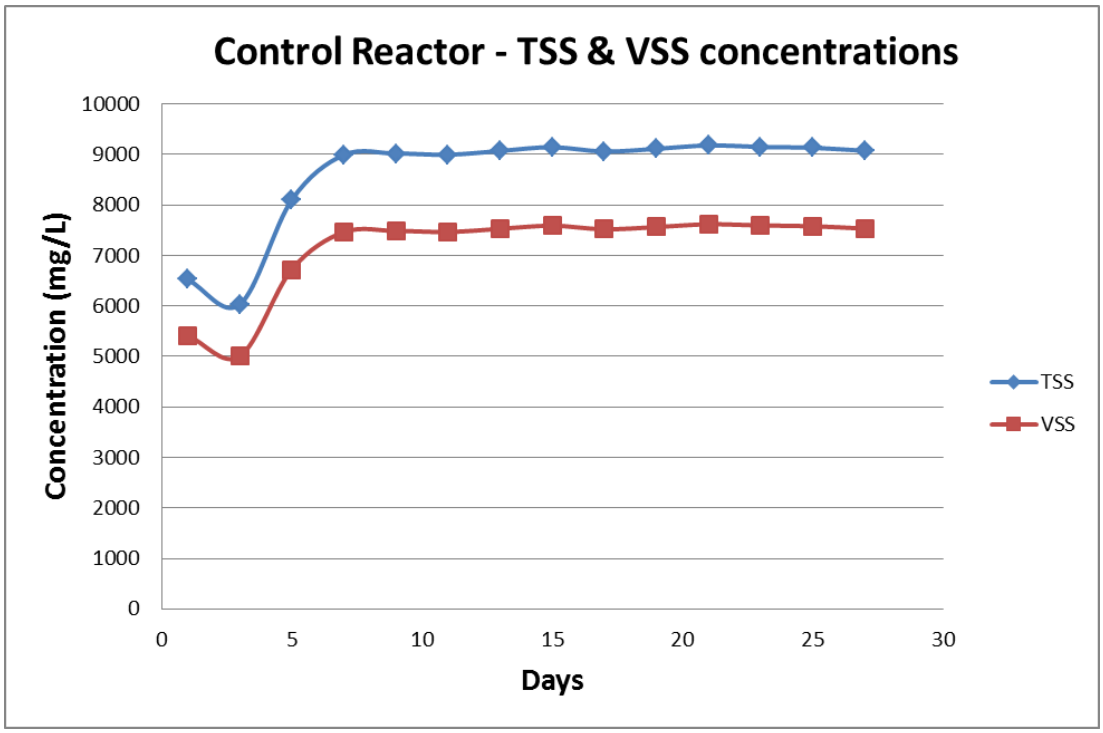

Figure 5.9 The VSS and TSS Concentrations in the control reactor

Applying 2.65 mg/g Ozone dose

The first ozone dose selected for RAS treatment was 2.65 mg/g, based on data presented in Figure 5.5. The excess sludge wastage was identical to the control reactor to maintain 25 days SRT. A 200 Ml Ozone-treated sludge was fed to the reactor once every other day, hence giving a volumetric rate of 100 mL/day. However there was a sharp decline in the biomass concentration in this reactor, as depicted in Figure 5.10, until the day when biomass stabilized at 0.8 g/L around 20th day. Evidently over ozonation disintegrated, and perhaps chemically oxidized the feed RAS, since TSS value of the ozonated sludge dropped to 4 g/L and VSS to 1.2 g/L; consequent upon ozonating at 2.65 mg/g. With the low cryptic growth rate of

the existing biomass in the absence of adequate carbon feed; and owing to the flushing effect of sludge wastage which evidently did not balance the daily wasted sludge from the reactor, biomass has rapidly declined in this reactor. A low VSS concentration as 0.8 g/L is clearly unacceptable in an anaerobic digester.

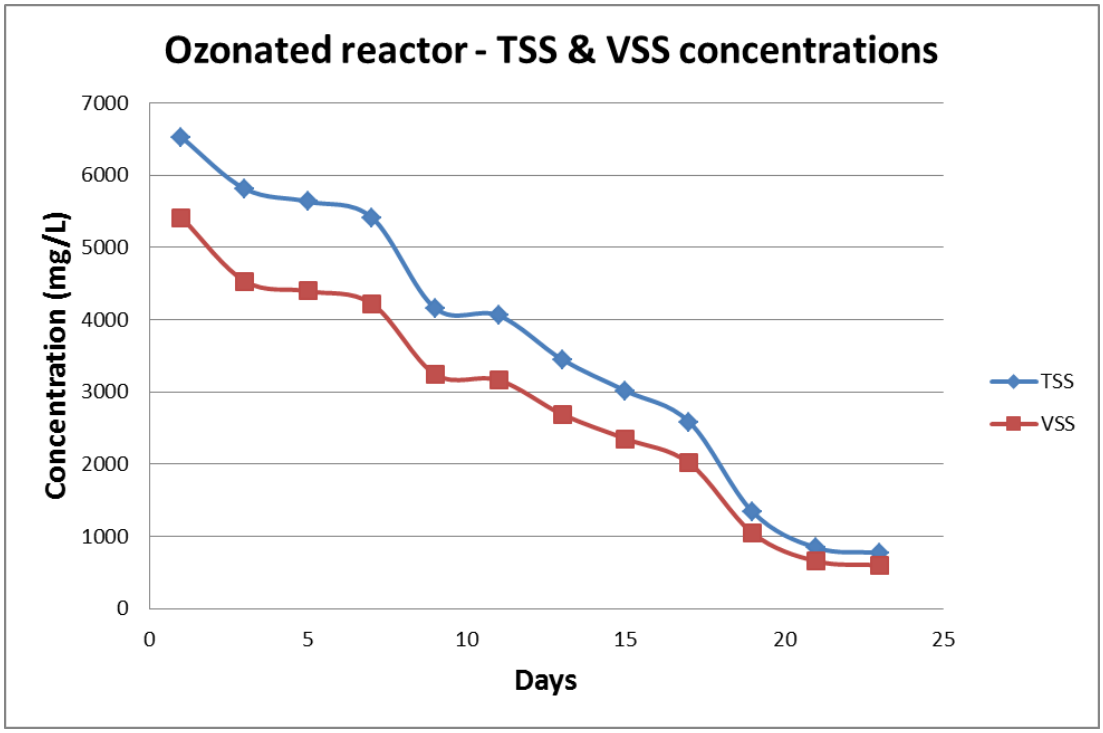

Figure 5.10 The VSS and TSS decline when feed RAS was dosed with 2.65 mg/g ozone. Sludge was routinely wasted in this rector.

Applying 0.66 mg/g Ozone dose

In order to maintain steady reactor excess sludge wastage from the reactor was stopped and ozone dose was reduced by half to 0.66 mg/g ozone. The biomass concentration in this reactor was 7.3 g/L TSS and 4.8 g/L VSS. The feed RAS on the other hand contained 3.9 g/L TSS and 2.4 g/L VSS and 0.7 g/L as sCOD after ozonation. When feed RAS was dosed with 0.66 mg/g ozone; reactor remained steady at 3 g/L in terms of biomass concentration, VSS, as shown in Figure 5.11. During this time excess sludge was not produced, therefore reactor was operated on

infinite SRT. Evidently the growth rate of microorganisms just balanced the decayed biomass

Applying 1.33 mg/g Ozone dose

When RAS samples were treated with 1.33 mg/g ozone before feeding into the reactor, the reactor was also more or less steady at 2.5 g/L biomass concentration, VSS, as shown in Figure 5.12, operating at infinitive SRT.

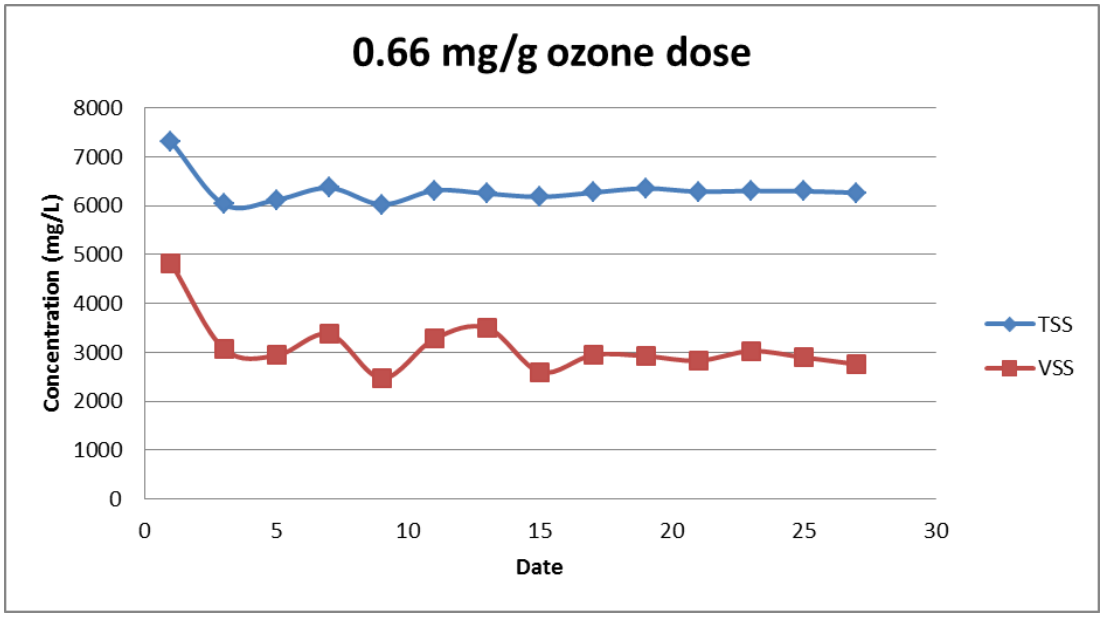

Figure 5.11 The VSS and TSS concentrations maintained in reactor when feed RAS was dosed with 0.66 mg/g ozone. No wastage of biomass from the reactor.

