EFFECTS OF MICROWAVE, H_2O_2, $S_2O_8^{2-}$, MW/H_2O_2 AND MW/$S_2O_8^{2-}$ PRE-TREATMENTS ON BIOCHEMICAL METHANE PRODUCTION POTENTIAL OF THE SEWAGE SLUDGES

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To my mom, dad and sister,

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EFFECTS OF MICROWAVE, H_2O_2, $S_2O_8^{2-}$, MW/H_2O_2 AND MW/$S_2O_8^{2-}$ PRE-TREATMENTS ON BIOCHEMICAL METHANE PRODUCTION POTENTIAL OF THE SEWAGE SLUDGES

The considerable increase in the sewage sludge production in wastewater treatment plants becomes a serious problem for a modern society. The treatment of excess sewage sludge is a significant issue. Many different methods and pre-treatment techniques can be applied for the stabilization of the sludge and enhancement of energy production from the sludge. This study investigated the effects of sludge pre-treatments (microwave (MW), H_2O_2, $S_2O_8^{2-}$, MW/H_2O_2 and MW/$S_2O_8^{2-}$) and also the presence of antibiotics on anaerobic stabilization and biochemical methane production potential of sewage sludges.

The pre-treatments applied to sludge samples prior to anaerobic digestion speeded up the hydrolysis step and improved the biodegradability of the organics by increasing their solubility. Application of MW, H_2O_2 and combined MW/H_2O_2 (1 g H_2O_2/g TS (total solids)) pre-treatments increased the methane yields by 65.5%, 20% and 40%, providing 626 mL CH4/g VS, 453 mL CH4/g VS and 529 mL CH4/g VS methane yields, respectively. However, persulfate pre-treatment (1 g $S_2O_8^{2-}$/g TS) decreased the biogas production and eliminated the methane production due to inhibiting effect of the $S_2O_8^{2-}$ dose on the survival of the methanogenic bacteria.

The presence of antibiotics (1 mg CIP/g TS) in sewage sludge samples decreased methane yields in the anaerobic digestion process. The methane yield, obtained in antibiotic contaminated sludge containing reactor, decreased 22% to 275 mL CH_4/g VS, while uncontaminated sludge containing control reactor had a methane yield of 352 mL CH_4/g VS. The presence of antibiotic in sewage sludge did not show negative effect on the sludge stabilization.

MİKRODALGA, H_2O_2, $S_2O_8^{2-}$, MD/H_2O_2 VE MD/$S_2O_8^{2-}$ ÖN-ARITIM METODLARININ BİYOKİMYASAL METAN ÜRETİM POTANSİYELİ ÜZERİNDEKİ ETKİLERİ

Atıksu arıtma tesislerinde toplanan arıtma çamuru miktarındaki önemli artış modern bir toplum için ciddi bir problem oluşturmaktadır. Fazla atık çamurun arıtılması önemli bir konudur. Çamur stabilizasyonunun ve çamurdan enerji üretiminin arttırılması için birçok farklı ön arıtma yöntemi uygulanabilir. Bu çalışmada çamur ön arıtımlarının (mikrodalga (MD), H_2O_2, $S_2O_8^{2-}$, MD/H_2O_2 and MD/$S_2O_8^{2-}$) ve ayrıca antibiyotik varlığının arıtma çamurlarının anaerobik stabilizasyonu ve biyokimyasal metan üretim potansiyeli üzerindeki etkileri araştırılmıştır.

Anaerobik arıtmadan önce çamur numunelerine uygulanan ön işlemler, hidroliz aşamasını hızlandırmış ve çamurdaki organik bileşiklerin çözünürlükleri artırarak biyolojik olarak parçalanabilirliklerini iyileştirmiştir. MD, H_2O_2 ve kombine MD/H_2O_2 (1 g H_2O_2/g TKM (toplam katı madde)) ön-arıtım uygulamaları, sırasıyla 626 mL CH_4/g UKM, 453 mL CH_4/g UKM ve 529 mL CH_4/g UKM metan üretim verimi sağlayarak, metan verimleri % 65.5, % 20 ve % 40 oranlarında arttırmıştır. Bununla birlikte, persülfat ön-arıtımları, $S_2O_8^{2-}$ dozunun (1 g $S_2O_8^{2-}$/g TKM) metanojenik bakterilerin hayatta kalması üzerindeki inhibe edici etkisinden dolayı biyogaz üretimini azaltmış ve metan üretimini ortadan kaldırmıştır.

Arıtma çamuru numunelerinde antibiyotik varlığı (1mg CIP/g TKM) anaerobik arıtma sürecindeki metan üretim verimini azaltmıştır. Antibiyotikle kirletilmemiş çamur içeren kontrol reaktörünün metan verimi 352 mL CH_4/g UKM (uçucu katı madde) iken, antibiyotikle kirlenmiş çamur içeren reaktörde elde edilen metan verimi %22 azalarak 275 mL CH_4/g UKM'ye düşmüştür. Arıtma çamurunda antibiyotik varlığı çamur stabilizasyonu üzerinde olumsuz bir etki göstermemiştir.

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LIST OF SYMBOLS/ABBREVIATIONS

Abbreviation	Explanation	Unit
AD	Anaerobic Digestion	
CH_4	Methane	mL/day
CIP	Ciprofloxacin	mg/g
COD	Chemical Oxygen Demand	mg/L
CST	Capillary Suction Time	seconds
GC	Gas Composition	
H_2O_2	Hydrogen Peroxide	
KHP	Potassium Hydrogen Phthalate	
MW	Microwave Irradiation	
MW/H_2O_2	Combined Microwave/H_2O_2 pretreatment	
MW/$S_2O_8^{2-}$	Combined Microwave/$S_2O_8^{2-}$ pretreatment	
NH_4-N	Ammonium Nitrogen	mg/L
NO_3^--N	Nitrite Nitrogen	mg/L
NO_2-N	Nitrate Nitrogen	mg/L
PO_4^{3-}	Phosphate	mg/L
$S_2O_8^{2-}$	Persulfate	
sCOD	Soluble Chemical Oxygen Demand	mg/L
SO_4^{2-}	Sulfate	mg/L
SS	Sewage Sludge	
TOC	Total Organic Carbon	mg/L
TKN	Total Kjeldahl Nitrogen	mg/L
TP	Total Phosphorous	mg/L
TS	Total Solids	g/L
TSS	Total Suspended Solids	g/L
VFA	Volatile Fatty Acids	mg/L
VS	Volatile Solids	g/L
VSS	Volatile Suspended Solids	g/L
WWTP	Wastewater Treatment Plant	

1. INTRODUCTION

The considerable increase in the amount of sewage sludge production in wastewater treatment plants (WWTP) becomes a serious problem for the healthy development of the cities. The amount of disposed sewage sludge should be reduced with the application of various treatment and pre-treatment methods. The wastewater sludge must be stabilized sufficiently to reduce their organic content so that it can be safely disposed of without causing odor problems and/or pathogen contamination.

Anaerobic digestion (AD) has been used to treat waste activated sludge by providing a reduction in mass and volume of input sludge materials including high organic loads. Anaerobic digestion has attractive benefits both on sludge stabilization and energy production from biomass in form of biogas; it makes the sludge harmless and the resources reusable (Phothilangka, 2008; Ahn et al., 2009; Weiland, 2010). Anaerobic digestion of sludges can be improved by the application of various physical (thermal, mechanical, ultrasonic, microwave), chemical (alkaline, hydrogen peroxide, ozone oxidation), and biological (enzymatic) pre-treatment methods or the combination of any two of these methods to the sludges. These pre-treatments increase the biodegradability of sludge and increase the efficiency of anaerobic digestion, and so the methane production.

There are number of studies and research activities dealing with the determination of the biogas potential of solid organic substrates which is an important parameter affecting the design and economic details of a biogas plant, to some extent (Angelidaki et al., 2009; Weiland, 2010; Climent et al., 2007).

In sludge treatment, there are important pollutants that should be considered, like antibiotics. Antibiotics are the organic micropollutants placed in the wastewater sludges. The treatment of these antibacterial micropollutants is significant since they spread into the environment in an uncontrolled way. Increasing concentrations of

antibiotics in water bodies create antibacterial resistance in microorganisms and may cause to secondary pollution.

Antibiotics can be removed from the waste water and waste water sludge with the application of various methods such as; UV irradiation, ozonation, chlorination, advanced oxidation techniques etc. The advanced oxidation processes not only degrade the antibiotics in sludge, but also used as pre-treatment method to solubilize the sludge solids and enhance their biodegradability. Ozonation is very effective advanced oxidation method for the degredation of antibiotics and solubilization of sludge (Oncu and Balcioglu, 2013). However, ozonation is expensive and this limits its large scale applications. Many other effective oxidants can be used for the antibiotic removal with the advance oxidation.

Hydrogen peroxide (H_2O_2) and persulfate ($S_2O_8^{2-}$) have strong oxidative power and they are used for the degradation of antibiotics and disintegration of sludge solids (Bilgin Oncu and Akmehmet Balcioglu, 2013). There are only a few studies on the use of persulfate in the degradation of antibiotics and in the disintegration of sewage sludges and investigation of their biogas production potential.

This study investigated the effects of hydrogen peroxide (H_2O_2) and persulfate ($S_2O_8^{2-}$) pre-treatments, applied for degradation of antibiotics, on the biochemical methane production potential and on anaerobic stabilization of sewage sludges. In the study, peroxide and persulfate pre-treatments were also applied to sludge samples combined with microwave irradiation (MW/H_2O_2 and MW/$S_2O_8^{2-}$) to see the effect of combined pre-treatments. Moreover, the effects of antibiotics presence on anaerobic stabilization and the potential of the biochemical methane production of the sewage sludges were investigated.

2. LITERATURE REVIEW

2.1. Sludge Pre-treatments

Wastewater sludges are pretreated for different purposes like sludge minimization, increasing biodegrability of sludge, increasing biogas production and removal of some micropollutants.

Various pre-treatment techniques are applied to wastewater sludges to enhance their digestion efficiency. These processes are mechanical (such as ultrasound, high pressure and cell lysis), thermal (microwave), chemical (alkali, advanced (hydrogen peroxide, ozone) oxidation) and biological (enzymatic) pre-treatments. Although pre-treatment processes increase the overall cost of operation, they can provide beneficial effects on digested sludge quality (Ariunbaatar et al., 2014)

Mechanical disintegration processes of sludge are mainly relying on the disruption of the microbial cell walls by the help of shear stress. The shear stress of the solids tenses and deforms the cell wall, and so causes the cell lysis. To create the cell lysis, the strength of the applied stress should be higher than the strength of the cell wall (Neyens and Baeyens, 2003).

Ultrasonic pre-treatment is one of the treatment alternatives for sludge disintegration. It is an energy-intensive process. Ultrasound is used to enhance the hydrolysis and solubilization of complex substrates preceding their digestion anaerobically (Bougrier et al., 2005; Tiehm et al., 1997). Ultrasonic pre-treatment comprises of the use of cyclic sound pressure with a variable frequency to wastes for the disintegration of complex and rigid structures. Microbubbles are formed during sonication, due to high-pressure applied to liquid. These microbubbles create violent collapses and high rates of energy release into a small area. As a result of the extreme conditions, certain radicals (·HO, ·H) can be generated, and they can disintegrate volatile compounds with the pyrolysis (Rincón et al., 2014).

Alkaline pre-treatment is advantageous since, it needs simple devices and it has convenient and highly efficient operation (Cassini et al., 2006). Sodium hydroxide (NaOH) is really effective even if it is used in low dosages and applied at ambient temperatures for the disintegration of the municipal waste activated sludge (Neyens et al., 2004).

With the help of hydroxyl anions, alkaline destructs the cell walls and the structures of flocs. Natural shape of proteins loses, saponification of lipid and hydrolysis of RNA occurred at excessively high pH (Wonglertarak and Wichitsathian, 2014).

In an alkaline pre-treatment study, treatment under thermophilic anaerobic condition removed 42.16% of TS, 43.15% of VS and 50.64% of COD, and provided higher gas production. With the effect of thermophilic and ambient conditions on anaerobic digestion, the alkaline thermophilic anaerobic reactors showed higher organic matter removal rate and gas production (Wonglertarak and Wichitsathian, 2014).

Pre-treatments can be applied to the different industries' sludge such as pulp and paper sludge like in the study of Lin et al. (2009). To evaluate the methane productivity in anaerobic digestion of pulp and paper sludge (PPS) an alkali pre-treatment process is done by using different concentrations of NaOH solution prior to AD in 42 days on 37 °C. The optimal amount of sodium hydroxide for organics solubilization was 8 g NaOH/100 g TS sludge. Under this optimal pre-treatment condition, methane productivity increased as 83% at a lower cost compared with other pre-treatments. SCOD increased up to 83% as well as the VFA concentration showed a peak value of 1040 mg acetic acid/L during AD. The results indicated that NaOH pre-treatment could be an effective method for improving methane yield with PPS (Lin et al., 2009).

Thermal hydrolysis includes high temperature thermal pre-treatment (>100°C) and low temperature thermal pre-treatment (<100°C). Most studies worked on high temperature thermal pre-treatment since, the higher temperature gives more

efficient treatment. However, when the temperature is higher than 180°C, the production of recalcitrant soluble organics or toxic/inhibitory intermediates occurs and these intermediates reduce the biodegradability (Wilson and Novak, 2009).

There are also some combined sludge pre-treatment applications like alkaline-thermal pre-treatment. For example, the combination of the low-temperature thermal pre-treatment and alkaline treatment achieves better treatment efficiency, because they have different mechanisms of sludge dissolution; their combination has the advantages of both methods (Yi et al., 2013).

In last years advanced oxidation techniques gain importance. Hydrogen peroxide (H_2O_2) and ozone (O_3) applications to the sludge have been studied by many researchers. Although, ozone is a very powerful oxidant, ozonation is an expensive process limiting its large scale application. In recent years, some other chemicals are used like persulfate ($S_2O_8^{2-}$) and peroxide (H_2O_2).

2.1.1. Microwave (MW) Pre-treatment

The microwave region of the electromagnetic spectrum corresponds to the wavelength of 1 mm to 1m with frequencies of 300 GHz to 300 MHz, respectively. Residential and industrial microwaves generally operate at a wavelength of 12.2 cm which corresponds to 2.45 GHz and energy of 1.02×10^{-5} eV. Materials can be heated with the energy of the microwaves by applying high frequency electromagnetic waves (Jacob et al., 1995).

Microwaves can induce a thermal effect depending on the dipole orientation that leads to possible breakage of hydrogen bonds and the disintegration of the floc matrix. After microwave treatment, an important release of organic components occurs such as soluble COD, soluble proteins and soluble carbohydrates. The COD concentration, proteins and carbohydrates in the liquid phase of the sludge increases. The temperature increase in microwave irradiation leads to the breakage of chemical bonds and denaturation of proteins and causes the soluble organic components release into the liquid phase of the sludge (Eskicioglu et al., 2007). MW-

irradiation created greater damage on microbial cells at similar applied temperatures compared to conventional heating. Disruption of the chemical bonds in the cell walls and membranes causes denaturation and destruction of the microbial cells.

Microwave irradiation provides heating of the materials by absorbing microwave energy with changing the molecules' vibrant energy. In other saying, the electric field generated by microwave irradiation and the polarizations of the dipoles in a polar solvent cause the friction that generates heat.

In addition, the use of microwave irradiation has a sterilization effect which is useful for further utilization. Furthermore, microwave irradiation related with hydrothermal systems has been studied for the growth of particles with different structural and morphological properties (Yu et al., 2010).