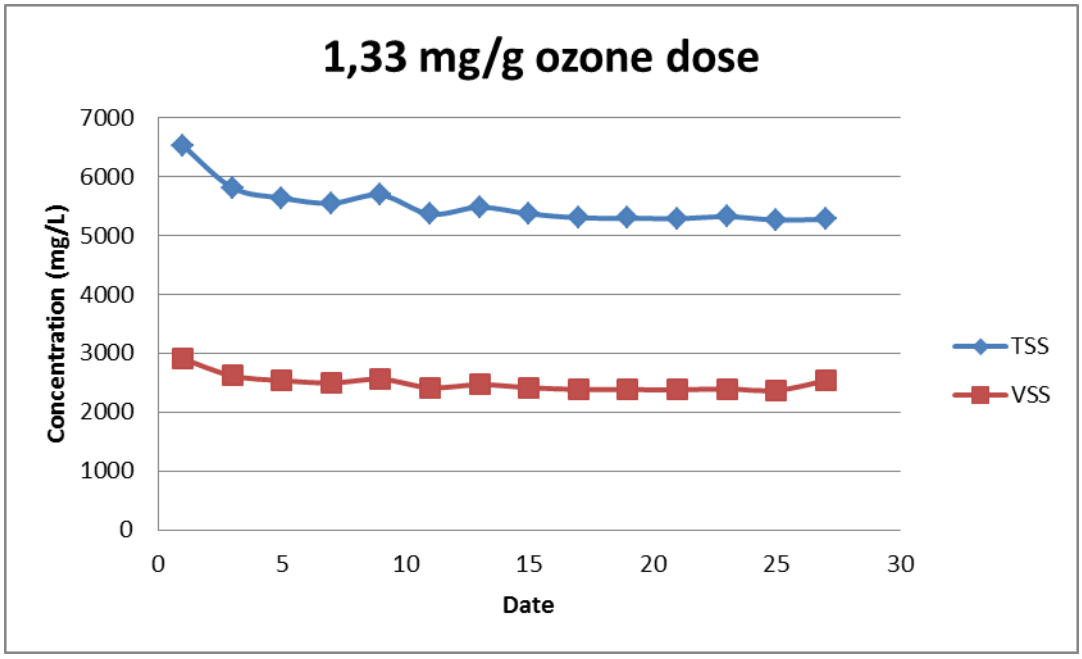

Figure 5.12 The VSS and TSS concentrations maintained in reactor when feed RAS was dosed with 1.33 mg/g ozone. No wastage of biomass from the reactor.

Treatment of EDCs in reactors

In an attempt to illustrate the possible role of ozone on the removal of EDCs in sludge, parallel experiments were carried out. Anaerobic sludge sample taken from Ankara Tatlar WWTP were initially spiked once with 20 ppb of the selected EDCs, and their disappearance were monitored by routine sample analysis for 27 days in the reactors. The aim of these analysis was to see the effect of both anaerobic digestion and ozone pre-treatment of the feed sludge on the degradation of emerging compounds. Degradation of EDCs are given in Figures.13-18.

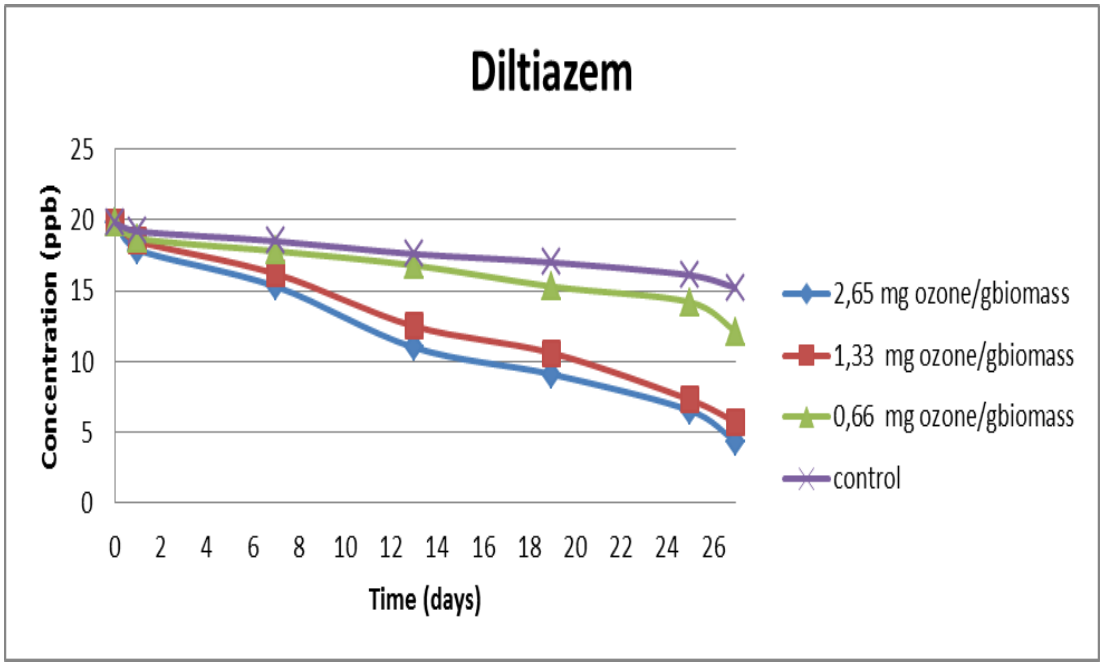

Figure 5.13 Change in the concentration of diltiazem in anaerobic digester during 27 days

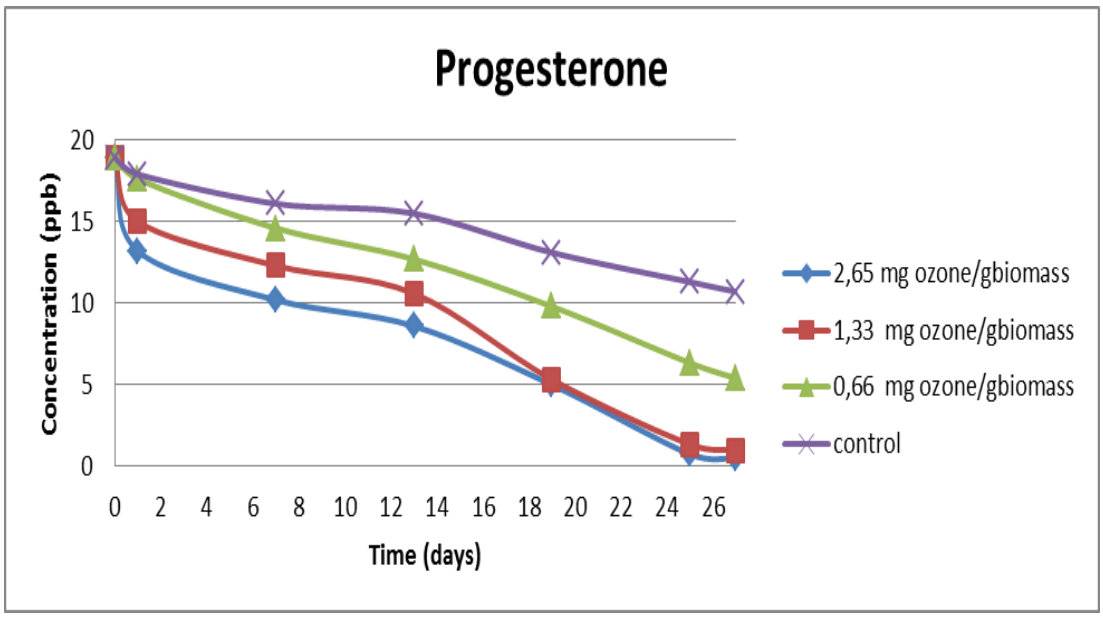

Figure 5.14 Change in the concentration of progesterone in anaerobic digester during 27 days

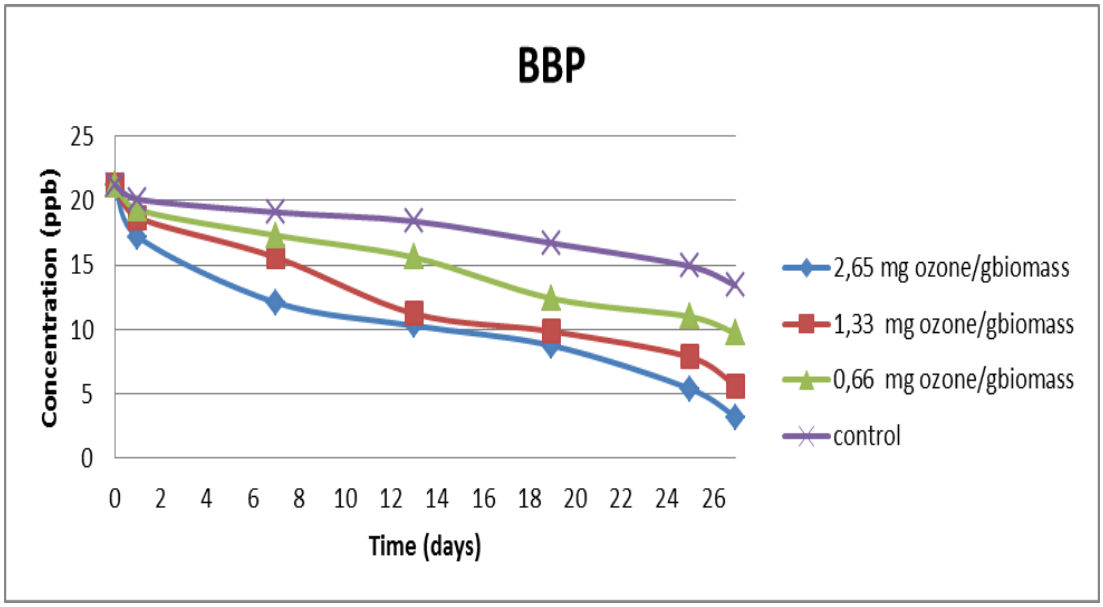

Figure 5.15 Change in the concentration of BBP in anaerobic digester during 27 days