Microwaves enhance the dewatering of sludge and the biogas production of the waste activated sludge with the anaerobic digestion. MW disintegration mechanism destroys cell bacteria. Microwave irradiation damages the cell membranes when the waves introduced into the bacteria cells. As the cell membranes are destroyed, DNA of the bacteria is damaged and so, bacterial activity decreases (Phothilangka, 2008).

2.1.2. Hydrogen Peroxide (H_2O_2) and Combined MW/H_2O_2 Pre-treatments

Hydrogen peroxide is the simplest peroxide and it has strong oxidative power. Hydrogen peroxide can be used as a disinfectant or bleaching agent, and also used in the water treatment applications. The disinfection steps takes place through the oxidation of hydrogen peroxide to highly reactive hydroxyl radicals.

$$H_2O_2 \rightarrow OH^- + OH^{\bullet} \qquad (2.1)$$

In the presence of catalysts, the reaction occurs rapidly in few minutes. However, the degradation can also occur without a catalyst slowly (Hannmann et al., 2012).

In the study of Kim et al. (2009), hydrogen peroxide oxidation was used for the reduction of activated sludge and the efficiency of hydrogen peroxide oxidation improved by applying alkaline pre-treatment method. The radicals of the hydrogen peroxide destruct the cell wall of microorganism, thus, provide solids and particle size reduction, and sCOD generation. In the experiment, different concentrations of hydrogen peroxide (35%) were applied at different pHs. The 1.6 M H_2O_2 dose at pH 11 was resulted 33% removal of total solids and 53.1% increase in sCOD/TCOD ratio. It was understood from the study that the H_2O_2 oxidation combined with alkaline pre-treatment improved the sludge reduction. Hydrogen peroxide was successful in the biodegradation of the sludge (Kim et al., 2009)

Hydrogen peroxide pre-treatment process was applied to increase the biodegradation performance and biogas yield of rice straw, in the study of Song et al. (2013). The rice straw was pretreated with a H_2O_2 concentration of 3% (w/w total solid), a total solid (TS) content of 5.1%, a pre-treatment time of 6 day, and substrate to inoculum ratio of 1:1, and then anaerobically digested. As a result, a methane yield of 290 mL/g VS, which was 88% higher than the untreated rice straw, was obtained. It was seen that the H_2O_2 pre-treatment of rice straw improved the methane yields during biogas production (Song et al., 2013).

Wong et al. (2006) focused the effects of combination of microwave heating and hydrogen peroxide on the advanced oxidation process (MW/H_2O_2–AOP) for the sludge treatment. This study showed that all of the COD was solubilized at temperatures above 80^oC by the MW/H_2O_2–AOP combined treatment method. Different concentrations of H_2O_2 (30 wt. %) and temperatures were carried out. With the MW temperature at 80^oC and the H_2O_2 concentration of 2 mL (30 wt. %) for 30 mL of undiluted sludge, having TS concentration of 0.35 – 0.40%, 100% of the COD was converted into the soluble form. That result showed that the MW/H_2O_2–AOP method improved the sludge biodegradation and could create an efficient pre-treatment method before anaerobic digestion for methane production. The MW/H_2O_2–AOP enhanced the sterilization of sludge by converting the all of the organic matters of sludge into soluble form. Likewise the COD, the phosphorus converted into the ortho-phosphate substantially. The MW/H_2O_2–AOP composed a

sludge management option by improving solubilization and also methane production (Wong et al., 2006).

In the study of Yin et al. (2007), microwave combined advanced oxidation processes (MW-AOP); such as MW/O_3, MW/H_2O_2 and MW/H_2O_2/O_3 were performed at 100^oC to see the efficiencies of the combination of oxidation processes. MW alone was set as the control. 1 mL of H_2O_2 (30 wt. %) was added to the 29 mL of sludge, having a TS concentration of 2.93%, at ambient temperature and the reaction time was 4 hour. For microwave combined peroxide (MW/H_2O_2) treatment, 1 mL of H_2O_2 was added to the 29 mL of sludge and microwaved at 100°C for 3 minutes. The results showed that both oxidation process could reduce suspended solids and release nutrients from sewage sludge. Best results were provided by the MW/H2O2/O3-AOP with the use of both hydrogen peroxide and ozone. More than 30% of TP, 20% of TKN and 37% of total COD were released into the solution. The second good option was the MW/H_2O_2-AOP. It showed really closed results to the MW/H_2O_2/O_3-AOP (Yin et al., 2007).

The H_2O_2 is usually applied together with the Fenton in many studies. The study of Neyens et al. (2003) the catalytic activation of H_2O_2 by Fenton (iron salts) was investigated. H_2O_2 oxidation was performed as 25 g H_2O_2/kg DS (dry solids) in the presence of 1.67 g Fe^{2+} ions/kg DS at pH 3 and at ambient temperature and pressure. The dry solids concentration of the untreated sludge was 6% DS (1.048 kg/L). That application enhanced the sludge reduction and the product quality. The reduction of DS of the sludge cake was increased to 47%, when the traditional sludge dewatering facility having a reduction of 20–25%. The dewaterability was enhanced with a 60% reduction of the sludge volume, the DS content of the sludge cake was enhanced 20% and the CST was reduced approximately 20 seconds as compared with ‘blank’ sludge sample (Neyens et al., 2003).

2.1.3. Persulfate ($S_2O_8^{2-}$) and Combined MW/$S_2O_8^{2-}$ Pre-treatments

The persulfate ($S_2O_8^{2-}$) is a strong oxidant, and it can be mostly used for swimming pools cleaning, hair bleaching, and as the initiative for polymerization

reactions. The persulfate used for the environmental purposes is obtained from the persulfate salts. The sodium form of the salts, sodium persulfate ($Na_2S_2O_8$), is the most commonly used one. It is a white crystalline solid and a reactive oxidant. It can be used for the degradation of organic compounds in contaminated soil and groundwater (Block et al., 2004).

The persulfate anion is the one of the strongest oxidant of the peroxygen family, and that is more stable than hydrogen peroxide and ozone. The standard reaction of the persulfate oxidation is below:

$$S_2O_8^{2-} + 2H^+ + 2e^- \rightarrow HSO_4^- \qquad (2.2)$$

In the presence of catalysts, the persulfate can form sulfate free radicals rapidly. The unpaired electrons occurred during the oxidation, creates a strong oxidizing agent (Hannmann et al., 2012).

The study of Bilgin Oncu and Akmehmet Balcioglu (2013) conducted combine MW irradiation with chemical oxidation for the degradation antibiotics in sewage sludge. Hydrogen peroxide and persulfate with MW were used for the antibiotics removal. Microwave-assisted hydrogen peroxide (MW/H_2O_2) treatment and microwave-assisted persulfate ($MW/S_2O_8^{2-}$) treatment of biological sewage sludge were compared in terms of antibiotic removal and sludge degradation. Persulfate anion ($S_2O_8^{2-}$) and hydrogen peroxide produce reactive sulfate ($SO_4^{\bullet-}$) and hydroxyl ($OH\bullet$) radicals. The generation of radicals from these oxidants can results in the oxidation of reduced species shown in equations below.

$$H_2O_2 + \text{MW irradiation} \rightarrow 2OH^{\bullet} \qquad (2.3)$$

$$S_2O_8^{2-} + \text{MW irradiation} \rightarrow 2SO_4^{\bullet-} \qquad (2.4)$$

$$H_2O_2 + Metals^{n+} \rightarrow 2OH^{\bullet} + Metals^{n+2} \qquad (2.5)$$

$$S_2O_8^{2-} + Me^{n+} \rightarrow SO_4^{\bullet-} + SO_4^{2-} + Me^{n+2} \qquad (2.6)$$

With the 1.2 g H_2O_2/g TS and 0.87 g $S_2O_8^{2-}$/g TS at 160°C for 15 min. in MW, (the total solid concentration of the sludge was adjusted to 10.0 g/L) both MW/H_2O_2

and MW/$S_2O_8^{2-}$ treatments provide more than 97% degradation of antibiotic. MW/$S_2O_8^{2-}$ provided 48% more metal solubilisation, 2 times higher increase in dewaterability, solubilisation of ammonia to nitrate in a shorter treatment period, lower TCOD degradation, and also it was more effective for the antibiotic degradation (CIP) relative to MW/H_2O_2 (Bilgin Oncu and Akmehmet Balcioglu, 2013).

The study of Bilgin Oncu et al. (2015) investigated persulfate oxidation effect for the antimicrobial micro pollutants degradation. The oxytetracycline (OTC), ciprofloxacin (CIP) and triclosan (TCS) antibiotics were used as the micro pollutants in the study. The concentrations of the antibiotics were adjusted to 1 mg/L (100 mg/kg TS in the sludge). The $S_2O_8^{2-}$ concentrations were 8.0, 15.4, 22.7 mM. The $S_2O_8^{2-}$ oxidation improved solubilisation of organic carbon (SCOD/TCOD). The highest dose of the $S_2O_8^{2-}$ gave the best results on the organic carbon solubilisation as 24% (Bilgin Oncu et al., 2015).

In the article of Zhang et al (2010); microwave assisted hydrogen peroxide (H_2O_2), peroxymonosulfate (PMS) and persulfate (PS) were used for the removal of COD from the landfill leachate. The study was conducted by changing the oxidant dosages and chloride concentrations. At the end of study, the organic materials in leachate were degraded by MW-assisted peroxides effectively. By using an oxidant concentration of 0.3 mol/L, COD removals of 47.5%, 64%, 30.8% were provided by H_2O_2, PMS and PS, respectively. When the MW irradiation applied together with these pre-treatments; 43.5%, 80.2%, 97.3% of COD removals with 0.3 mol/L oxidant concentration were provided with the MW-assisted H_2O_2, PMS and PS, respectively. Therefore, it can be seen that MW is a really effective way for the enhancement of persulfate activation (Zhang et al., 2010).

The study of Sun et al. (2012) investigated the effect of sulfate radical ($SO_4^{-\bullet}$) pre-treatment on mesophilic anaerobic digestion of sewage sludge. A persulfate concentration of 0.1 g $K_2S_2O_8$/g SS was applied to the sludge, having a SS concentration of 25.8 g/L, at 90^oC water bath for 90min. Then, the sludges were anaerobically digested in batch reactors at 35^oC. At the end of the AD, the removal

rate of TCOD was increased by 11.5% and the concentration of VSS was decreased by 6.9%. VFA concentrations increased together with alkalinity, and so the system stayed stable. The pre-treatment improved the methane yield to 44.9% over the control. The results showed that $SO_4^{-\bullet}$ pre-treatment enhanced the performance of the AD and the methane yield (Sun et al., 2012).

In the study of Song et al. (2016a), the effect of persulfate and zero valent iron (ZVI) on the dewaterability of the anaerobically digested sludge (ADS) was investigated. Combinations of the different concentrations of $S_2O_8^{2-}$ (0 - 1.0 g/g TS) and ZVI (0 - 4.0 g/g TS) at neutral pH were applied on ADS. The TS concentration of ADS was 28.5 g/L. The dewaterability of ADS was measured by capillary suction timer (CST). The dewaterability was highly enhanced with the presence of persulfate. The best results were obtained with at 2.0 g ZVI/g TS and 0.5 g $S_2O_8^{2-}$/g TS with a reduction percentage of 90% in CST. By increasing the concentration of $S_2O_8^{2-}$ from 0 to 0.5 g/g TS, the reduction was improved from 20% to 90%. The combined persulfate-ZVI treatment provided a high efficiency in the improvement of ADS dewaterability (Song et al., 2016a).

There are some other studies investigated the persulfate effects on the dewaterability of wastewater sludges (Zhen et al., 2012; Zhou et al., 2015; Lee et al., 2016; Kim et al., 2016). All these studies show that persulfate treatment improves the dewaterability.

2.2. Sludge Disintegration and Biogas Production

The anaerobic digestion (AD) process is used for the sludge disintegration. The performance of the AD can be enhanced by applying pre-treatments for the biogas production. Biogas production from the sludges is a good way to gain energy from the sludge.

2.2.1. Anaerobic Digestion

Anaerobic digestion (AD) is the most known method for sludge stabilization. AD treatment is used for obtaining energy with the biogas production. It produces methane by using anaerobic bacteria (Priadi et al., 2014).

Anaerobic digestion is a conversion process of biodegradable material in microorganisms into biogas in the absence of oxygen, and it comprises mainly methane (CH_4) and inorganic end-products like carbon dioxide (CO_2). Anaerobic digestion of organic material occurs in four stages, hydrolysis, acidogenesis, acetogenesis, and methanogenesis. At the first stage of hydrolysis, fermentative bacteria convert the soluble complex organic matter and high molecular weight compounds into soluble molecules (Wu-Haan, 2008; Climent et al., 2007; Lastella et al., 2002). The hydrolysis stage is usually defined as the rate limiting step. The limitations of anaerobic digestion are long retention times and low overall degradation efficiency of the organic matter. Since, the most organic matters are in cells in the sewage sludge, the cell membrane of microorganisms consists of a semi-rigid structure to protect the cell from osmotic lysis (Yi et al., 2013). Thus, application of pre-treatment to the sludge accelerates the hydrolysis step by solubilizing organics in sludge and increases the performance of the anaerobic process.

Anaerobic digestion has several advantages. It needs less amount of energy for the process, provides favorable stabilization and produces biogas. However, it has some disadvantages like; being slow, sensitive to shock loads and toxic materials, and complicated (Lin et al., 1999).

2.2.1.1. AD of Antibiotic Contaminated Sludges. Antibiotics can affect negatively to the operation of AD process. The presence of antibiotics in sludges during anaerobic digestion can destroy the biogas production. Sanz et al. (1996), studied on the effects of the antibiotics on the anaerobic digestion process. Different types of antibiotics were used in the study, and their effects on biogas production were investigated by observing the antibiotics' impacts on methanogenic and acetogenic bacteria. According to the results of their study, the antibiotics can harmed to the

some types of the methanogenic bacteria and decreased the biogas production (Sanz et al., 1996).

In the study by Lallai et al. (2002), effects of the amoxicillin, oxytetracycline and thiamphenicol concentrations on the methane production were investigated. They found that the higher concentrations of antibiotic resulted the lower the methane production due to the inhibitory effect of antibiotic concentrations on the methane production (Lallai et al., 2002). The study of Ince et al. (2013) also investigated the effect of oxytetracycline (OTC) in biogas production, and found that OTC presence in the manures used in biogas plants caused 50-60 % decreases in biogas productions (Ince et al., 2013). In the study of Arikan et al. (2006), manure from medicated calves (containing OTC) was used in anaerobic digestion. The presence of OTC decreased biogas production by 27% during the anaerobic batch experiments, however it had no significant effects on the system performance (Arikan et al., 2006).

2.2.1.2. AD Applications for the Enhancement of the Biogas Production. Anaerobic digestion helps sludge stabilization by reducing odors, pathogens and the sludge amount. AD also provides the recovery of renewable energy in the form of methane.

Vranitzky and Lahnsteiner states that, in the anaerobic digestion, only about 50% of the organic matter in sludge can be degraded into biogas, the remaining organic can be degraded very slowly. To increase the efficiency of AD, some disintegration techniques, like ozone, can be combined with AD process (Vranitzky and Lahnsteiner, n.d.).

Shehu et al. (2012) studied on the performance of anaerobic digestion of cow dung for biogas production in batch and semi-continuous systems. They found that the biogas production from the cow dung was increased with the stable performance of the AD system. While increasing the biogas productions, AD showed an enhanced performance on the removals of contaminants (Shehu et al., 2012).