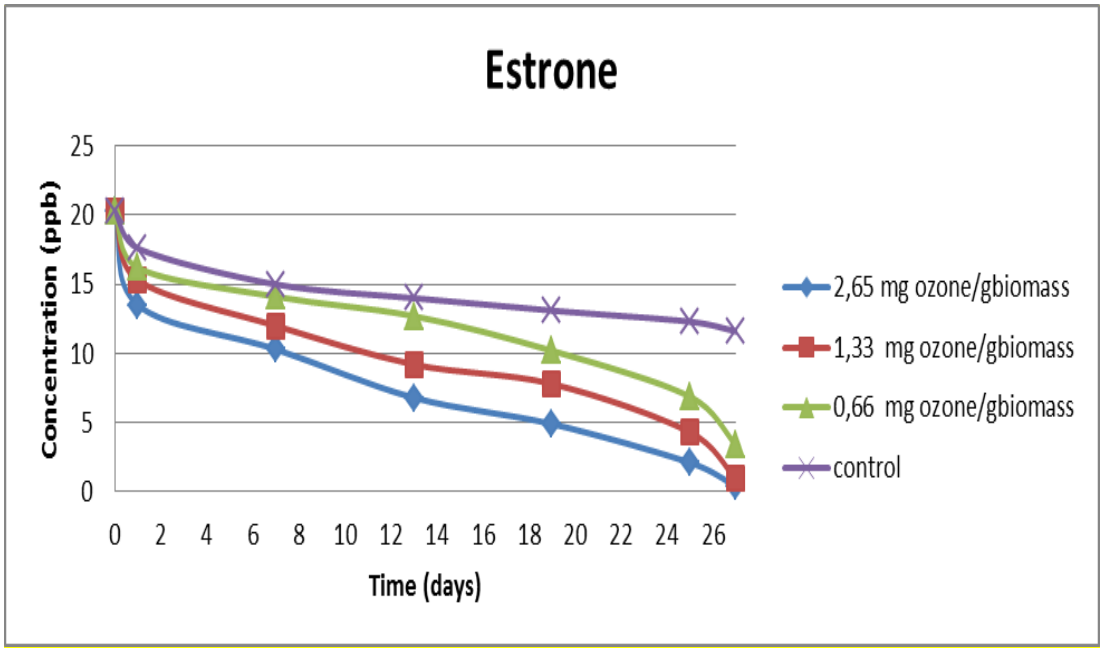

Figure 5.16 Change in the concentration of estrone in anaerobic digester during 27 days

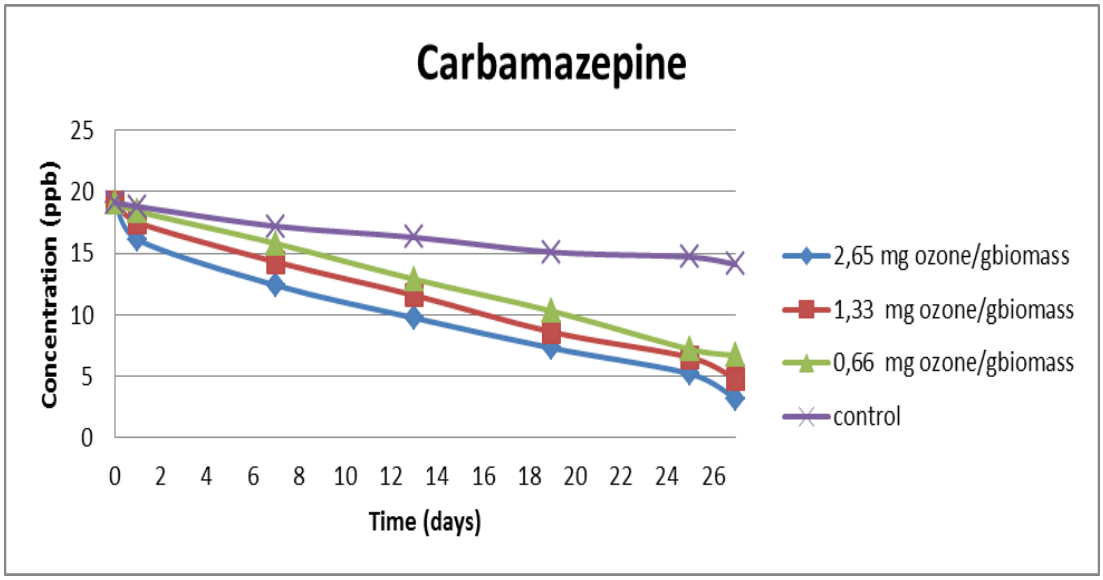

Figure 5.17 Change in the concentration of carbamazepine in anaerobic digester during 27 days

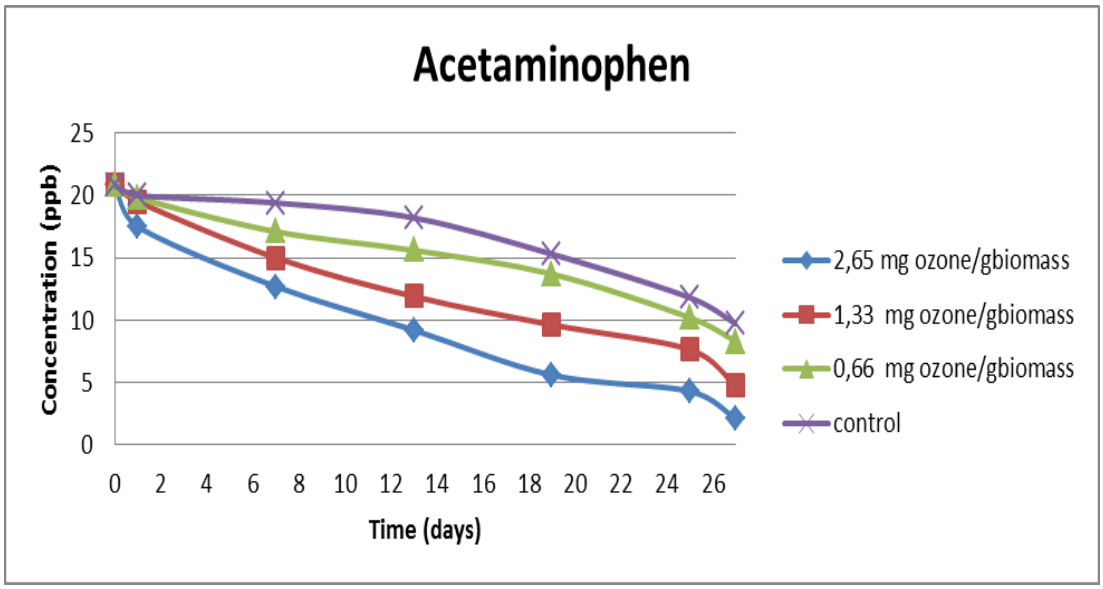

Figure 5.18 Change in the concentration of acetaminophen in anaerobic digester during 27 days

As these figures imply ozone pre-treatment of the feed has a significant effect on the removal of EDCs which were initially present within the sludge. Figures show that there were obvious changes in the EDC content of the sludge in the reactors. Therefore, these significant changes can be attributed to the effect of ozone such that

control did not affect as much removal of EDCs as the ozone treated feed receiving reactors. Higher the ozone dose, yielded higher EDCs removal . Highest removal was observed in 2.65 mg ozone dosed reactor. However, much of the EDC was evidently removed from this this reactor with the biomass wastage to maintain 25 days SRT and the remainng due to the ozone effect. The same is also applicable to the control, since this was operated at 25 days SRT too. Whereas difference between 2.65 mg/g ozone and control is clearly due to ozone effect. Biomass concentration within the reactor was washed out because of high ozone. Hence the the reduction in concentration of EDCs could be the results of both high amount of sludge wastage and effect of ozone pre-treatment. For the other ozonated reactor, on the other hand, the sludge wastage was terminated and at infinite SRT still reduction in concentration of EDCs were observed. Therefore, it can be said that using ozone as a pre-treatment technique is a good way to treat emerging compounds in sludges. Control reactor or as in the real case anaerobic reactors as itself, treated EDCs noticeably. Although the reduction rate observed in control reactor can not be underestimated, depending on the future regulation on emerging compounds, these removal rates may remain incapable.

At the end of the digestion period, as mentioned before for the ozonated reactors, important amount of EDCs previously spiked to reactors were treated. In Figure 19 the degree of EDCs reduction was given visually.

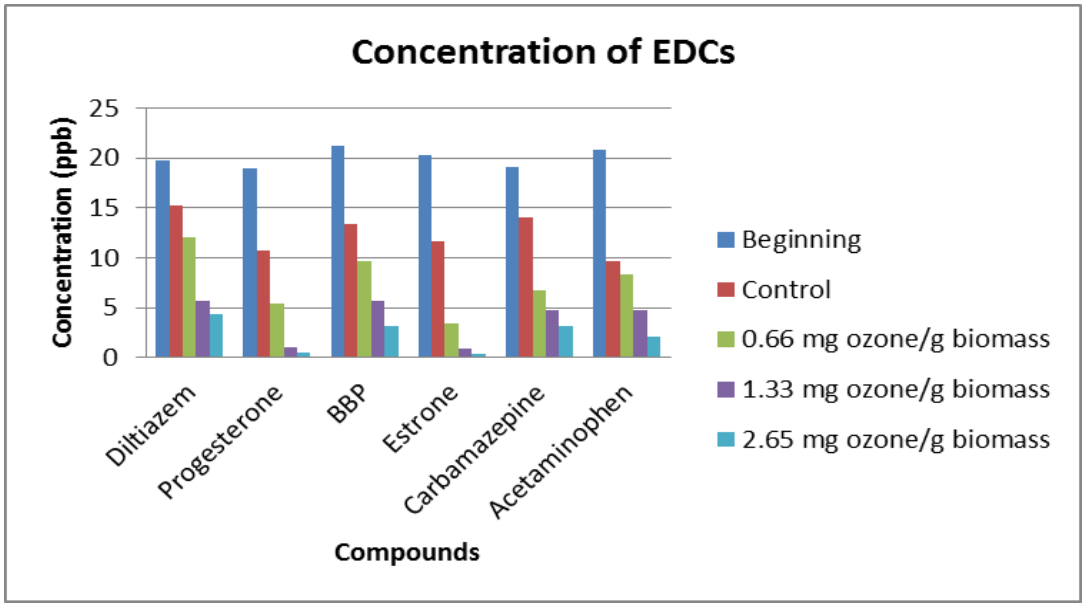

Figure 5.19 Change in concentrations of EDCs at the end of digestion period according to different ozone doses

In Figure 19, column named 'beginning' represents the starting concentrations of EDCs after spiking. The other columns represents the concentration of EDCs athe end of digestion period. This figure reveals the fact that pharmaceuticals except acetaminophen have lower reduction rates than that of natural hormones. This situation can be related to the complex structures of pharmaceutical compounds. The heat in the ractors can be other reason for this difference in removal of EDCs such that natural hormones are less durable to heat than pharmaceuticals. However, the effect of ozonation on anaerobic reactors was obvious such that the great difference in the end concentrations of EDCs between control reactors and other ozonated reactors have evidential values of this situation.

Gas production in the reactors

The cumulative gas production results obtained upon ozonation of the feed sludge is summarized in Figure 5.13. Experiments were stopped when gas production rate leveled off after 27th day, indicating onset of steady-state conditions governing the reactor operation. In this figure cumulative gas production values for all the reactors, except for 2.65 mg/g dose, represent steady-state conditions. In the case of 0.66 and

1.33 mg/g ozone SRT was infinitive. The SRT for the control reactor was steady at 25 days.

Percentages of methane in the biogas were given in Figure 5.14. It was seen from this figure that percentage of methane also increased after treatment of feed RAS with ozone; highest being at 2.65 ozone /g biomass dose. From Figure 5.13, it can be seen that biogas produced by the ozone-treated sludge fed reactor was almost two folds, or 200 %, of the control rector at 1.33 mg ozone dose. In the case of 0.66 mg/g ozone increase was 33 %. In both cases reactors operated at steady-state with infinitive SRTs and without sludge wastage. In the case of control 4 % of the existing sludge (1/SRT) in the reactor had to be wastes daily. Whereas in the case of 2.65 mg O3/g application, it was evident that standing biomass concentration in the reactor seriously reduced resulting in shorter SRTs, as given in Figure 5.8. This may be compensated by increasing the Organic Loading Rate (OLR) and stopping sludge wastage. This remains to be explored.

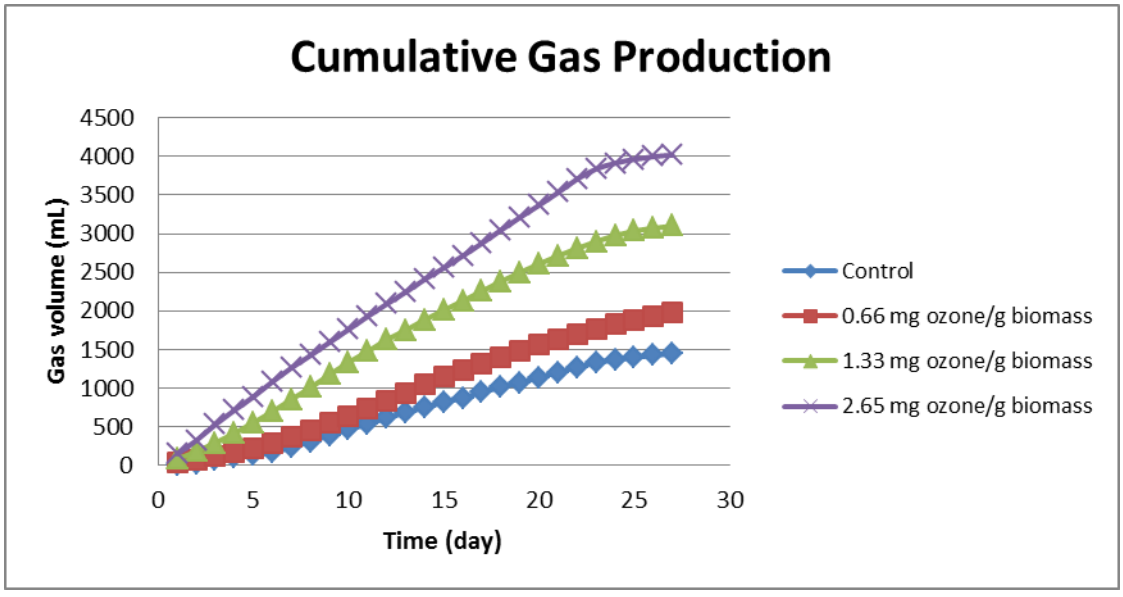

Figure 5.20 Total gas volume comparison at different ozone doses

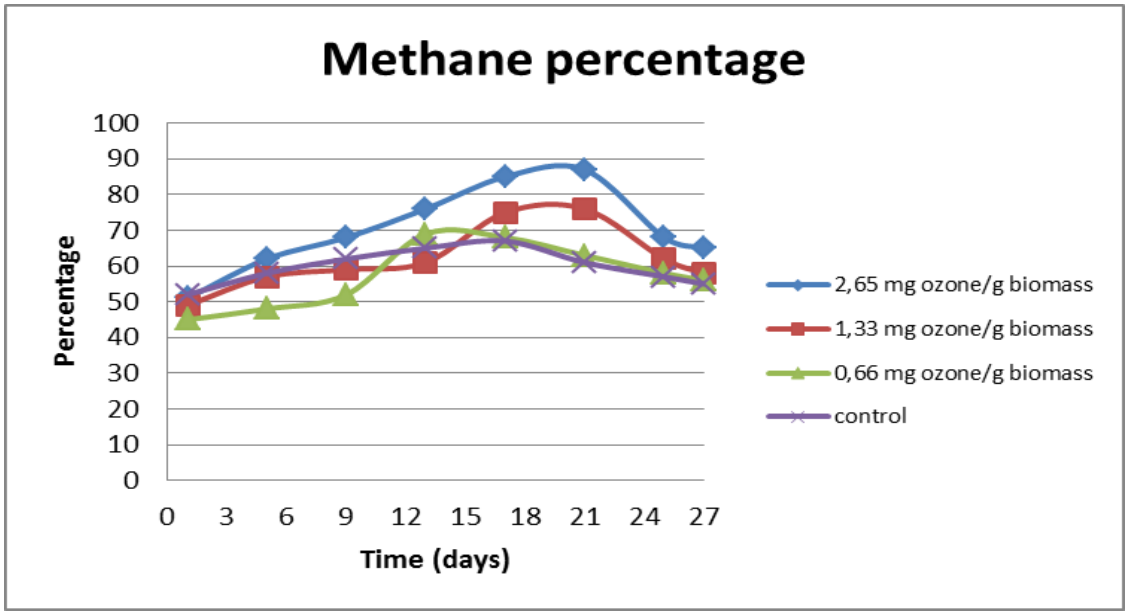

Figure 5.21 Comparison of methane percentage of biogas at different ozone dose

Material Balance

A material balance was tried to be established for the steady-state reactors fed with 1.33 and 0.66 mg ozone/g biomass doses. The 2.65 ozone/g biomass reactor was excluded from this balance because of its earlier non-steady characteristic. It was reasoned that a material balance on this reactor might be misleading since the reactor was unsteady for most of the time.

To do the material balances, first cumulative methane produced by the reactors were calculated as depicted in Figure 5.15.

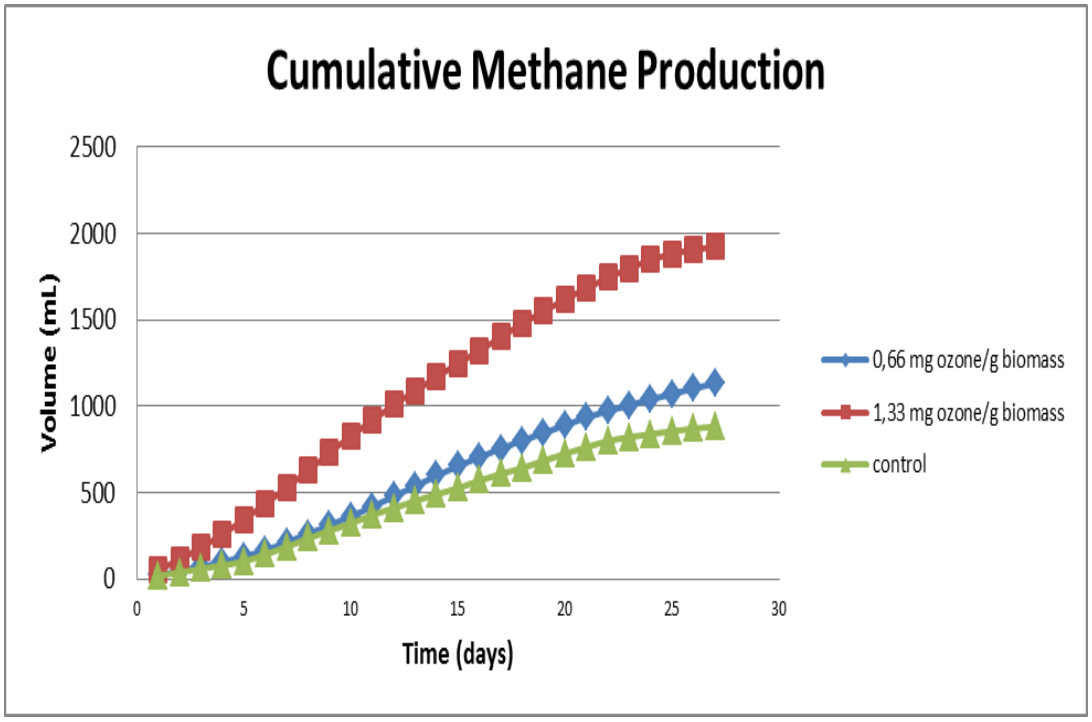

Figure 5.22 Cumulative methane production during the operation of reactors

The total COD fed to the reactors was taken as 3400 mg/L from Table 5.6, since when 2.65 mg ozone / g biomass was fed, all the biomass have disintegrated and COD was directly analyzed in the homogenate. Therefore 3400 mg/L COD figure represents total COD concentration in the RAS feed.