2.2.2. Biogas and Methane Production

Anaerobic digestion of solid organic material is a two-stage process consisting of acidogenesis and methanogenesis. In these processes usually the acidogenic and methanogenic microorganisms are in dynamic equilibrium. Methanogenic bacteria grow much slower than acidogenic bacteria. In the case of imbalance between the processes, acidification can occur and so anaerobic digestion process cannot continue. AD failure can also be arisen from the high organic loading rates (Vavilin and Angelidaki, 2005).

Biogas consists mainly of methane and carbon dioxide, and small amounts of hydrogen sulfide and ammonia, and water vapor (Weiland, 2010). Biochemical methane potential (BMP) test helps to determine the biogas productions from the sludge samples. The anaerobic digestibility of sludge samples can be evaluated by biochemical methane potential (BMP) test. The BMP assay process was first established by Owen et al. (1979) as a simple and inexpensive procedure to monitor relative anaerobic biodegradability of substrates (Owen et al., 1979).

In the study Doğan and Sanin (2009), pH-10, pH-12, MW (alone), MW + pH-10 and MW + pH-12 pre-treatments of sludge were done before the anaerobic digestion in small scale batch reactors. BMP method was applied and the best results for total gas and methane productions were reached with MW + pH- 12 with an increase of 16.3% and 18.9% (Doğan and Sanin, 2009). In the study of Yi et al. (2013); combined pre-treatment of alkali and thermal with the addition of 0.05 g NaOH/g TS for 9 hours at 70°C, having TS and SS concentrations of 20300 mg/L and 19750mg/L, then BMP test were applied. After the pre-treatments, the SS removal was reached as 21%, VSS reduction rate was 27.1% and soluble chemical oxygen demand (SCOD) was more than 200 times of control group. The amount of biogas production at the end of AD period was 45 mL for the control sample, and 329 mL for pretreated sludge. The biogas production was obtained nearly 6 times higher than the control and the average amount of methane content of biogas production is 64 %. Thus, NaOH pre-treatment was found efficient for solubilization and biogas production (Yi et al., 2013).

After a chemical pre-treatment application, an effective improvement in the gas production and in the removal efficiencies of COD and VS relative to unpretreated samples can be obtained. In the study of Lin et al (1999); Municipal waste activated sludge was treated with NaOH to solubilize the particulate organic matter in order to improve the digestibility of sludge. The sludge of 1% total solids treated with 20 and 40meq/L NaOH at ambient temperature for 24h. The SCOD/TCOD ratio increased as 55%. After the chemical pre-treatment and AD, the efficiency was determined with BMP (biochemical methane potential) tests. The results of BMP tests resulted that the methane production was 349 mL (at 1 atm and 35°C) for 1 g of COD removed. 41% increase in VS removal, 30% increase in COD removal and 34% increase in the gas production were obtained with the 40 meq/L of NaOH treatment (Lin et al., 1999).

In the study of Teghammar et al. (2010), hydrothermal pre-treatment combined with 2% NaOH and 2% H_2O_2 applied to the paper tube residuals at 220^oC for 10 min. to improve the biogas production. After 45 days of digestion period, methane production of 493 N mL/g VS was obtained (Teghammar et al., 2010). In the study of Takashima and Tanaka (2014), acidic thermal post-treatment (ATPT) (with pH 2, 4 and 6 by adding hydrochloric acid, at 25^oC, 100^oC and 180^oC) was applied to enhance anaerobic digestion of sewage sludge in the continuous and the batch reactors. The TS, VS and COD concentrations of sludge were 26.1 g/L, 15.4 g/L and 27.1 g/L, respectively. The volatile suspended solids reduction, COD destruction and methane production were increased as 30-46%, 66-75% and 14-21%, respectively with the application of the pre-treatment (Takashima and Tanaka, 2014).

3. STATEMENT OF THE PROBLEM

The aim of this study is to investigate the effects of microwave, H_2O_2, $S_2O_8^{2-}$, and combined MW/H_2O_2 and MW/$S_2O_8^{2-}$ pre-treatments on the anaerobic stabilization and the biochemical methane production potential of the sewage sludge. The sludge pre-treatments were applied to the sewage sludge samples prior to anaerobic digestion to enhance the biogas and methane production rates, and also improve the biodegradability, organic matter and volume reduction rates of the sludges.

The spread of antibiotics to the environment is a significant issue. If any residues of antibiotics persist in environment, there may grow some antibiotic-resistant bacteria. The hydrogen peroxide and persulfate oxidations had produced reasonable results for antimicrobial degradation in sewage sludge. This study also investigated the effects of antibiotic presence on the anaerobic stabilization and the biochemical methane production potential of the sewage sludge.

4. MATERIALS AND METHODS

In this chapter, specifications of sewage sludge and inoculum, their preparation methods for anaerobic reactors, analytical methods performed to determine biochemical methane production potential were explained, respectively.

4.1. Materials

4.1.1. Sewage Sludge and Inoculum Sludge

The sewage sludge samples were obtained from recirculation unit of a biological wastewater treatment plant located in Istanbul. The inoculum sludge used in this study was supplied from the anaerobic digesters of a biological wastewater treatment plant in Istanbul.

Raw sewage sludge and the inoculum sludge were characterized by different parameters listed in Table 4.1. This table shows average values of parameters for the initial characteristics of the sludge samples.

Table 4.1. Characteristics of sewage sludge and inoculum.

Parameter	**Unit**	**Sewage Sludge**	**Inoculum**
TS	g/L	13.6	45
VS	g/L	8.4	21
TSS	g/L	13	42
VSS	g/L	8.3	20
pH	-	6.65	7.6
Alkalinity	mg $CaCO_3$/L	1231	8386
COD	mg/L	18159	40743
sCOD	mg/L	253	1040
TOC	mg/L	228	234
TKN	mg/L	870	1920
NH_3-N	mg/L	161	1322
NO_3^--N	mg/L	0.6	6.55
NO_2^--N	mg/L	2	12
P	mg/L	300	700
PO_4^{3-}-P	mg/L	910	2130
P_2O_5-P	mg/L	680	1590
SO_4^{2-}	mg/L	70	15
VFA (as acetic acid)	mg/L	3.84	17.27
Particle Size d(0.5)	µm	44.257	38.043
CST	seconds	70	-
Viscosimetry	cP	(%9.5) 36.8	(%41.2) 161.2

4.1.2. Chemicals and Instrumental Equipment

All chemicals used in this study were at analytical grade and mainly supplied from Sigma-Aldrich, Hach and Merck.

The analytical methods and some special instruments that were used in this study are given in Table 4.2.

Table 4.2. The parameters, methods and some special instruments and chemicals used in the study.

Parameter	Method and Special Instruments
pH	4500-H B Method Electrometric (APHA, AWWA-WEF-2012) WTW Inolab pH meter
CST (s)	Instrumental method, CST Instrument
COD (mg/L)	5220 D Dichromate Closed Reflux Method (APHA, 2012) HACH COD Digester, HACH DR/2010 Spectrophotometer
sCOD (mg/L)	Centrifugation and 5220 D Dichromate Closed Reflux Method (APHA, 2012) Hettich Universal 16A Centrifuge, HACH COD Digester HACH DR/2010 Spectrophotometer
Viscosity	Brookfield Rvdv I Prime Model Viscosimeter
TKN (mg/L)	Gerhardt Vapodest Digester Apparatus 4500-N B Digestion Method (APHA, AWWA-WPCF-2012)
Ammonia-N (mg/L)	Gerhardt Vapodest Distillation Apparatus 4500-NH_3 B&C Distillation & Titrimetric Method (APHA, AWWA-WPCF-2012)
Nitrate-N (mg/L)	4500-NO_3^- C Spectrophotometric Method (APHA, AWWA-WPCF-2012) HACH DR/3 Spectrophotometer
Nitrite-N (mg/L)	4500-NO_2 C Spectrophotometric Method (APHA, AWWA-WPCF-2012) HACH DR/3 Spectrophotometer
TP (mg/L)	4500-P E Method Ascorbic Acid (APHA, AWWA-WEF-2012) HACH DR/3 Spectrophotometer
Alkalinity (mg/L)	2320 B Method Titration (APHA, AWWA-WPCF-2012)
TS (g/L)	2540 B (APHA, AWWA-WEF-2012)
VS (g/L)	2540 B and E (APHA, AWWA-WEF-2012)
TSS (g/L)	2540 G (APHA, AWWA-WPCF-2012)
VSS (g/L)	2540 D and E (APHA, AWWA-WPCF-2012)

Particle Size Distribution	Malvern Mastersizer 2000 Particle Size Analyzer
VFA (mg/L)	Perkin Elmer Clarus 600
TOC	Shimadzu TOC-V CSH
Microwave	Berghoff MWS+3 Speedwave Microwave
Total Gas Production	LUTRON Electronic Manometer (PM-9107)
CH_4	Agilent HP 6850 Gas Chromatograph (GC)
H_2O_2	Sigma-Aldrich, Hydrogen peroxide solution, 30 % (w/w) in H_2O
$Na_2S_2O_8$	Sigma-Aldrich, Sodium persulfate, reagent grade ≥ 98%

4.2. Methods

4.2.1. Sludge Pre-treatments

In this study, sludge samples were pretreated by microwave (MW) irradiation (as thermal pre-treatment), hydrogen peroxide (H_2O_2) and persulfate (S_2O_8) oxidations (as chemical pre-treatment) and their combinations (MW/H_2O_2, MW/S_2O_8) before the anaerobic digestion.

The pre-treatment techniques were tried to observe their effects on the biogas production rate.

4.2.1.1. Microwave Irradiation. Microwave treatment of sewage sludge was performed by using bench scale microwave irradiation system, Berghoff MWS+3 Speedwave Microwave System shown in Figure 4.1.



Figure 4.1. Berghof Speedway MWS+3 microwave system.

The microwave system has a power supply of 230 V/50 Hz/1,350 W, microwave output of 1000 W and frequency of 2450 Hz weight/dimensions (W x D x H). Temperature measurement range is 50-260 ºC and the maximum pressure is 150 bars.

Microwave (MW) has 12 teflon vessels each having a capacity of 60 mL. Five staged temperature program of the MW were adjusted by using the digital screen of MW.

Sludge samples were treated at 160ºC for 15 minutes. In this study, two different oxidants, hydrogen peroxide (H_2O_2) and persulfate ($S_2O_8^{2-}$) were used for pre-treatment of sludge. After the addition of either hydrogen peroxide or persulfate in the closed vessels, they were treated at 160ºC for 15 minutes in MW. Maximum 40 mL sample was irradiated in each vessel.

4.2.1.2. Hydrogen Peroxide (H_2O_2) Pre-treatment. Applying hydrogen peroxide oxidation to the sewage sludge is used for the stabilization of the sludge. This treatment can be also used for the removal of antibiotics from the sludge. Peroxide activation was achieved by thermally.

The peroxide oxidation process was conducted with the conventional heating and microwave heating with a peroxide concentration of 1 g H_2O_2/g TS. This concentration was determined by doing literature search.

The study of Bilgin Oncu and Akmehmet Balcioglu (2013) investigated microwave-assisted chemical oxidations for the micropollutant degradation and sludge solubilisation. They used 0.6 H_2O_2/g TS combine with MW at 140°C and 1.2 g H_2O_2/g TS combine with MW at 160°C (Bilgin Oncu and Akmehmet Balcioglu, 2013) The study of Song et al. (2016b) used the combination of Fe(II) with 0-100 mg/g TS concentrations and H_2O_2 with 0-1000 mg/g TS concentrations under pH 3 to enhance the dewaterability of anaerobically digested sludge (Song et al., 2016b). Jung et al. (2014) investigated the effects of H_2O_2 oxidation of WAS on solubilisation and biogas yield in AD. They used H_2O_2 concentrations of 0-200mM at 60-90°C and found the optimum conditions as 74.2mM & 60°C (Jung et al., 2014). In the studies of Wong et al. (2006) and Yin et al. (2007), MW and H_2O_2 combined advanced oxidation process were applied. They used 1 mL or 2 mL of H_2O_2 (30 wt.%) for 30 mL of sludge samples (Wong et al., 2006; Yin et al., 2007) In the study of Kim et al. (2009), H_2O_2 oxidation and alkaline hydrolysis were applied as post treatment processes for sludge disintegration. They used H_2O_2 without and with alkaline hydrolysis. Four different concentrations of 35% H_2O_2 (0.4, 0.8, 1.2 and 1.6 M) were used in the study (Kim et al., 2009).

After these pre-treatments applied, sludge samples were digested anaerobically in batch reactors.

4.2.1.2.1. Conventional H_2O_2 Pre-treatment. The sludge samples were heated in polytetrafluoroethylene (PTFE) tubes that have capacity of 50 mL. Pre-determined amounts of H_2O_2 (1 gram H_2O_2 (30% w/w) per gram total solids) were added to 30 mL of the sludge, which were preheated to the desired temperature in order to initiate chemical reaction into the PTFE tubes. The tubes were subjected to heat treatment in a temperature controlled water bath (Julabo, SW22) at 75°C for 90 minutes.

4.2.1.2.2. Combined MW/H_2O_2 Pre-treatment. The peroxide oxidation process was applied to sludge with 1 gram H_2O_2 (30% w/w) per gram total solids (1 g H_2O_2/g TS). Combined MW/H_2O_2 treatment consisted of a preheating stage. First, the samples were heated at 120 °C for 15 minutes in MW to destruct the biological enzymes in the sludge, in order to prevent the excessive consumption of hydrogen peroxide (Wang et al., 2009). After preheating stage, the predetermined amounts of H_2O_2 (1 g H_2O_2/g TS) were added to 40 mL of the sludge in closed vessels and samples were irradiated at 160 °C for 15 minutes in MW.

4.2.1.3. Persulfate ($S_2O_8^{2-}$) Pre-treatment. Applying activated persulfate oxidation to the sewage sludge is used for the stabilization of the sludge. Persulfate activation was also achieved by thermally.

The persulfate oxidation process was conducted with the conventional heating and microwave heating with a peroxide concentration of 1 g $S_2O_8^{2-}$/g TS. This concentration was determined by doing literature search.

The studies of Bilgin Oncu and Akmehmet Balcioglu (2013) and Akmehmet Balcioglu et al. (2016) investigated chemical oxidations and microwave-assisted chemical oxidations for the micropollutant degradation and sludge solubilisation. They used 0.44 g $S_2O_8^{2-}$/g TS concentration combined with MW at 140°C and 0.87 g $S_2O_8^{2-}$/g TS combined with MW at 160°C; and 0.22, 0.43 & 0.87 g $S_2O_8^{2-}$/g TS concentrations at 75 ^{o}C conventional heat (Bilgin Oncu and Akmehmet Balcioglu, 2013; Akmehmet Balcioglu et al., 2016). The study of Song et al. (2016b) used the combination of Fe(II) with 0-100 mg/g TS concentrations and $S_2O_8^{2-}$ with 0-1000 mg/g TS concentrations under neutral pH to enhance the sludge dewaterability of anaerobically digested sludge (Song et al., 2016b). Another study of Song et al. (2016a) investigated the effects of the persulfate & ZVI treatments on the dewaterability by using the combinations of ZVI (0-4.0 g/g TS) and $S_2O_8^{2-}$ (0.5 g/g TS), and $S_2O_8^{2-}$ (0-1.0 g/g TS) and ZVI (2.0 g/g TS) concentrations (Song et al., 2016a). In the study of Zhou et al. (2015), the combination of ZVI (0-30 g/L) and $S_2O_8^{2-}$ (0-6 g/L) under pH 7 were used to increase the dewaterability (Zhou et al.,

2015). Another study, aimed to improve the dewaterability again, used thermally and alkali activated persulfate with a concentration of 25 mM of $S_2O_8^{2-}$ (Lee et al., 2016).

After these pre-treatments applied, sludge samples were also digested anaerobically in batch reactors.

4.2.1.3.1. Conventional $S_2O_8^{2-}$ Pre-treatment. The sludge samples were heated in polytetrafluoroethylene (PTFE) tubes that have capacity of 50 mL. Pre-determined amounts of $S_2O_8^{2-}$ (1 gram $S_2O_8^{2-}$ per gram total solids), which was prepared with sodium persulfate solution ($Na_2S_2O_8$), were added to 40 mL of the sludge in PTFE tubes. The tubes were subjected to heat treatment in a temperature controlled water bath (Julabo, SW22) at 75ºC for 90 minutes.

4.2.1.3.2. Combined MW/$S_2O_8^{2-}$ Pre-treatment. The persulfate oxidation process was applied to sludge with 1 gram $S_2O_8^{2-}$ per gram total solids (1 g $S_2O_8^{2-}$ /g TS) at 160°C in 15 minutes. 40 mL of the sludge samples were put into the closed vessels and 1 g $S_2O_8^{2-}$/g TS was added into the vessels. Samples were irradiated at 160 °C for 15 minutes in MW.

The representations of sludges after pre-treatments are given in Figure 4.2 below.

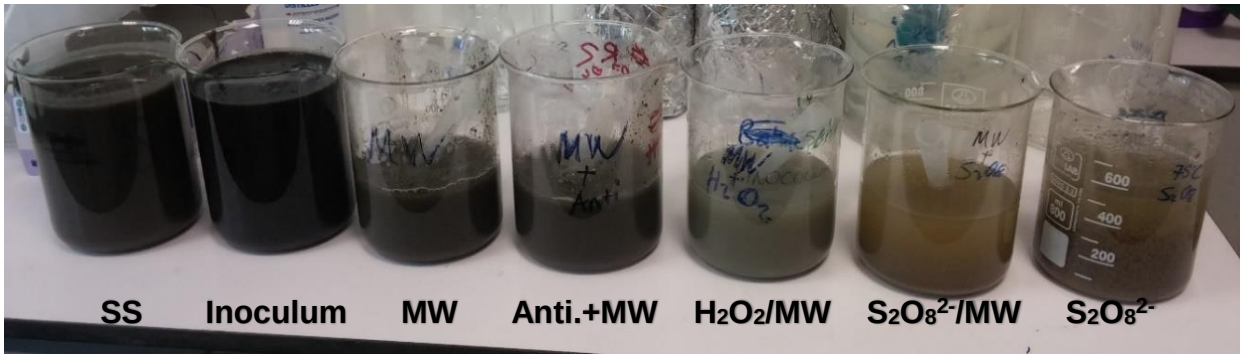

Figure 4.2. Picture of the slugdes after pre-treatments.

4.2.2. Antibiotic Contamination

In this part of the study, to see the effect of the antibiotic existence to sludge disintegration and the methane production; sewage sludge was synthetically contaminated by using CIP (ciprofloxacin) antibiotic as micro-pollutants. The antibiotic CIP was preferred since it is a human sourced antibiotic type. Micro-pollutant concentration was 1 mg CIP/g TS. After spiking the micro-pollutants into the sludge, microwave pre-treatment applied to both the raw and the contaminated sewage sludges. The raw and antibiotic contaminated sewage sludges alone, their mixture with inoculum sludge and microwave pretreated ones mixed with inoculum were anaerobically digested for 40 days in batch reactors. The anaerobic reactors were set up as explained in section 4.2.2.

The micro-pollutant concentration of 1 mg CIP/g TS was determined by doing literature search. Oncu and Balcioglu (2013) studied antibiotics degradation in WAS. Two types of antibiotic were used at high and low concentrations as: 20 mg/L of OTC and 2-8 mg/g TS of CIP and 0.08 mg/g TS of OTC & CIP (Oncu and Balcioglu, 2013). In their second study in 2013, they used 2 mg/g TS of OTC and CIP (as the antibiotic concentration of already present in the sludge), and 0.08 mg/g TS of OTC and CIP (as the antibiotic concentration of environmentally relevant) (Bilgin Oncu and Akmehmet Balcioglu, 2013)

Giger et al. (2003) stated that the concentrations of CIP of sewage sludge in WWTP was ranged from 1.4 to 2.4 mg/kg of dry matter (Giger et al., 2003). Fick et al. (2009) stated that a high concentrations of CIP as 14 mg/L were found in the effluents of a facility in India (Fick et al., 2009). Hannmann et al. (2012) studied persulfate oxidation treatment for the 20 mg/L CIP solution (Hannmann et al., 2012).

4.2.3. Anaerobic Reactors

Anaerobic batch reactors were used to digest pretreated and non-pretreated sewage sludge to observe their biodegradability. Before setting up the batch

reactors, sewage sludge were pretreated by the use of single and combined pre-treatment methods as explained in the sludge pre-treatments section.

After pre-treatments applied to the sewage sludge, the inoculum and the pretreated sewage sludges were mixed with an inoculum to substrate ratio (I:S) of 1:1 VS based. The sewage sludge used in the study had 8.4 g/L VS and the inoculum had 21 g/L VS. By calculation, it was determined to mix 57 mL of sewage sludge plus 23 mL of inoculum to have a total of 80 mL volume in 120 mL serum bottles. The reactors were named with the letters and the contents of the each reactors were given in the Table 4.3 below.

Table 4.3. The contents of the anaerobic reactors.

Symbols	**Explanations of the Ingredients (abbreviations)**
A	Sewage Sludge (SS)
B	Inoculum Sludge
C	SS + Inoculum
D	Microwave pretreated SS + Inoculum (MW)
E	$S_2O_8^{2-}$ and Microwave pretreated SS + Inoculum (MW/$S_2O_8^{2-}$)
F	H_2O_2 and Microwave pretreated SS + Inoculum (MW/H_2O_2)
G	$S_2O_8^{2-}$ and heat pretreated SS + Inoculum ($S_2O_8^{2-}$)
H	H_2O_2 and heat pretreated SS + Inoculum (H_2O_2)
K	SS + Antibiotic
L	SS + Antibiotic + Inoculum
M	Microwave pretreated antibiotic contaminated SS + Inoculum (Antibiotic MW)

After preparation of all the reactor sets, the pH values and the alkalinity concentrations of the contents of each reactors were measured, and adjusted. The pH values of the reactors adjusted to be in the favorable range of 7-7.2 for AD by adding 1 or 2 drops of 6 N HCl or 6 N NaOH (Appels et al., 2008). After the addition of acid or base and alkalinities of the reactor contents were measured. Alkalinities of the reactors should be between 2000-5000 mg/L (as $CaCO_3$) for an effective anaerobic digestion (Turovskiy and Mathai, 2006). Alkalinity addition was necessary

according to the initial alkalinity contents in some of the reactors. The initial alkalinity concentrations of the reactor contents were adjusted to be around 3000-4500 mg/L as $CaCO_3$ by adding $NaHCO_3$ in to the reactors which were out of the range.



Figure 4.3. Reactors during flushing with nitrogen gas.

When the adjustments of the pH and alkalinity were completed, the serum bottles were closed and crimped with rubber stoppers with metal caps. After sealing of the reactors, each of the reactors were flushed with nitrogen gas for 2 minutes to remove traces of oxygen and create an anaerobic environment as shown in Figure 4.3. The batch reactors were anaerobically digested for 40 days at a constant temperature of 37°C by using a water bath shown in Figures 4.4.



Figure 4.4. The anaerobic batch reactors at 37°C in water bathes.

The experiment was conducted in six parallel sets for every reactor type. Changes in sludge characteristics were determined weekly throughout the anaerobic digestion (AD) process by opening one of the six parallels of the each reactors to take sludge sample for the analyses. Total biogas productions in bottles measured every day by using a manometer (pressure method) and the biogas compositions of the reactors were analyzed periodically by using a gas chromatography (GC) at predetermined days of each week. Biogas samples were taken manually using a GC syringe from each reactor to determine the gas composition.

4.2.4. Biochemical Methane Production (BMP)

The biodegrability of the sludge samples can be measured with the biochemical methane production (BMP). The BMP tests also provide information about the bio-methanation of the substrates.

The BMP tests in the study were conducted according to the procedure that described by Owen et al. (1979) in 100 mL serum bottles (Owen et al., 1979).

4.2.4.1. Total Biogas Production Measurement Method. Daily gas productions in the anaerobic reactors were measured by pressure method using a manometer, the produced biogas accumulates inside the reactor and hence generates proportional overpressure. That pressure measured every day with Lutron PM-9107 Electronic Manometer and then converted into the volume. At the end of the anaerobic digestion, the cumulative biogas production of the reactors were obtained.

4.2.4.2. Methane Production Measurement Method. The biogas compositions of the anaerobic reactors were analyzed at predetermined days of each week during the AD period of batch reactors. Biogas samples were collected from the reactors by Hamilton Agilent 2.5 mL gas syringe. 0.5 mL of gas sample was taken from the each reactor and immediately injected to the Agilent HP 6850 Gas Chromatograph (GC). In GC, helium gas was used as the carrier gas. The compositions of biogas

were obtained as the areas of O_2, N_2, CO_2 and CH_4 gases. The percentages of each gas type into the biogas were calculated by the areas obtained from the GC.

4.2.5. Analytical Methods

All analytical methods used in this study were based on standard methods and explained in detailed in this part.

4.2.5.1. pH & Alkalinity. pH measurements were conducted by using WTW pH 3110 portable pH meter. The pH meter was calibrated regularly with pH standard buffer solutions.

Alkalinity analyses were conducted according to the Standard Methods (APHA, 2012). Reagents needed for the method were prepared and then the alkalinity of the samples were measured by the titration method (Method 2320 B) described in Standard Methods for the Examination of Water and Wastewater (APHA, 2012).

Sludge samples were added to a beaker with a magnetic stirrer. The initial pH of the sample was measured with pH meter and the sample was titrated with 0.02N sulfuric acid (H_2SO_4) until its pH reached to 4.5. Alkalinity concentrations of the samples were found by calculations as mg $CaCO_3$/L.

4.2.5.2. Total Solids and Volatile Solids (TS & VS). TS and VS analyses were performed according to the 2540 B and 2540 E methods of Standard Methods for the Examination of Water and Wastewater (APHA, 2012). For TS and VS measurements, first, evaporating dishes were brought to a constant weight by heating in muffle furnace (Protherm PLF 110/8) for 1 hour at 550ºC. Then, well-mixed homogeneous sludge samples were put in evaporating dishes, their weights were saved and then evaporated and dried in oven (Nüve FN 500) for 24 hours at 105ºC. After that, evaporating dishes were cooled in desiccator for 30 minutes and weighed. The weights were saved again. For VS measurement, dishes were ignited in the muffle furnace at 550ºC for 1 hour, cooled in desiccator and weighed. Weights

were reported. TS and VS concentrations of the samples were calculated by using the reported values.

In order to determine suspended solids content, methods 2540 B and 2540 G in Standard Methods (APHA, 2012) were used. Sludge samples were filtered through 0.45 µm Millipore filter papers. Filter papers were dried at 105 ˚C for 24 hour in drying oven before filtration of sludge samples. Filter papers with filtrated sludge samples were dried at 105ºC for 24 hours first. They were cooled in desiccator and weighed. Then, they were put into the crucibles, which had been brought to a constant weight previously. Crucibles with filter papers and samples were ignited at 550 ˚C for 1 hour. Crucibles were cooled in desiccator and weighed. All weights were reported. Volatile or fixed solids contents were found by the calculations of the reported values.

4.2.5.3. Chemical Oxygen Demand and Soluble Chemical Oxygen Demand (COD & sCOD). COD and sCOD analyses were conducted by using the dichromate closed reflux colorimetric method according to the Standard Methods for the Examination of Water and Wastewater, Method 5220 D (APHA, 2012). The sludge samples were digested for 2 hour at 150°C in Velp Scientifica COD Digester (Eco 25 Thermoreactor) after adding sulfuric acid (H_2SO_4) and potassium dichromate ($K_2Cr_2O_7$). Sludge samples were diluted before the digestion. For sCOD analyses, samples were centrifuged in Hettick Rottina 380 centrifuge. After centrifugation, supernatant portion of the sample were taken, diluted if necessary, and then digested by reflux colorimetric method. After digestion, samples were cooled and the absorbances of the digested samples were determined at 600 nm in HACH DR/2010 Portable Data Logging Spectrophotometer. TCOD and sCOD concentrations are determined by using the potassium hydrogen phthalate (KHP) calibration curve, which was prepared before with deionized water by same method. All analyses were performed triplicate.

4.2.5.4. Total Kjeldahl Nitrogen (TKN). TKN analyses were performed by using Nessler Method (Method 8075) of HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997) subsequently to the digestion of the sample. Sludge samples were

digested first at 440°C using a Digesdahl Digestion Apparatus. 5 mL of sludge sample and 4 mL of concentrated sulfuric acid were heated to 440ºC and after 4 minutes 15 mL of hydrogen peroxide was added. When the digestion ended, the digesdahl flask was cooled and then it was diluted to 100 mL. After digestion, samples were analyzed by using the Nessler Method (Method 8075) for TKN measurement according to Hach DR/2010 Procedures Manual (Hach, 1997). TKN indicator, KOH solution, Mineral Stabilizer, Polyvinyl Alcohol Dispersing Agent and Nessler Reagent were added on the determined digested sample volume respectively. After each addition, sample was shaken slightly to mix. Same steps were followed for the blank sample by using deionized water as sample to zero in the spectrophotometer. Samples were poured into 25 mL sample cells, and the TKN of the samples was read as mg/L at 460 nm in Hach DR/2010 spectrophotometer. The real TKN concentrations were calculated by using the following formula (Hach, 1997):

$$\mathrm{TKN\ (mg/L)} = \frac{\mathrm{A} \times 75}{\mathrm{B} \times \mathrm{C}}$$

where,

A : concentration, reading from instrument, mg/L.

B : sample amount (sample volume used for digestion), mL.

C : analysis volume, mL. (4.1)

4.2.5.5. Total Phosphorus. The digested samples in Digesdahl Digestion Apparatus for TKN measurement were used for TP experiment also. Digested samples were analyzed by using Ascorbic Acid Method (Method 8048) in accordance with Hach DR/2010 Spectrophotometer Handbook (Hach, 1997). Phenolphthalein, KOH solution and Sulfiric Acid (1N) were added on the determined digested sample volume respectively. After each addition, sample was shaken slightly to mix. Sample was poured into 10 mL Hach cell and then read into the spectrophotometer, saved as zero. After zeroing, PhosVer 3, Phosphate Powder Pillow kit was poured into the 10 mL cell and cell was shaken vigorously for 2 minutes reaction period. Then, TP content of the sample was measured as mg/L at 880 nm in Hach DR/2010

spectrophotometer. TP concentrations were calculated by using the following formula (Hach, 1997):

$$\mathrm{TP\ (mg/L)} = \frac{\mathrm{A} \times 1000}{\mathrm{B} \times \mathrm{C}}$$

where,

A: concentration, reading from instrument, mg/L.

B: sample amount (sample volume used for digestion), mL.

C: analysis volume, mL. (4.2)

4.2.5.6. Ammonia Nitrogen (NH_3-N). Ammonia analysis of sludge samples were performed with distillation step and titrimetric method according to the Standard Methods for the Examination of Water and Wastewater, 4500-NH_3 B Preliminary Distillation Step & 4500-NH_3 C Titrimetric Method (APHA, 2012). For the distillation step, borate buffer solution, 6 N NaOH and indicator boric acid solution reagents were prepared. Distillation of the sludge samples were done by the Gerhardt Vapodest Distillation Apparatus.