Taking that at 37 ^{o}C and at atmospheric pressure 25.5 mL of volume is occupied per mmole of methane; and 1 mmole of methane produces 64 mg COD from stoichiometry. Considering that RAS feed contained 3400 mg/L total COD from Table 5.6 the following calculation should hold:

$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$

Accordingly,

16 g methane $\rightarrow$ 64 g COD

$$22.4\ ml\ x\ {}^{310}/_{273} = 25.4\ mL$$

$$\begin{pmatrix} COD\ of\ daily \\ feed\ stock\ after \\ ozonation \\ (g/d) \end{pmatrix} x \begin{pmatrix} Operation\ duration \\ of\ reactors \\ (d) \end{pmatrix} = \begin{pmatrix} COD\ equivalent \\ of\ total\ methane \\ production \\ (g) \end{pmatrix}$$ Eqn. 5.1

Feed COD:

$$3400 \frac{mg}{L\ x\ d} x\ 0.1\ L\ x\ 27\ d = 9180\ mg\ COD$$

Methane produced in 1.33 mg Ozone flask from Figure 5.15 was 2000 mL:

$$2000\ mL \Big/ 25.4 \frac{mL}{mmole}\ x\ 64 \frac{mg}{mmole} = 5040\ mg\ CH4\ as\ COD$$

% COD converted into methane in 1.33 mg Ozone flask:

$$\frac{5040\ mg\ methane\ as\ COD}{9180\ mg\ COD}\ x\ 100 = 55\%$$

% COD converted into methane in 0.66 mg Ozone flask:

Methane produced in 0.66 mg Ozone flask from Figure 5.15 was 1200 mL;

$$1200\ mL \Big/ 25.4 \frac{mL}{mmole}\ x\ 64 \frac{mg}{mmole} = 3024\ mg\ CH4\ as\ COD$$

$$\frac{3024\ mg\ methane\ as\ COD}{9180\ mg\ COD}\ x\ 100 = 33\%$$

It can be concluded that 55 % of the feed sludge, in COD units, fed into the 1.33 mg Ozone reactor was converted into methane. Remaining 45 % COD was evidently converted into CO_2. This figure is rather low when compared to typical anaerobic reactor figures, where typically 70 % methane is recorded in the off gas in a good operating reactor. The low figure may be attributed to infinitive SRT employed in this reactor, since biomass produced was totally decayed due to long SRT. Whereas

in a typical reactor, such as the control reactor, SRT is set to 25 days; hence reactor is a faster reactor

However in spite of low methane percentage in the off gas, still the volume of methane produced is much greater in 1.33 mg Ozone reactor, as compared to the control reactor.

Cost Calculation of the Ozone Pre-treatment of Anaerobic Digestion

When applying ozone to feed stock, for the 2.65 mg ozone/g biomass dosed reactor, RAS was ozonated for 5 minutes. The duration was calculated using the calibration curve given in Figure 4.4. The other reactors receiving 1.33 and 0.65 mg ozone/g biomass feed stock were ozonated for 2.5 and 1.3 minutes, respectively. Again the ozonation durations were calculated according to calibration curve of ozone generator.

Electrical consumption of the ozone generator was given as 1.1 kWh/kg ozone in the manual. In total, 2.65 mg/g ozone dosed reactor received 173 mg ozone. Thus, the electrical consumption of ozonation was calculated as 0.2×10^{-3} kWh. The unit price of electricity is 0.65 TL/kWh. Therefore, cost of ozonation of 2.65 mg ozone/ g biomass ozone dosed reactor was 1.3×10^{-4} TL. It can be concluded that the cost of ozone pre-treatment can be neglected when other pre-treatment techniques such as chemical, mechanical, are considered.

CHAPTER 6

CONCLUSION

With more and more stringent regulations on sludge treatment and disposal, pursuits of new technologies and processes have become crucial. Nowadays, simply reduction of water content, heavy metals and microbiological content of sludge are not considered enough. Micro pollutants like EDCs in sludge are an important issue for disposal of sludge. Hence the EDC content of anaerobic sludge was analyzed in this study. Also, treatability of these compounds in sludge has been investigated. For this aim, ozone treatment of anaerobic sludge was considered. Anaerobic digester and belt filter sludge samples were ozonated for 3 different durations: 20, 30 and 45 minutes. However, at the end of these experiments no significant reduction in TSS or EDCs could be obtained. To improve treatability the waste sludge directly from the activated sludge process was treated with ozone prior to anaerobic digestion. For this reason, setting-up of semi-continuous anaerobic reactors was decided. Ozone was applied at three different doses to sludge being fed to the reactors and one reactor was set as the control reactor. Target EDCs were spiked at 20 ppb to the feed sludge and the removal of these compounds during anaerobic digestion were followed. It was seen that a significant treatment of EDCs have materialized in the reactors. Anaerobic reactor performance was also monitored by observing total gas production, methane percentage and TSS-VSS. At first, reactors were operated at 25 days SRT. However, upon realizing that TSS value in the reactor steadily declined at this SRT; SRT was set to infinity by stopping wastage from the reactors. This way a steady biomass level could be maintained in the reactors. Total gas production and percentage of methane in the off gas was substantially higher than the control reactor. There seems to be a direct proportionality between the ozone doses applied and total gas produced within the spectrum tested. At the end of the operation period for all the reactors, degradation of EDCs was achieved. In control reactor, this reduction was highest for acetaminophen which was degraded from 20.8 ppb to 9.7 ppb. Therefore anaerobic reactor itself, without any pre-treatment can degraded EDCs up to 50%. However, when ozone was applied as a pre-treatment technique to

anaerobic reactor, removal rates for EDCs increased dramatically. Natural hormones, for this study progesterone and estrone, treated with the highest degradation rate. Removal rates of other compounds, which were diltiazem, BBP, carbamazepine and acetaminophen, were close to each other. When the applied ozone dose increased, removal rates also increased. The difference in then end concentrations of EDCs in sludge shows the effect of ozonation of RAS prior to the anaerobic reactor.

In addition to the removal of EDCs in sludge, performance of anaerobic reactor was also investigated. The results of total gas production of the reactors showed that ozonation of RAS prior to anaerobic reactor increased the performance as well. Such that, control reactor produced approximately 1500 mL of gas during 27 days at 25 day-SRT. The ozonated reactors, on the other hand, produced approximately 2000, 3100 and 4000 mL of gas with ozone doses of 0.65, 1.33 and 2.65 mg/g, respectively. This huge difference in total gas productions can be attributed to the effect of ozone. Since the ozone is an oxidizing agent, it solubilizes the most of the constituents in sludge and makes it available for methanogenesis bacteria. Therefore, total gas production increases dramatically.

This study shows that anaerobic digestion of waste sludge is a good option for energy recovery and the treatment of emerging compounds when it is coupled with ozone pre-treatment. It was obvious that ozonation of the anaerobic reactors increased both the performance and the treatability of EDCs. Therefore, this technique might be an efficient modification for the existing anaerobic digesters; especially when and if a legislation for the emerging compounds are implemented.