After distillation, the distilled products were titrated with 0.02 N H2SO4. The amounts of acid volume used in titration were saved and NH_3-N concentrations were calculated by using the following formula (APHA, 2012):

$$\mathrm{NH_3 - N\ (mg/L)} = 280 \times \frac{\mathrm{V_{acid}}}{\mathrm{V_{sample}}} \qquad (4.3)$$

4.2.5.7. Nitrite and Nitrate Nitrogen (NO_2^--N & NO_3^--N). For nitrite and nitrate analyses, sludge samples were centrifuged first and the supernatants of the centrifuged samples were used. The supernatants diluted if necessary and the concentrations were measured in HACH/DR 2010 Spectrophotometer.

For nitrite measurements, Ferrous Sulfate Method (Method 8153) described in the HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997) was used. Sample was poured into 10 mL Hach cell and then read into the spectrophotometer, saved as zero. After zeroing, standard NitriVer 2 Nitrite Reagent Powder Pillow kit was

added into the Hach cells, and the cell was shaken vigorously to provide dissolution. After 10 minutes of reaction period, cell was placed in the spectrophotometer again at a wavelength of 585 nm, and sample was analyzed.

For nitrate measurements, Cadmium Reduction Method (Method 8039) in HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997) was used. Sample was poured into 10 mL Hach cell and then read into the spectrophotometer, saved as zero. After zeroing, standard NitraVer 5 Nitrite Reagent Powder Pillow kit was added into the Hach cells, and the cell was shaken vigorously to provide dissolution. After 5 minutes of reaction period, cell was placed in the spectrophotometer again at a wavelength of 500 nm, and sample concentration was measured.

4.2.5.8. Sulfate (SO_4^{2-}). SO_4^{2-} analysis was conducted in accordance with the Method 8051 of HACH/DR 2010 Spectrophotometer Handbook (Hach, 1997). For sulfate analyses, the supernatants of the centrifuged sludge samples were used. The supernatants diluted if necessary. SulfaVer 4 Reagent powder pillow were added to the samples in 10 mL Hach cell and sample cells were shaken vigorously. After 5 minute reaction period, the concentrations were measured in spectrophotometer at 880 nm.

4.2.5.9. Total Organic Carbon (TOC). TOC of the samples were measured by Shimadzu TOC-V SSM-5000 A total organic carbon analyzer. Before the analyses, sludge samples were dried in furnace. The samples that were in solid form were weighted in the SSM boats. Each sample was prepared in two boats with same weights. One of the boats was covered with the ceramic fiber and placed into the TC (total carbon) part. The second boat was placed into the IC (inorganic carbon) part and during the IC measurement phosphoric acid was added into the boat. The analyzer measured the concentration of TC and IC, and gave directly the TOC concentration as mg/g.

4.2.5.10. Volatile Fatty Acids (VFA). VFA analyses were conducted in Perkin Elmer Clarus 600 Gas Chromatograph equipped with a flame ionization detector (FID) and an ID DF column. Samples were centrifuged at 10000 rpm for 30 minutes and then

supernatant parts of sludge samples filtered from Sartoris Stedim Minisart syringe filter (0.2 µm pore size). After filtration, 1.35 mL of the filtrated sample was poured into the eppendorf tubes and 0.15 mL of 10 N phosphoric acid (H3PO4) was added on it. This mixture was put into the GC vials having a volume of 1.5 mL and the vials were placed into the Gas Chromatograph (GC) for analyzing. If the measurement couldn't be performed immediately, samples can be stored at +4oC with the addition of phosphoric acid. After the analyses of GC were completed, the concentrations of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and, heptanoic acids were obtained as mmolar (mM). The VFA concentration is given in terms of acetic acid concentration by converting the all types of these acids into acetic acid.

4.2.5.11. Particle Size. Particle size analyses were carried out by using Malvern Mastersizer 2000 with the wet dispersion unit of Hydro2000MU. Laser diffraction technique is used to measure the size of particles in the Mastersizer 2000. A scattering pattern is created during the measurement period to calculate the size of the particles. The dispersant liquid was the deionized water. The refractive indexes of water and the sludge samples were 1.33 and 1.5, respectively. The stirrer and pump were operated at a speed of starting 600 rpm to 1100 rpm. Higher speeds like 2500-3000 rpm were set for cleaning the cells operating only with water. For analyses, sludge samples were diluted in a dispersion tank which was introduced into the measuring cell. The dispersion tank is a beaker that has 800 mL deionized water. While measuring the sample, the stirrer and pump were set 600 rpm first, then the sample was slowly added into the water using a Pasteur pipette and the speed was set to the 1100 rpm. Each sample was analyzed in triplicate and between every sample measurement cleaning was done at least two times. The particle size distribution was measured in three diameters d(0.1), d(0.5) and d(0.9). The measurement data were reported as the average values of particle size diameters (µm) and the plots of particle size distribution (volume (%) vs. particle size (µm)).

4.2.5.12. Viscosity. Viscosity measurements were done by using a Brookfield RVDV-IPRIME digital viscometer. For making measurements, the level of the viscometer was adjusted with the three feet of the viscometer by controlling the

bubble on the top of viscometer. After leveling, viscometer was auto zeroed. The viscosity was obtained using RV Guardleg Spindles number 2 of the RV spindle set. 400 mL of sludge samples were filled in 600 mL beaker and the operating speed was set to 100 rpm. All measurements were done at ambient temperature. The results were reported as cP values.

4.2.5.13. Capillary Suction Time. CST analyses were carried out by using Triton Electronics Type 304M capillary suction timer. For each sample different CST papers were used. 2 mL sample was poured into the CST sample cylinder with pasteur pipettes. The CST apparatus has two concentric electrodes as sensor at two different diameters (inner and outer electrodes). When the water from the sludge starts to wet the paper and reaches to the first circle on the CST paper, the sensor bleeps and timer starts to count; and then, if the sample can reach to the second circle, it bleeps again and timer stops. The elapsed time between two circles is saved as saved as capillary suction time (CST) in seconds.

5. RESULTS AND DISCUSSION

This study investigated the effects of sludge pre-treatments (MW, H_2O_2, $S_2O_8^{2}$, MW/H_2O_2 and MW/$S_2O_8^{2-}$) and also the presence of antibiotics on anaerobic stabilization and biochemical methane production potential of sewage sludges.

5.1. Results of Pre-treatments Applied Anaerobic Reactors

The pre-treatments were applied to the sewage sludge (SS), then they were mixed with the inoculum sludge, and anaerobically digested for 40 days in batch reactors.

In this part, the results obtained from the pre-treatment applied anaerobic reactors are presented.

5.1.1. Sludge Properties of Pre-treatments Applied Anaerobic Reactors

One of the six parallels of the eight different reactor sets was opened weekly to take sludge samples for pH, alkalinity, TS, VS, COD, sCOD, NH_3-N and volatile fatty acids (VFA) analyses.

The reactor set A containing only sewage sludge and B, containing only inoculum sludge were set up as control reactors. Rest of the reactor sets contained sewage sludge and inoculum sludge with an inoculum to substrate ratio (I:S) of 1:1 on VS basis. The reactor set C contained inoculum and unpretreated sewage sludge. The rest of the reactors D to F contained inoculum plus pretreated sewage sludges.

5.1.1.1. pH & Alkalinity. Table 5.1 presents the pH values in the reactors. The initial pH values of reactors' contents were ranging between 3.4 and 7.6. pH values in the reactors were adjusted to be in the favorable range of 7 - 7.2 for AD.

Table 5.1. pH values in the reactors.

	pH values						
	Days						
Reactors	**$0_{initial}$**	**$0_{adjusted}$**	**6**	**12**	**18**	**28**	**40**
A	6.65	7.1±0.1	7.14	7.25	7.55	7.60	7.84
B	7.60	7.1±0.1	7.51	7.82	7.68	7.99	8.01
C	7.48	7.1±0.1	7.10	7.28	7.38	7.56	8.10
D	7.50	7.1±0.1	7.15	7.46	7.45	7.59	7.56
E	3.40	7.1±0.1	7.12	7.50	7.76	7.77	7.85
F	7.05	7.1±0.1	7.15	7.32	7.63	7.86	8.31
G	5.00	7.1±0.1	7.06	7.18	7.48	7.77	8.79
H	7.21	7.1±0.1	7.08	7.45	7.61	7.94	8.38

(A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

The pH values in the reactors showed an increasing trend throughout the AD process and remained in a range between 7 and 8.8.

The reactors should have an alkalinity between 2000 - 5000 mg/L for a successful anaerobic digestion (Turovskiy and Mathai, 2006). The initial alkalinity concentrations in the reactors were adjusted to be around 3230 - 4360 mg/L as $CaCO_3$ by adding $NaHCO_3$, and stayed in the favorable range for AD. The alkalinity addition was done for the pH adjustment during the AD. Figure 5.1 shows the alkalinity variations in the reactors throughout the AD process.

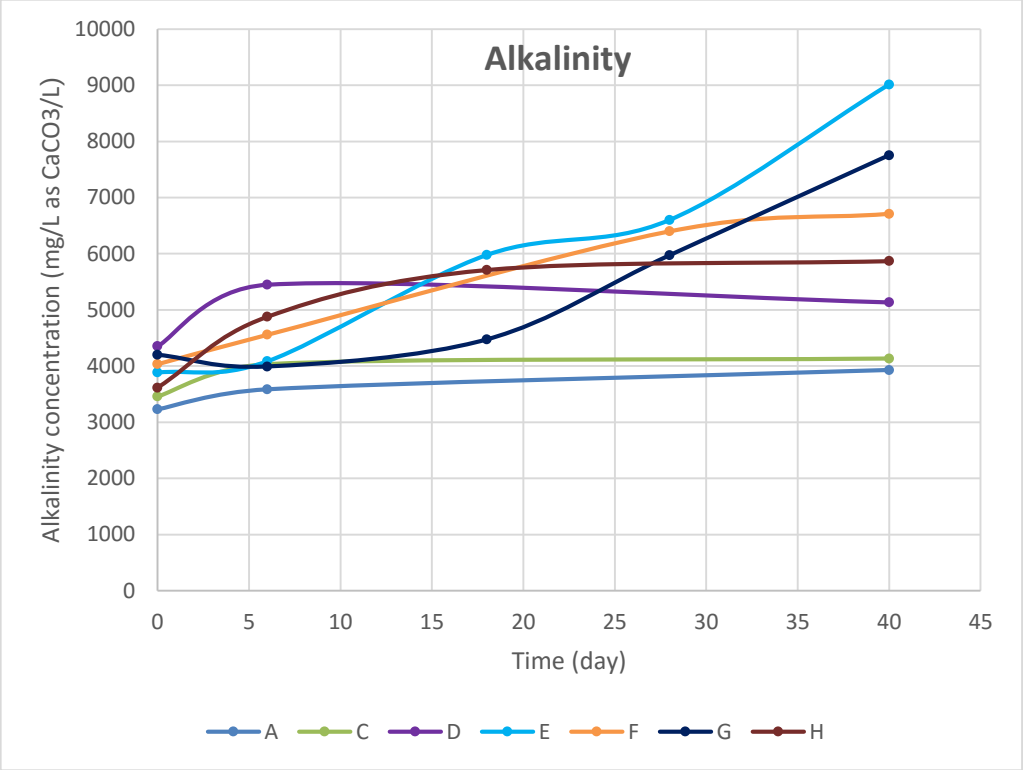

Figure 5.1. Variations of alkalinity concentrations in the reactors.

(A = SS, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

During the AD process alkalinity values showed a similar trend with pH. The initial increase of alkalinity was because of the activity of the methanogenic bacteria that produced alkalinity in the form of carbon dioxide, ammonia and bicarbonate to provide a balance with the acidogens (Turovskiy and Mathai, 2006). It was expected due to the H^+ ions consumption in the anaerobic digestion. The pre-treatments increased the alkalinity concentrations slightly. Accordingly to the study of Chie et al. (2011), with the combined microwave and alkaline pre-treatment (MAP) on the thermophilic anaerobic digestion process (TADP), 7900 mg $CaCO_3$/L alkalinity was obtained when the control TADP had an alkalinity concentration of 5600 mg $CaCO_3$/L (Chi et al., 2011). Moreover, accordingly with the studies of the Song et al. (2013), studying the H_2O_2 pre-treatment of rice straw in AD, and Sun et al. (2012), studying the sulfate radical pre-treatment of sewage sludge in AD, similar alkalinity trend was observed with the results obtained in this study (Song et al., 2013; Sun et al., 2012). At the end of AD process, the alkalinity concentrations started to decrease due to the alkalinity consumption. MW/$S_2O_8^{2-}$ and $S_2O_8^{2-}$ pretreated reactors have an increase in alkalinity concentrations, since the methanogenesis

step of AD in those reactors failed, the alkalinity couldn't be consumed. The inoculum sludge had a really high alkalinity concentration as expected. On the other hand, alkalinity concentrations of sewage sludge samples were low.

5.1.1.2. Total Solids and Volatile Solids (TS & VS). The total and the volatile solids concentrations of the reactors were measured weekly. The variations in TS and VS concentrations are given in Figure 5.2 and Figure 5.3, respectively. The removal efficiencies of organic matters in the anaerobic reactors were found at the end of the biological degradation by VS analyses.

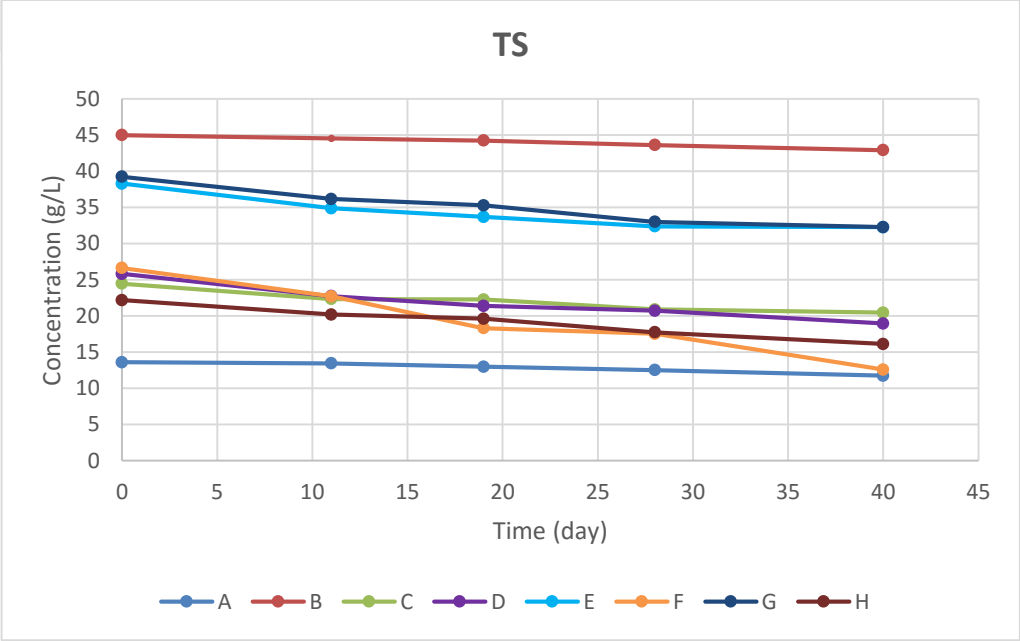

Figure 5.2. TS concentration variations in the reactors.

(A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

The initial TS concentrations of sewage sludge in reactor A and inoculum sludge in reactor B were measured to be 13.6 g/L and 45 g/L, respectively. Their mixture in reactors C, D, F and H had initial TS concentrations in the range of 22-26.6 g/L, except the $S_2O_8^{2-}$ pretreated reactors E and G. The increase observed in the initial TS concentrations of sludge samples in reactors E and G might be caused due to the addition of $Na_2S_2O_8$ for $S_2O_8^{2-}$ pre-treatment.

The pre-treatments can change the initial total and volatile solid concentrations in the reactors. Fabregat et al. (2011) studied the reduction and stabilization of the sludge. Different concentrations of H_2O_2 applied at different temperatures were tried in their study. The TS and VS concentrations in the reactors increased or decreased based on the dose of H_2O_2 and the temperature (Fabregat et al., 2011). Their study showed that the pre-treatments can change the TS and VS concentrations of sludge samples.

At the end of the AD process; reactors D, H and F, containing microwave, H_2O_2 and combined MW/H_2O_2 pretreated sludges, showed higher TS removal efficiencies. The highest TS removal rate was obtained in reactor F as 53% and followed by the TS removal rates of 27.3% and 26.6% in reactors H and D. TS removal efficiency in the control reactor C was 14.5%. The lowest TS removal efficiency of 9.3% was obtained in the reactor G containing $S_2O_8^{2-}$ pretreated sludge sample due to the increase in initial TS concentration of sludge. Applying combined MW/H_2O_2 pre-treatments before AD process resulted in a 3.6-fold increase in TS removal rates.

In the study of Astals et al. (2013), seven different mixed sewage sludge samples (having TS concentrations of between 18.4 - 48.4 g/L) were digested anaerobically at 37°C, and at the end of the AD at least 34% TS removal was achieved (Astals et al., 2013). Kim et al. (2009) investigated the sludge disintegration of hydrogen peroxide oxidation applied waste activated sludge (having 16200 mg/L TS concentration). By increasing concentration from 0 M to 1.6 M 33% TS removal was obtained (Kim et al., 2009).

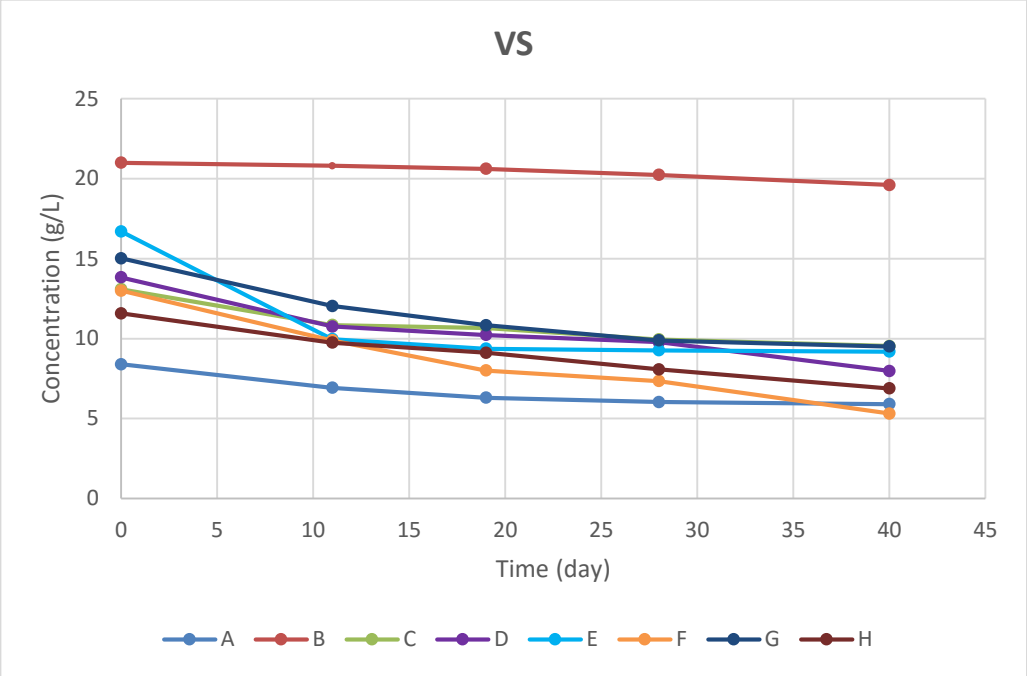

Figure 5.3. VS concentration variations in the reactors.

(A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

Volatile solids reduction is an important parameter for the anaerobic digestion, since VS are expected to be converted into biogas at the end of AD. However, since not all the sludge samples have the same VS compositions, different rates and degrees of biodegradation are obtained during the anaerobic digestion.

The initial VS concentrations of sewage sludge in reactor A and inoculum sludge in reactor B were measured to be 8.4 g/L and 21 g/L, respectively. Their mixture in the rest of the reactors had initial VS concentrations in the range of 11.5-16.7 g/L. The initial VS concentrations of the $S_2O_8^{2-}$ pretreated reactors E and G were higher due to the increase in their initial TS concentrations.

At the end of the AD, the highest reduction rate was obtained in the combined MW/H_2O_2 pretreated sludge containing reactors F as 59.2%. VS removal rates in reactors E, D, H and G were 45%, 42.4%, 40.6%, and 37%, respectively while it was 24% in control reactor C. As an improvement, combined MW/$S_2O_8^{2-}$ and MW/H_2O_2 pre-treatments increased the VS removal rates in reactors.

In the study of Astals et al. (2013), investigating the anaerobic digestion of different sludge samples (having TS and VS concentrations of between 14.3 - 40.5 g/L and 18.4 - 48.4 g/L, respectively) at 37ºC, at least 44% of VS reduction was achieved (Astals et al., 2013). In another study, the waste activated sludge (having initial concentrations of 1% TS and 3550 mg/L VS) treated with 40 meq/L of NaOH, and 41% removal of VS provided (Lin et al., 1999). Furthermore, in the study of Takashima and Tanaka (2014), the sewage sludge, having 26.1 g/L TS and 15.4 g/L VS concentrations, anaerobically digested. With the application of acidic thermal post-treatment process, the VS removal rates of sludge were increased more than two times from 30-42% to 66-67% (Takashima and Tanaka, 2014).

When the initial and the final VS/TS values of the reactors' contents compared, the final VS/TS values had a lower ratio than the initial ones. This can be explained by that the reactors had an increasing soluble matter concentration, together with a decreasing particulate matter concentration in anaerobic digestion. This was arisen from that the particular fraction of sludge became more mineral with disintegration methods like in the study of Bourgrier et al. (Bougrier et al., 2005).

5.1.1.3. Chemical Oxygen Demand and Soluble Chemical Oxygen Demand (COD & sCOD). COD and sCOD concentrations in the reactors were analyzed weekly. The variations of the COD and sCOD concentrations during the anaerobic digestion are illustrated in Figure 5.4 and Figure 5.5. Reactors having higher total solid content have higher COD concentrations as expected. sCOD concentrations of the organic compounds is a parameter for the sludge solubilisation. It can be used for representing the hydrolysis degree of sludge in AD process.

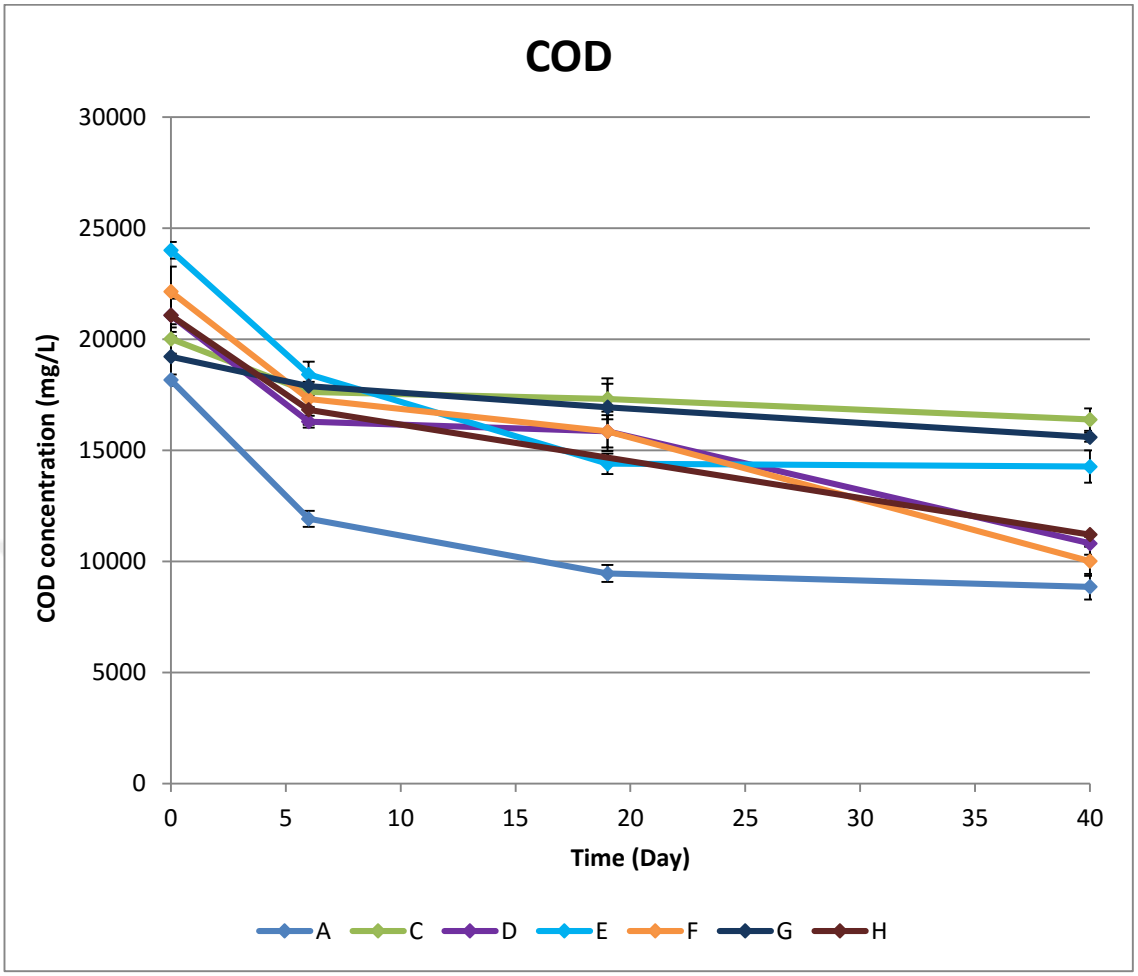

Figure 5.4. Variations of COD concentrations in the reactors.

(A = SS, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

The inoculum sludge used in the study had a high COD concentration as 40743 mg/L (B), and sewage sludge had 18159 mg/L (A) of COD concentration. Their mixture in control reactor C had 20011 mg/L COD concentration. The pre-treatments improved the initial total COD concentrations in the reactors, except the $S_2O_8^{2-}$ pretreated reactor (G).

At the end of the sludge degradation in anaerobic digestion process, the MW and H_2O_2 pre-treatments improved the COD removal efficiencies. The lowest final COD concentration was recorded in reactor F, and reactors D & H followed. The highest COD removal efficiency was 54.8%, belonged to reactor F. Then, 48.7%, 46.9% and 40.5% removal rates were obtained in reactors D, H and E, respectively, when the removal efficiency of the control reactor C were 18.1%. The lowest COD

removal rate was occurred in the $S_2O_8^{2-}$ pre-treatment (G). However, in reactor E, removal rate was increased by the contribution of microwave.

In the study of Lin et al. (1999), waste activated sludge (having 3910 mg/L COD) treated with 20 and 40meq/L NaOH at ambient temperature and, COD removal improved by 30% over the control sludge sample with 40 meq/L of NaOH treatment (Lin et al., 1999). Astals et al. (2013) studied on anaerobic digestion of different sludge samples (having COD concentrations of between 25.3 - 70.8 g O_2/L) at 37°C, and obtained COD removal efficiencies between 58 to 65% (Astals et al., 2013). Moreover, in Takashima and Tanaka's study, with the application of acidic thermal post-treatment process to improve anaerobic digestion of sewage sludge (having COD concentration of 26.6 g/L), 67-71% COD reduction was obtained, when the control process had 31-46% COD removal rate (Takashima and Tanaka, 2014). Another study conducted by Zhang et al. (2010) used MW-assisted H_2O_2, peroxymonosulfate (PMS) and persulfate (PS) treatments for the COD removal from landfill leachate. The COD concentration was 4062.8 mg/L initially in the study, and by applying the MW-assisted H_2O_2, peroxymonosulfate (PMS) and persulfate (PS), with a 0.3 mol/L oxidant concentration, 43.5%, 80.2%, 97.3% COD removal were obtained, respectively (Zhang et al., 2010). In this study; COD removal efficiencies were tripled up by applying MW/H_2O_2 pre-treatments to the sludge samples before AD.

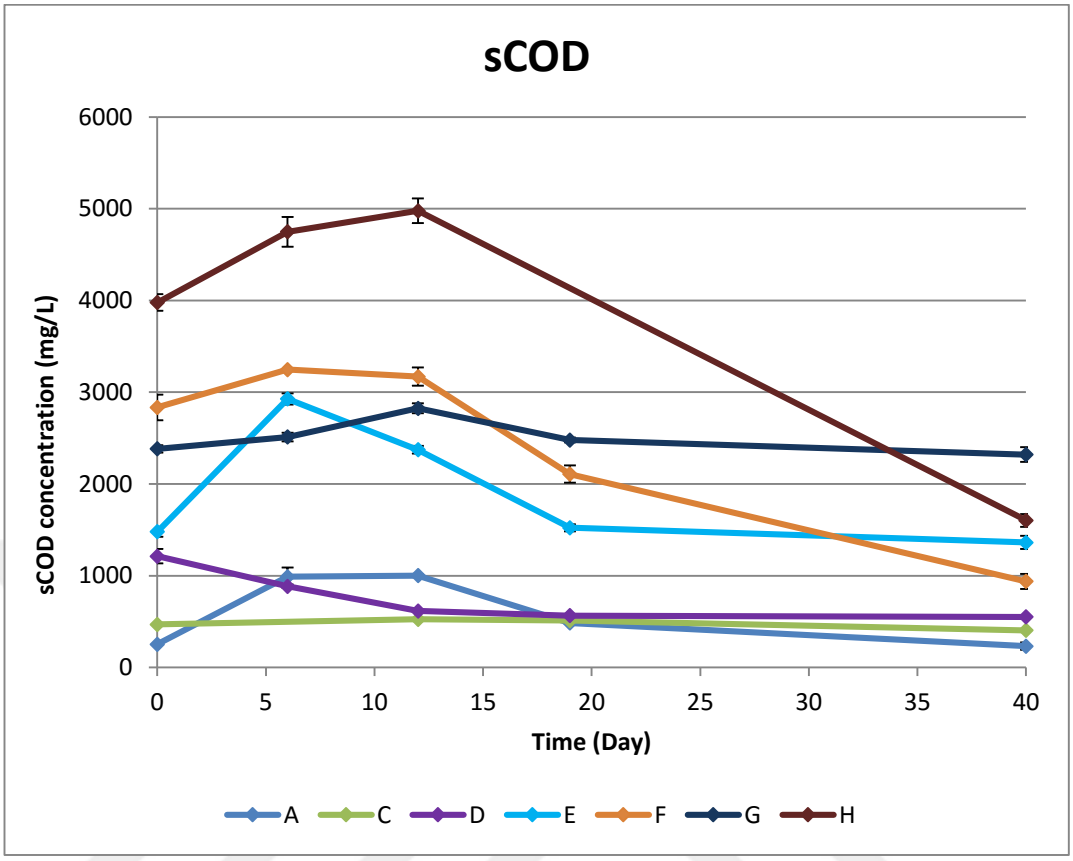

Figure 5.5. Variations of sCOD concentrations in the reactors.