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APPENDIX A

OPTIMIZATION OF LC (ESI) MS/MS

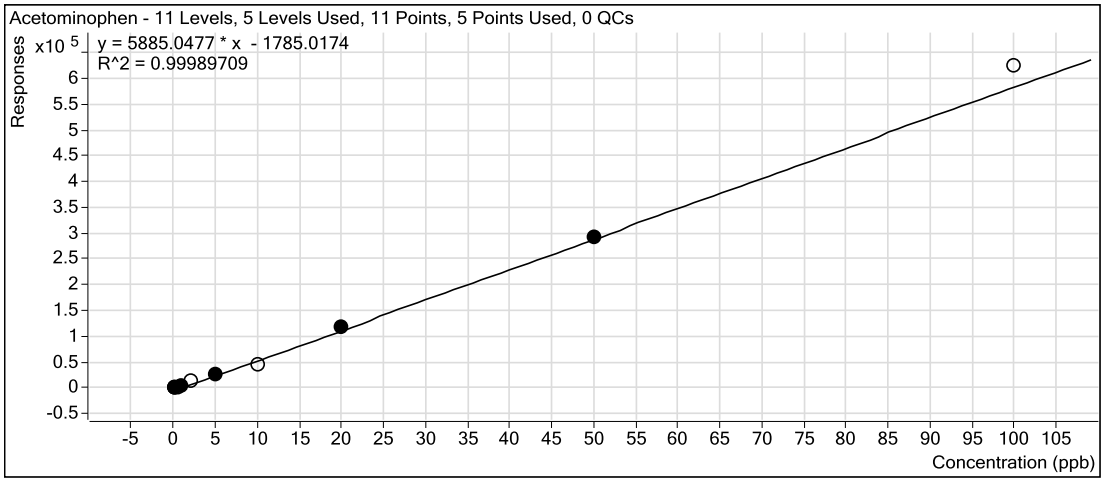

Figure 0.1 Calibration curve for acetaminophen

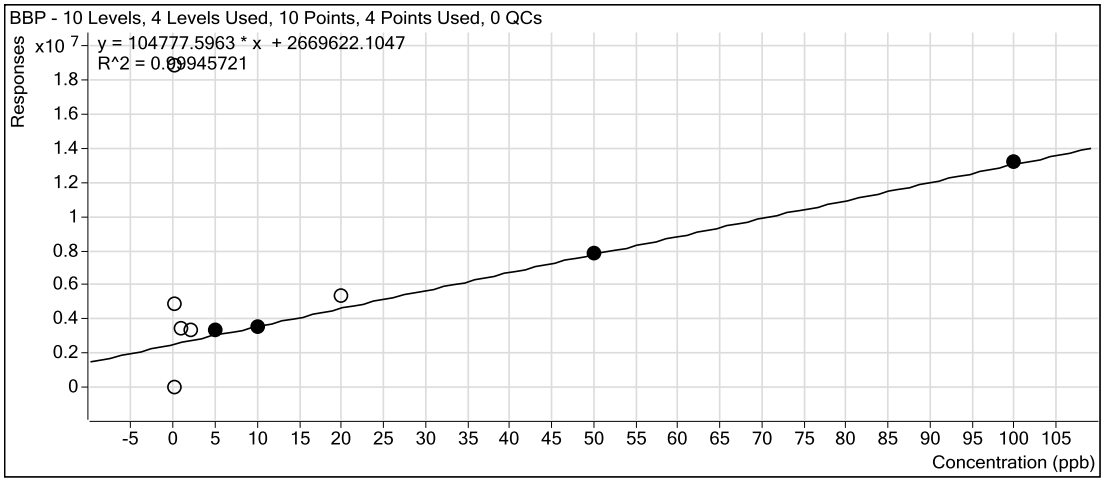

Figure 0.2 Calibration curve for BBP

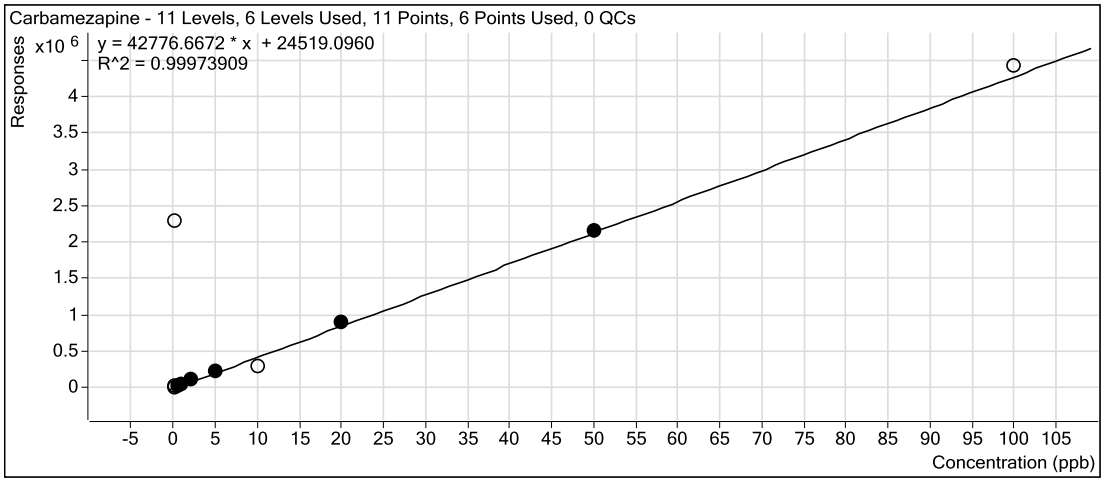

Figure 0.3 Calibration curve for carbamazepine

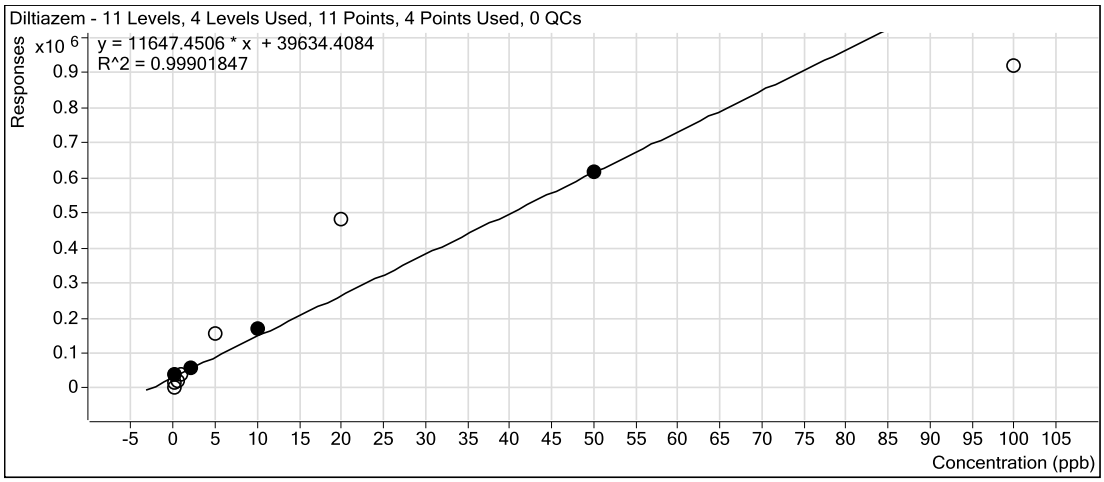

Figure 0.4 Calibration curve for diltiazem

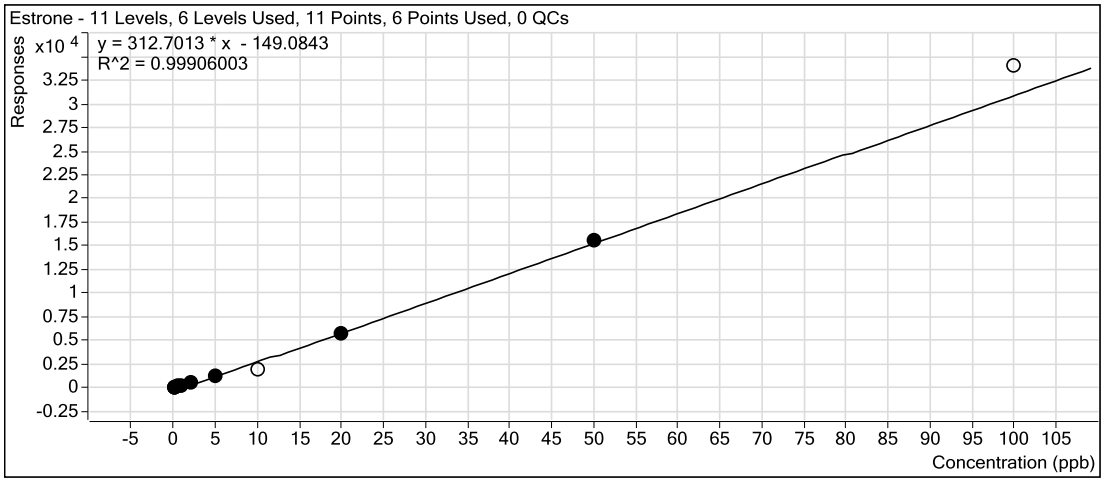

Figure 0.5 Calibration curve for estrone

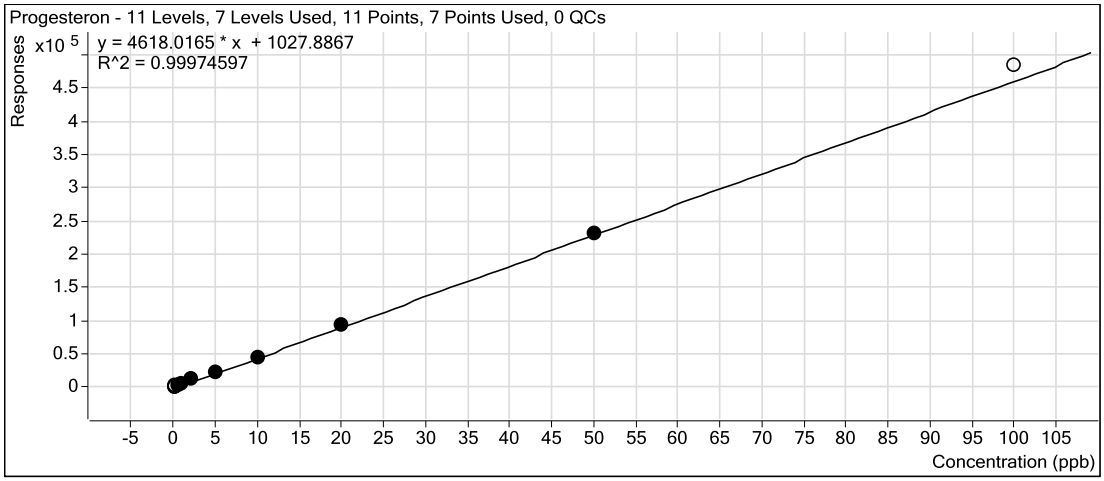

Figure 0.6 Calibration curve for progesterone

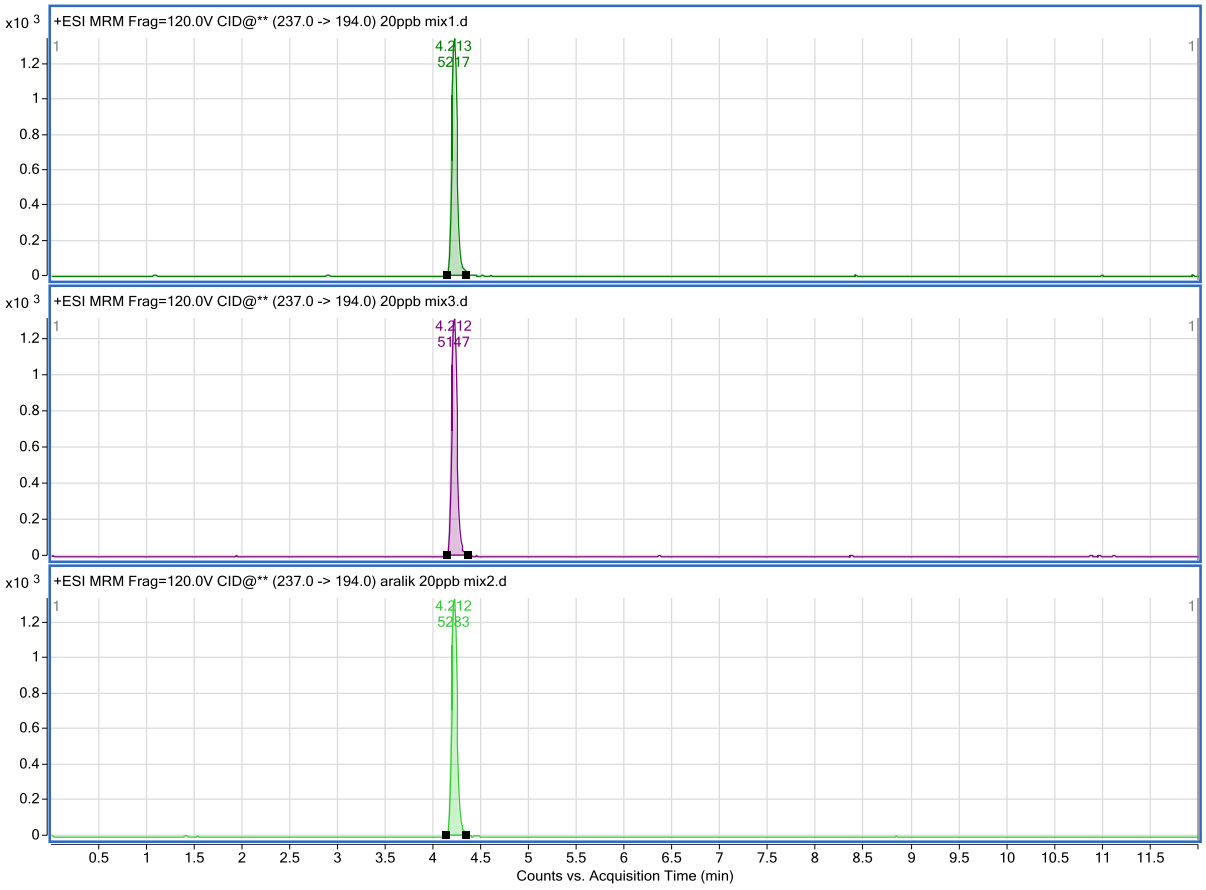

Figure 0.7 Sample chromatogram of standard injection at 20 ppb concentration

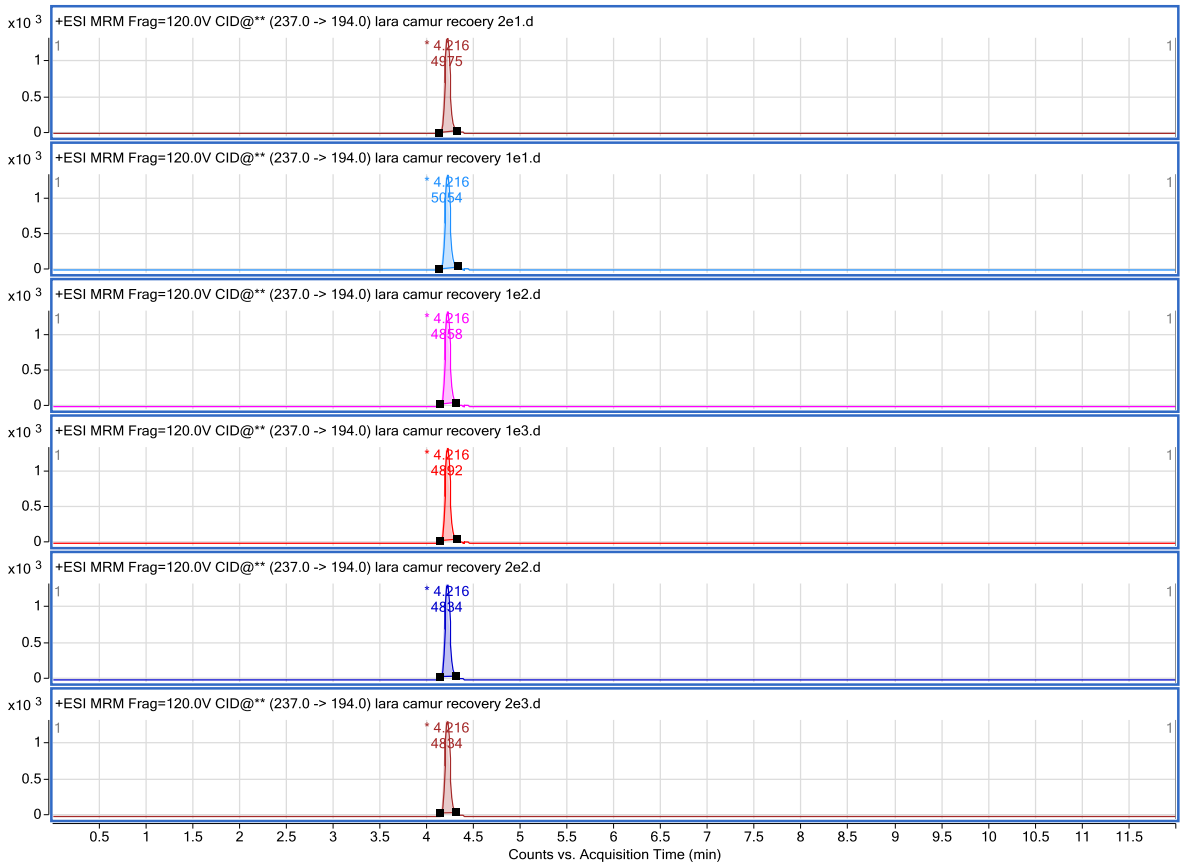

Figure 0.8 Sample chromatogram of 20 ppb compound injected sludge after sludge extraction

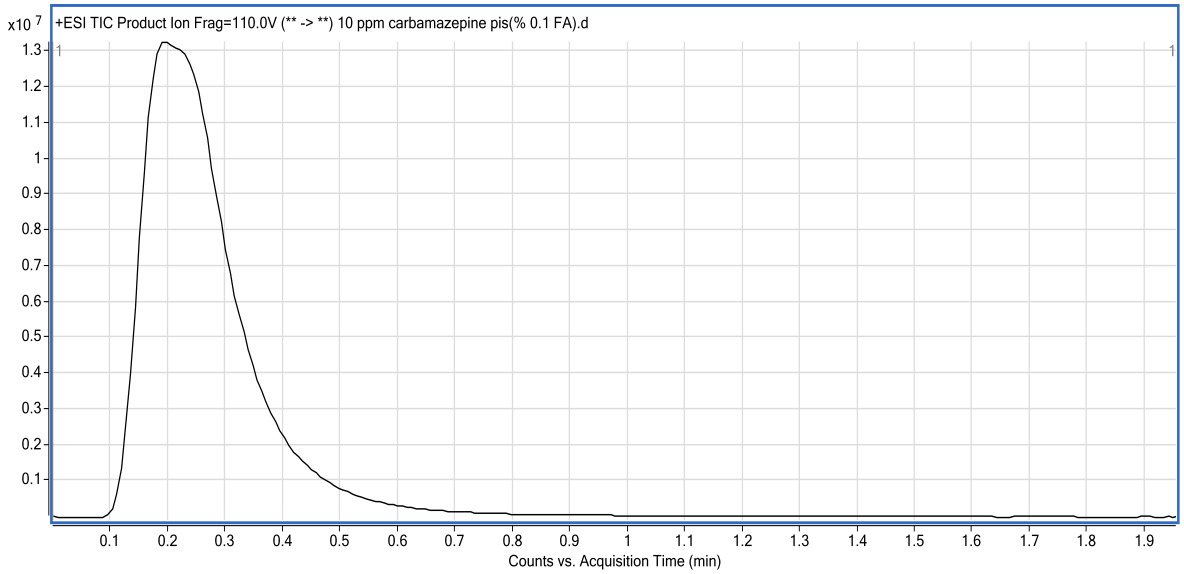

Figure 0.9 Signal of carbamazepine in mobile phase with 0.1% FA

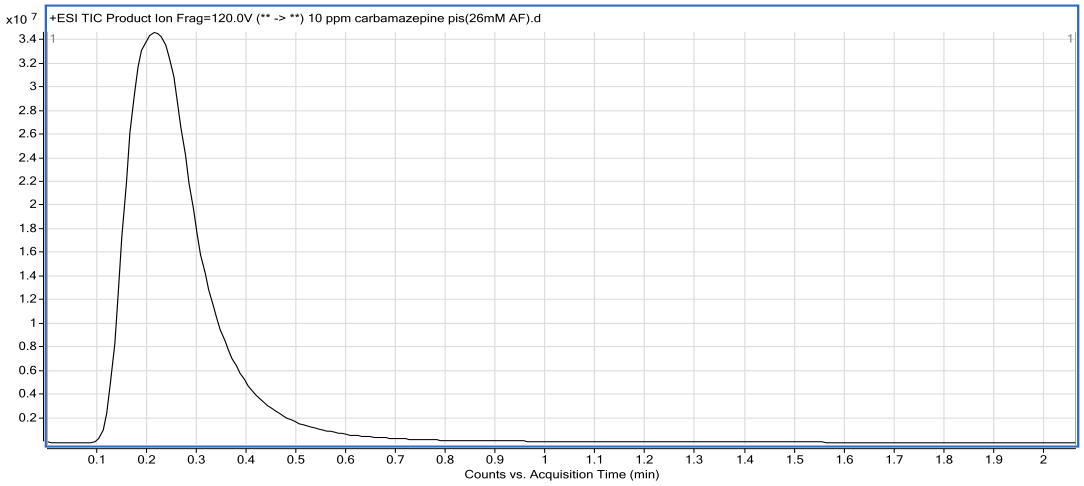

Figure 0.10 Signal of carbamazepine in mobile phase with 26 mM A.F.