(A = SS, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

The initial sCOD concentrations of sewage sludge (A) and inoculum sludge (B) were measured as 253.3 mg/L and 1040.4 mg/L, respectively. Their mixture in control reactor C had 469 mg/L sCOD concentration. The pre-treatments increased the initial sCOD concentrations of sludge samples in the reactors by solubilizing the particulate organics.

At the end of the AD, the highest sCOD removal efficiency was achieved in reactor F as 66.9%. A removal rate of 59.7% and 54.6% were obtained in reactors H and D respectively, as the control reactor had a removal rate of 13.7%. In reactors E & G, removal rates of sCOD were not improved. That was believed to be happening due to the sudden decrease in pH of sludge samples. After the application of 1 mg $S_2O_8^{2-}$/g TS to the sludge samples, the pH of reactor content decreased sharply to pH values of 3.4 and 5, and probably caused the death of the heterogenic and the methanogenic bacteria and led to survival of acid formers only.

MW and H_2O_2 pre-treatments enhanced the sCOD removal considerably. Microwave irradiation achieved solubilisation of the particulate COD by transferring materials from solid phase to liquid phase, and so created an important enhancement in biodegradability of the sludge. The hydrogen peroxide resulted with the same effect in chemical way. Therefore, their combined effect created the highest removal efficiency. The study of Wong et al. (2006) explained that the combination of MW and H_2O_2 pre-treatments increased the solubilisation of COD. At the end of the MW/H_2O_2 advanced oxidation process, nearly 80% of the COD was converted into soluble COD (Wong et al., 2006). In this study; sCOD removal efficiencies were increased 4.9 times higher than the control by applying MW/H_2O_2 pre-treatments to the sludge samples before AD.

5.1.1.4. Total Kjeldahl Nitrogen (TKN). TKN concentrations in the reactors were analyzed and the results are given in Table 5.2.

TKN is the sum of organic nitrogen, ammonia and ammonium. Organic nitrogen consists mostly of the proteins. In anaerobic digestion, with the hydrolysis of proteins, ammonia is produced. Proteins turn into peptides and amino acids that are reduced to short-chain fatty acids (Wilson and Novak, 2009).

The pre-treatments increased the initial TKN concentrations in the reactors as shown in Table 5.2 below. Accordingly Neyens et al. (2003), which investigated the H_2O_2 catalytic activation by Fenton oxidation process of thickened sludge, the peroxidized sludge had higher TKN concentrations (150 mg N/L) than the untreated sludge (104 mg N/L) (Neyens et al., 2003).

Table 5.2. TKN concentrations in the reactors.

Reactors	TKN (mg/L)
A	735
B	1808
C	1043
D	1290
E	1410
F	1305
G	1395
H	1140

(A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

5.1.1.5. Ammonia Nitrogen (NH_3-N). Ammonia concentrations showed mostly an increasing trend during the anaerobic digestion, as expected. Inorganic ammonia nitrogen exists in two forms as ammonium ion and free ammonia in the aqueous solution. Ammonium ion in the aqueous phase increased due to the hydrolysis of ammonia during to the disintegration of bacterial cells in anaerobic digestion. Ammonium provides a buffer capacity in the anaerobic reactors by stabilizing the pH values in the reactors with concentrations of up to 1000 mg/L. In addition to this, ammonium has an inhibitory effect when its concentrations in sludge are in a range of between 4000 and 6000 mg/L (Fricke et al., 2007). Likewise, according to the study of Chen et al. (2008), ammonia can be an inhibitor for methanogenesis, if the concentrations of ammonia in sludge increase up to 4051-5734 mg NH_3-N/L (Chen et al., 2008). However, in this study, ammonia concentrations in the reactors did not exceed 930 mg/L during the anaerobic digestion process, except inoculum (Inoculum had 1730 mg/L concentration at most.). Anyhow, the measured level of ammonia concentrations didn't create an inhibitory effect on methanogens in this study. The pre-treatments did not have an impact on the ammonia concentrations. The ammonia measurements were done for the control of anaerobic digestion staying in favorable range of ammonia concentrations for methanogenesis.

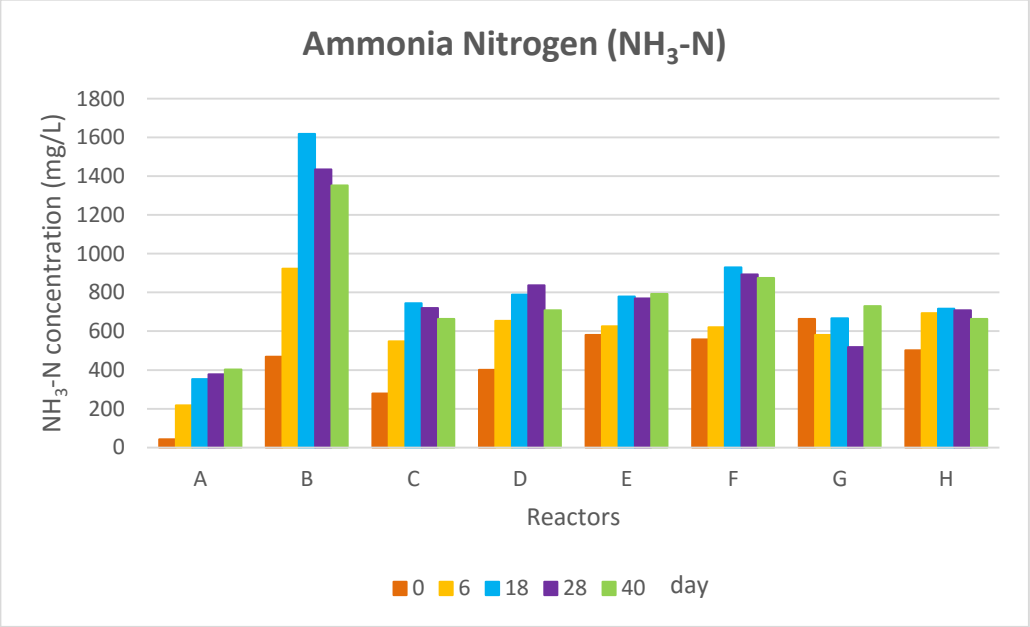

Figure 5.6. Variations of ammonia nitrogen (NH_3-N) concentrations in the reactors. (A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

5.1.1.6. Total Phosphorus. Phosphorus concentrations of the sludge samples in reactors were analyzed and the results are given in Table 5.3.

Phosphorus concentrations of the sludge samples increased relatedly with the increase of ortho-phosphate, which was formed as a result of the phosphorus solubilisation in the hydrolysis stage of the sludge in anaerobic digestion. As shown in Table 5.3; pre-treatments increased the initial ortho-phosphate concentrations in the reactors. At the end of AD, ortho-phosphate concentrations in all reactors were increased. A higher rate of increase in soluble phosphorus concentrations were seen in the pre-treatment applied reactors.

Table 5.3. Phosphorus concentrations in the reactors.

	Phosphorus (mg/L)		
Reactors	**PO_4^{3-}**	**P_2O_5**	**P**
A	910	680	300
B	2130	1590	700
C	1310	980	430
D	1520	1140	500
E	1200	900	390
F	1150	860	380
G	1300	970	420
H	1340	1000	440

(A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

5.1.1.7. Total Organic Carbon (TOC). TOC concentrations of the sludge samples in reactors were analyzed before and after the AD process, and the results are given in Figure 5.7.

Carbon fractions in the reactors also decreased with the pre-treatment applications during the anaerobic digestion similar to COD values. At the end of AD, highest reduction rates in TOC concentrations were obtained in reactors F, D, H and E as 29.6%, 25.1%, 21.8% and 20.2%, respectively, as the control reactor C having a reduction rate of 17.4%. MW pre-treatment obtained a reasonable increase in the removal efficiency of TOC as expected, and the efficiencies were improved by combining MW with the chemical oxidations.

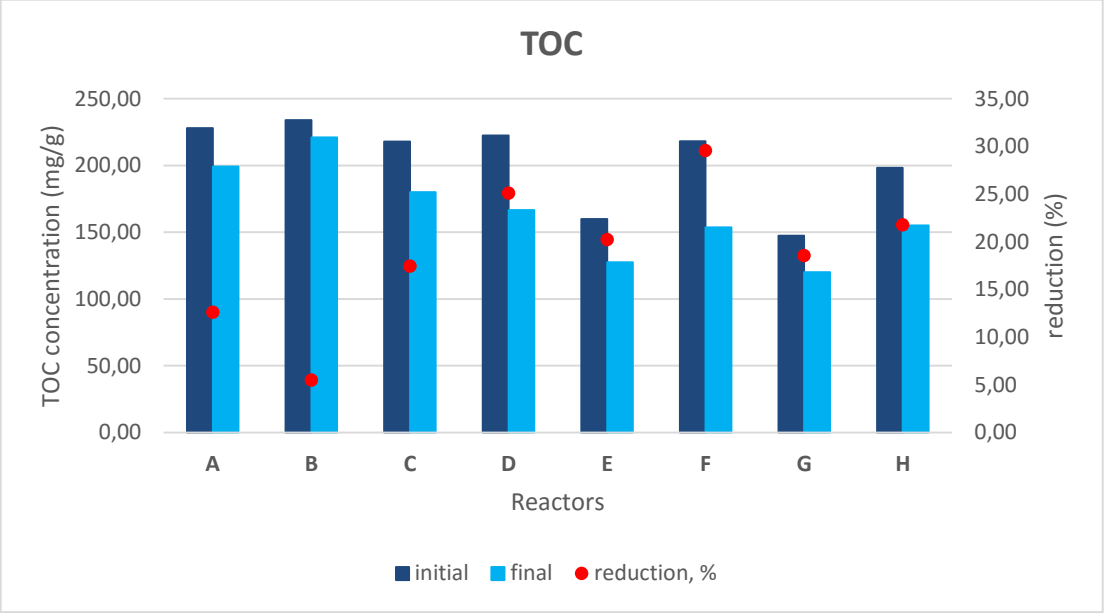

Figure 5.7. Initial and final TOC concentrations in the reactors and the reduction rates.

(A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

5.1.1.8. Volatile Fatty Acids (VFA). The volatile fatty acids (VFA) concentrations in the reactors were analyzed weekly during the AD. The variations of VFA concentrations are shown in Figure 5.8.

VFA is an important indicator in the AD process. The acids are degraded by the acetogens and the methanogenic bacteria that consuming hydrogen. If the concentrations of acetate and propionate are high, the degradation of the acids are inhibited. High amount of VFA can create an inhibitory effect on pH and so on the methane production during the anaerobic digestion (Appels et al., 2008).

Initially, acid concentrations in the reactors were lower, than the concentrations increased. Towards the end of the anaerobic digestion, acid concentrations decreased due to the degradation of the acids into methane and carbon dioxide. The changes of volatile fatty acids concentration during the anaerobic digestion can indicate the performance of the digestion. The methanogens produce biogas by consuming the VFAs during AD.

Nine types of VFAs were analyzed with the GC. In this study, all the measured types of VFAs were converted into the acetic acid and represented in Figure 5.8 below. The VFA concentrations in the anaerobic reactors changed between 1.3 to 778 mg/L as acetic acid. Only, the reactor H had a high concentration as 1343 mg/L once. The pre-treatments increased the VFA concentrations in the reactors before the anaerobic digestion. The highest amount of VFAs occurred in the H_2O_2 pretreated reactor (H), however towards the end of the AD the concentration of VFA decreased considerably due to the destruction of microbial cells and the biogas production.

It is known that, the volatile acids that accumulate in the digestion system have an inhibitory effect on methanogenic bacteria. In this study, the produced VFA concentrations did not create any inhibitory effect in AD, since the concentrations in the reactors were below the inhibitory range. Öztürk (1999), states that VFA concentrations for anaerobic treatment should not exceed 1000-1500 mg/L, and that the potential toxicity effect can be seen above these values. Sufficient alkalinity must be present in the environment to prevent this situation, and the ratio of VFA/alkalinity should not exceed 0.1. If this ratio is exceeded, the production of methane will be limited (Öztürk, 1999). In this study, the necessary amount of alkalinity was added into the each reactors, and so sufficient alkalinity was provided, the VFA/alkalinity ratio in all reactors were far below the limit 0.1. In another study, Wang et al. (2009) explained the different effects of VFAs in anaerobic digestion on bacteria and archaea. They stated that 2400 mg/L acetic acid and 1800 mg/L butyric acid concentrations do not create an important inhibition effect on the methanogens activity. VFA is a significant indicator for the reactor failure in anaerobic digestion (Wang et al., 2009).

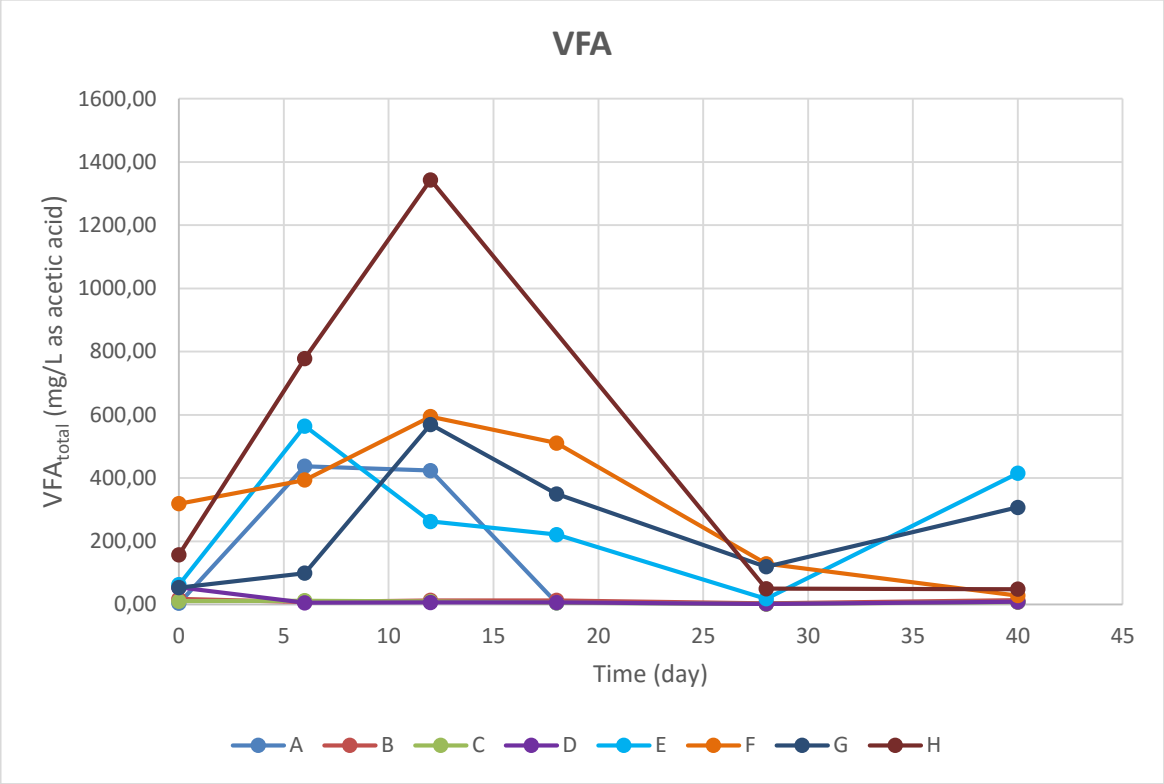

Figure 5.8. Variations of Volatile Fatty Acids (VFA) concentrations in the reactors. (A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

5.1.2. Biochemical Methane Production (BMP) of Pre-treatments Applied Anaerobic Reactors

In this part of the study, biochemical methane production of the sludge samples were tried to be improved by applying various pre-treatment methods before the anaerobic digestion in batch reactors at 37°C for 40 days. The BMP tests in the study were conducted according to the procedure that described by Owen et al. (1979) in serum bottles (Owen et al., 1979).