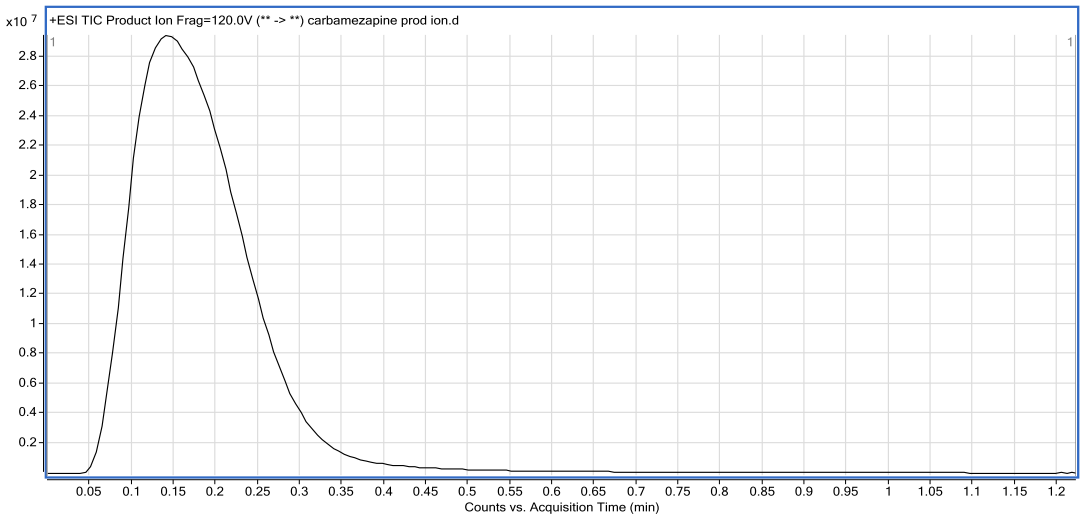

Figure 0.11 Signal of carbamazepine in mobile phase with 0.1% F.A. AND 5mM A.F.

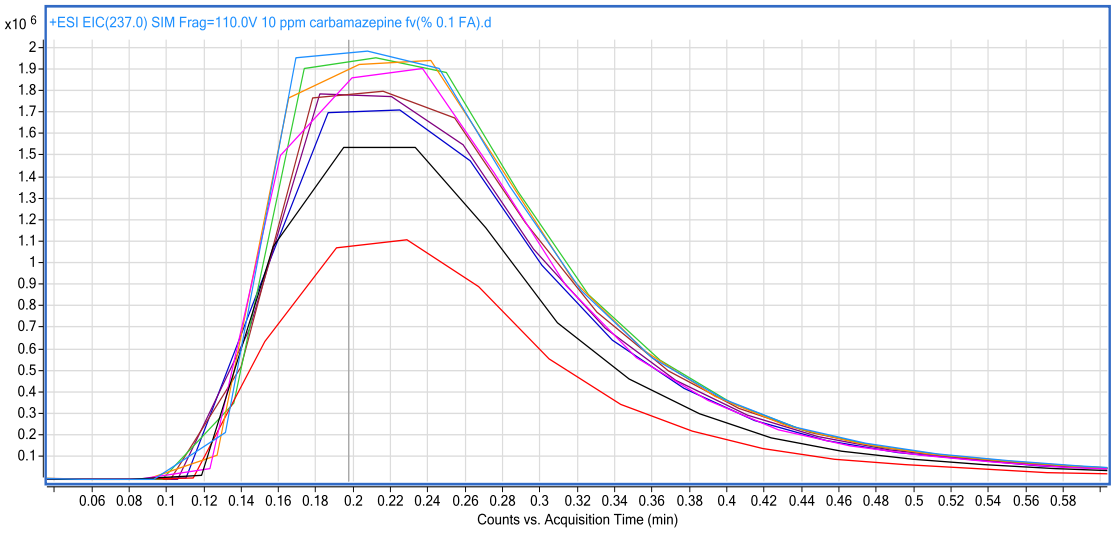

Figure 0.12 Signals of carbamazepine at different fragmantor voltages in mobile phase with 0.1% F.A.

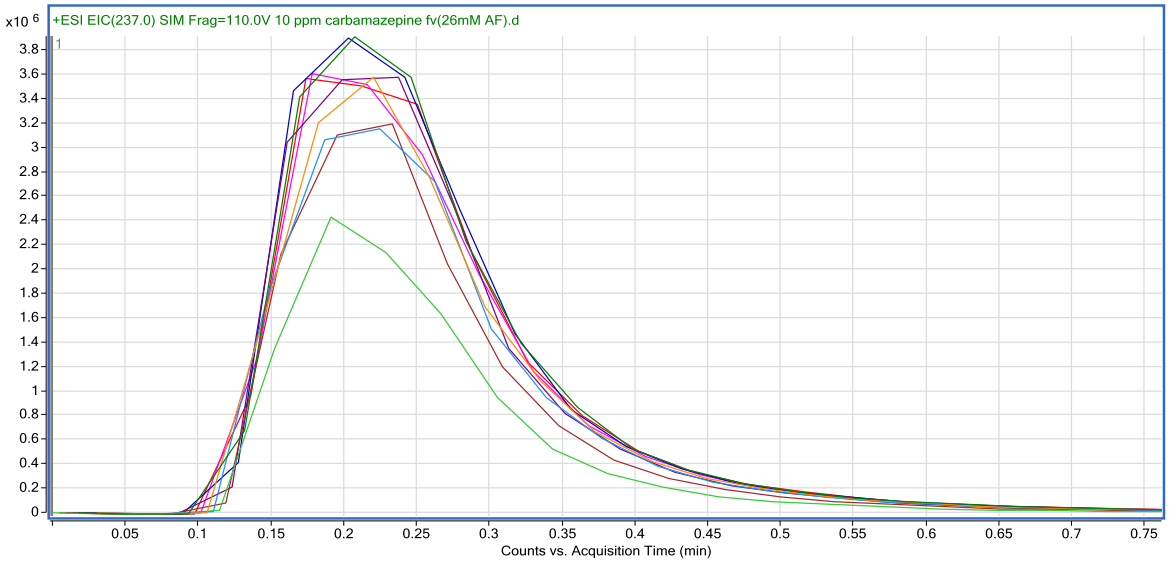

Figure 0.13 Signals of carbamazepine at different fragmantor voltages in mobile phase with 26mM A.F.

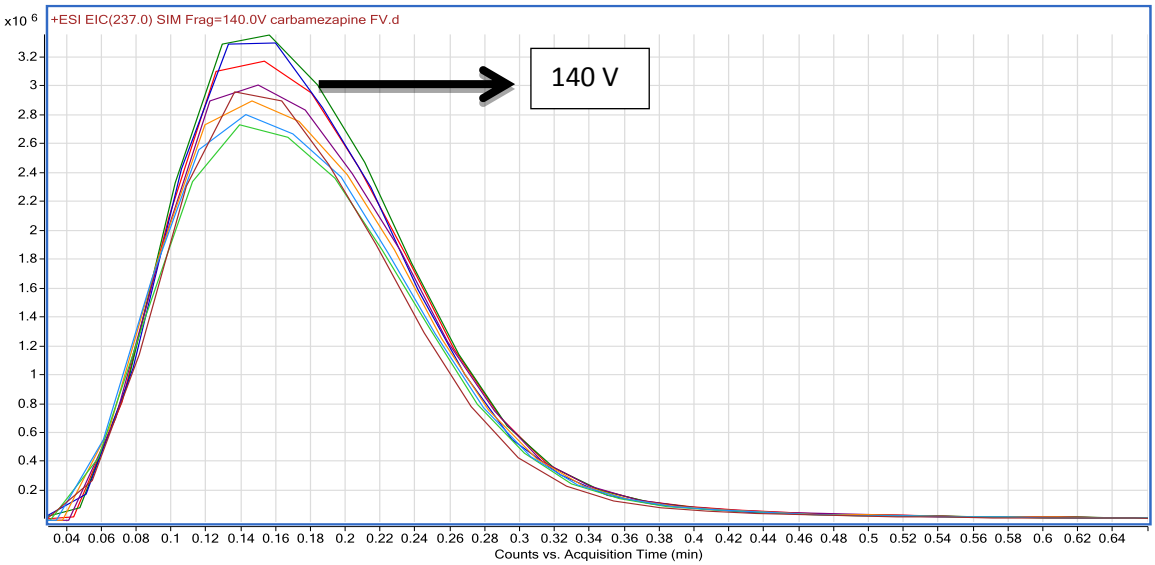

Figure 0.14 Signals of carbamazepine at different fragmantor voltages in mobile phase with 0.1% F.A. and 5mM A.F.