Biogas and methane amounts produced in the anaerobic reactors were measured periodically, and results are given in the Figure 5.9, Figure 5.10 and Figure 5.11 as the cumulative biogas and methane production in the reactors, and the methane yields in terms of organic removal, respectively.

5.1.2.1. Biogas Production. Total gas productions in the anaerobic reactors were measured daily by pressure method with the manometer. The pressure results of the produced gas were then converted into gas production volume.

Biogas consists of mainly methane (CH_4) and carbon dioxide (CO_2), and lower amounts of water vapor, hydrogen, nitrogen, hydrogen sulphide, unsaturated hydrocarbons and other gases (Gray, 2005).

The total gas production in the reactors represented the biogas productions in the anaerobic reactors. The amounts of cumulative biogas productions in the anaerobic reactors were given in Figure 5.9. All results were presented as the average values obtained from the parallel reactors.

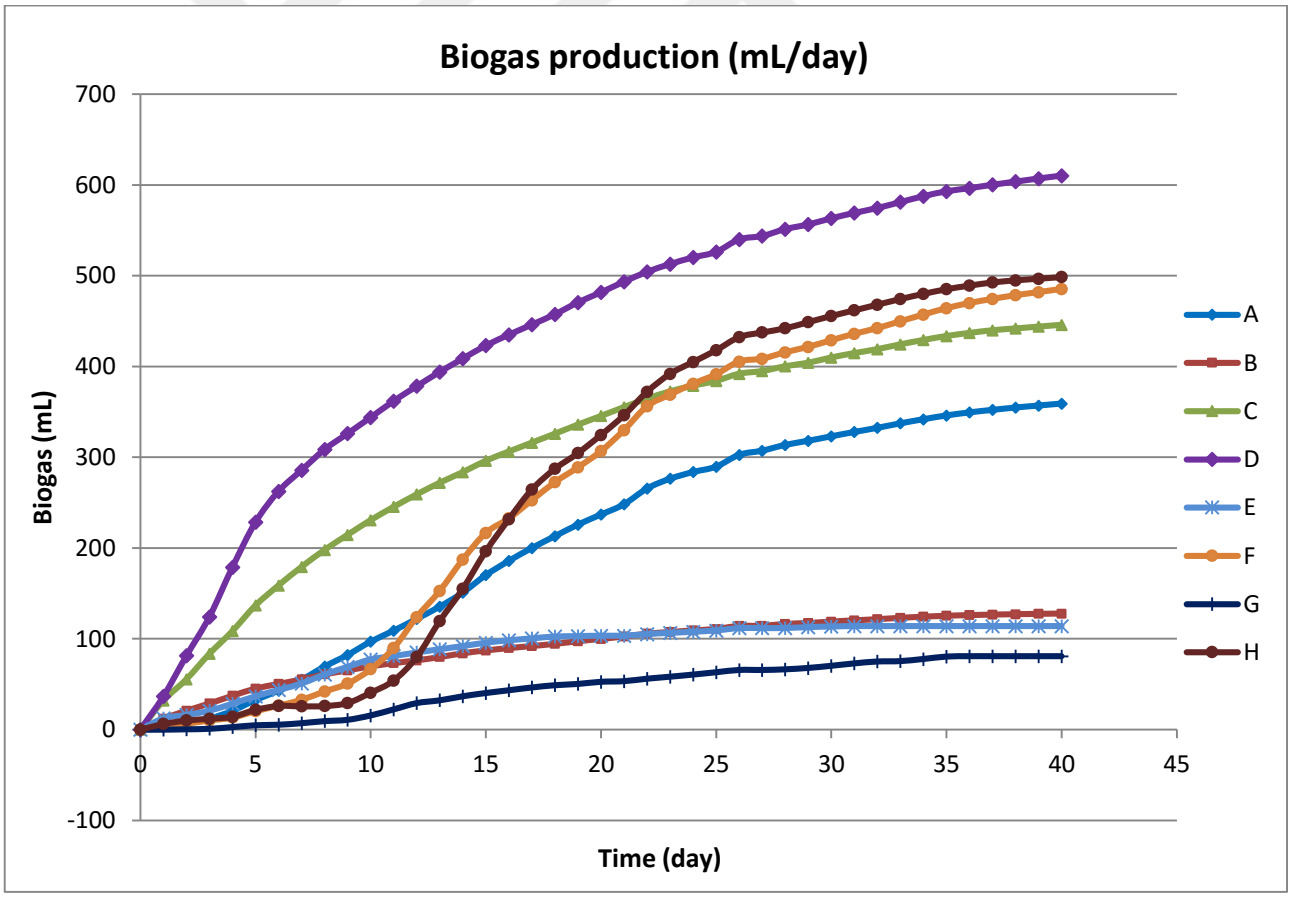

Figure 5.9. Cumulative biogas production in the reactors.

(A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

At first 10 days, only the reactors D and C showed an increasing gas production rate. The remaining reactors' acclimatization period in that time lasted longer. At the start and the end of the anaerobic digestion, biogas productions were slower. This slowness was related to specific growth of methanogenic bacteria that was linked to biogas production rate of batch reactors (Nopharatana et al., 2007). After the acclimatization period (lag phase), an exponential growth of methanogens were observed, and so biogas productions started to increase. After the day 11, the biogas production rates for the peroxide treated reactors were accelerated.

As the Figure 5.9 indicates, reactors D, H and F produced more biogas than the control reactors (610 mL/d, 499 mL/d and 485 mL/d, respectively). These results showed that the MW and H_2O_2 pre-treatment applications improved the biogas production. The contribution of the inoculum sludge was subtracted from each reactor, and it was found that the cumulative biogas productions were increased by 51.7%, 16.6% and 12.4% in reactors D, H and F, respectively.

In the study of Eskicioğlu et al. (2008), the effects of microwave pre-treatment on mesophilic digestion of sludge by using household and bench scale industrial microwaves at different temperatures, were analyzed. At the end of the anaerobic digestion, the 65ºC, 75ºC and 85ºC of microwave pre-treatments provided 10.8%, 10.9% and 16.2% increase in overall biogas production compared to controls, respectively. The MW pretreated sludge at 175°C showed 31% more biogas production (Eskicioglu et al., 2008).

The study of Yi et al. (2013) investigated different combinations of alkaline and low-temperature thermal pre-treatments for AD. With 0.05 g NaOH/g TS at 70°C an efficient solubilisation and biogas production were obtained at the end of the digestion. The accumulated biogas production for the control sample was 45 mL, and it was 329 mL for pretreated sludge. The average methane content of the produced biogas was 64 % (Yi et al., 2013).

Another study investigated the impact of alkali (KOH), thermal and oxidant (hydrogen peroxide or Fenton's reagent) pre-treatments on waste activated sludge

solubilisation and anaerobic biodegradability of waste activated sludge. The highest improvement in biogas production was provided as 54% at 130°C and pH = 10. The biogas production in 150 mmol/dm^3 H_2O_2 pre-treated digesters at 90°C was approximately same as raw sludge. However, the biogas production was increased 17% with 150 mmol/dm^3 H_2O_2 and 5 mmol/dm^3 $FeSO_4$ treatment at 90°C (Valo et al., 2004).

In the study Doğan and Sanin (2009), pH-10, pH-12, MW (alone), MW + pH-10 and MW + pH-12 pre-treatments of sludge were done before the AD in small scale batch reactors. Best results for total gas and methane productions were reached with MW + pH- 12 with an increase of 16.3% and 18.9% over control.

MW pre-treatment provided a significant improvement in the biogas production of the sludge sample. It is known that biogas production in the AD improves as volatile solids removal increases. However, even the VS removal was enhanced with persulfate oxidation; this outcome was not sustained with the biogas production of persulfate treated reactors. The biogas production was also in correlation with sCOD due to the biodegradation of organic matter for the generation of methane. sCOD removal couldn't achieved effectively with the persulfate treatments so as to biogas production.

The role of Fe(II)/$S_2O_8^{2-}$ oxidation on AD of waste activated sludge was investigated in the study of Zhen et al. (2013). The use of Fe(II)/$S_2O_8^{2-}$ oxidation introduced the dissolved sulfur compounds as $S_2O_4^{2-}$ along with S^{2-}. The dosages of Fe(II)/$S_2O_8^{2-}$ affected the production potential hydrogen sulfide (H_2S). It was seen from the study that the shock load of Fe(II)/$S_2O_8^{2-}$ oxidation pre-treatment had inhibitory effects on AD of waste activated sludge and H_2S production. TSS and VSS removals were also lower with the higher Fe(II)/$S_2O_8^{2-}$ dosages (0.8 mmol $S_2O_8^{2-}$/g VSS and 1.0 mmol Fe(II)/g VSS & 1.2 mmol $S_2O_8^{2-}$/g VSS and 1.5 mmol Fe(II)/g VSS). Fe(II)/$S_2O_8^{2-}$ pre-treatment on anaerobic digestion was dosage dependent, with high dosages inhibition occurred, and with some dosages H_2S production in biogas increased (Zhen et al., 2013).

In this study, persulfate treatments were not be able to achieve biogas production. This result can occurred due to the inhibition effects of $S_2O_8^{2-}$ oxidation dosages on methanogenic bacteria in AD of wastewater sludge (will be explained in next section in detail).

5.1.2.2. Cumulative Methane Production. In the anaerobic digestion, methane and carbon dioxide are the major components of biogas, including 55-70% and 30-45% by volume of the biogas, respectively. Methane is the most important gas due to its usage in energy production, and also the methane content of the biogas indicates the stability and performance of the anaerobic reactors (Gray, 2005).

The methane content of the biogas produced in the anaerobic reactors were analyzed every week during the AD period. In biogas production, the main important point is the methane percentage of the produced biogas. Except the reactors E and G, containing $S_2O_8^{2-}$ pretreated sludge samples, the methane percentages of the biogas were between 55% - 65% on average. These amounts of methane in the biogas show that the quality of biogas was good. MW and H_2O_2 pre-treatment methods had an additional positive effect on the methane content of biogas produced in the system. In reactor E, the methane percentages of the biogas were between 20% - 30% on average. Methane percentage of the biogas was 0% for G, so no methane production occurred in reactor G.

The amounts of cumulative methane productions in the anaerobic reactors were given in Figure 5.10. All results were presented as the average values, obtained from the parallel reactors.

As the Figure 5.10 indicates, reactors D, F and H produced more methane than the control reactor containing untreated sludge (387 mL/d, 340 mL/d and 304 mL/d, respectively). These results showed that MW and H_2O_2 pre-treatments improved the methane production. The contribution of the inoculum sludge was subtracted from each reactor, then, it was found that the cumulative methane productions were improved by 65.5%, 39.8% and 19.8% in reactors D, F and H, as compared to the control reactor.

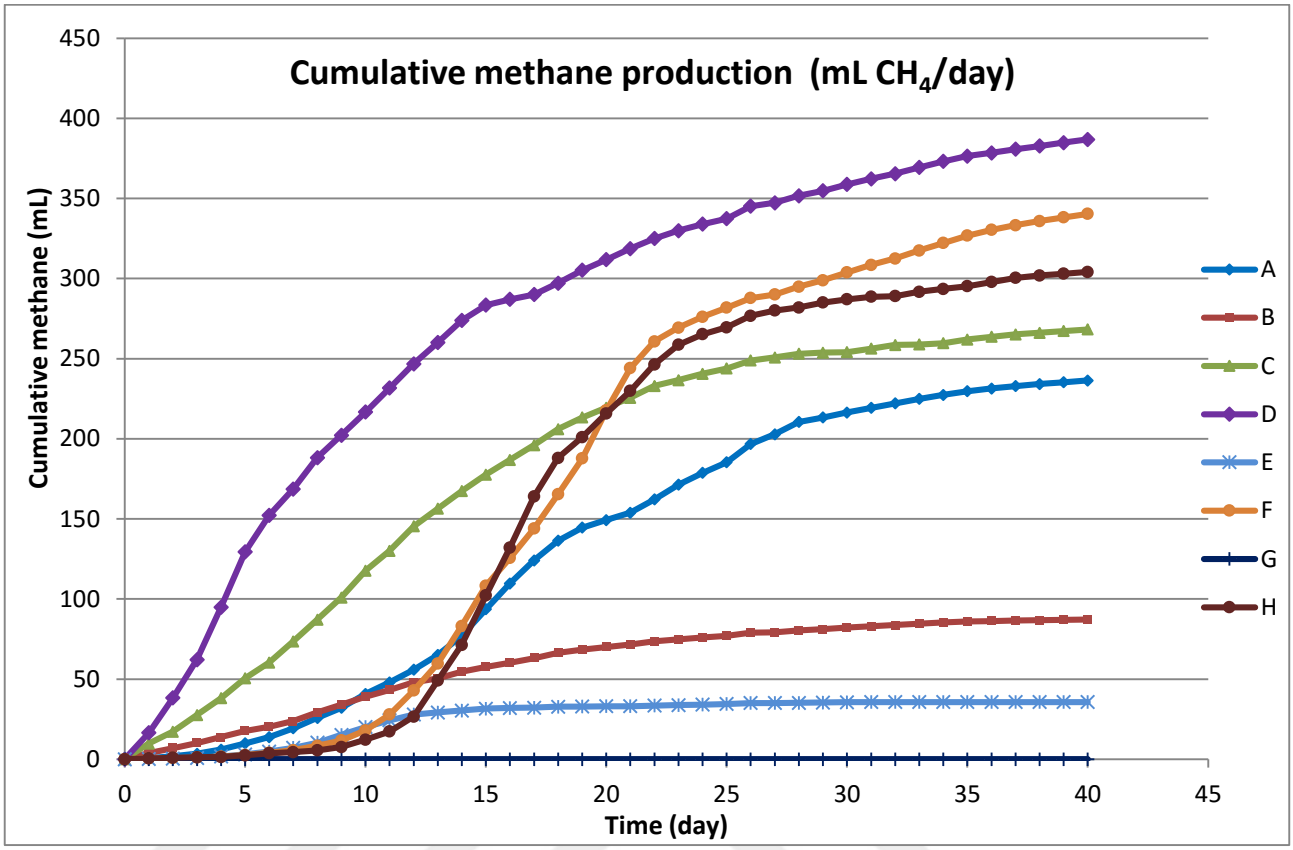

Figure 5.10. Cumulative methane production in the reactors.

(A = SS, B = Inoculum, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

The maximum amount of cumulative methane productions were obtained in the MW and the combined MW/H_2O_2 pretreated reactors. MW showed higher production amount than the combined treatment. It could be related to addition of chemicals can limit the positive effect of high temperature thermal pre-treatments. In the study of Valo et al. (2004), it was found that chemical additions with MW pre-treatments limit the effect of pre-treatment methods. In their study, MW pre-treatment had higher methane production over combined MW and alkaline pre-treatment (Valo et al., 2004).

In the study of Takashima and Tanaka (2014), acidic thermal treatments with pH 2, 4 and 6 at 25°C, 100°C and 180°C were applied to sewage sludge as the post-treatment of AD. This process obtained 14–21% more methane production compared to the control (Takashima and Tanaka, 2014). The study of Lin et al.

(1999) showed that the methane gas production increased by 34%, over the control with 40 meq/L of NaOH treatment (Lin et al., 1999).

The study of Wong et al. (2006) showed that microwave treatment process limited the microbial activity, and with hydrogen peroxide, the sterilization of pathogens were achieved. The solubilisation of COD was increased by MW, and also with the addition of H_2O_2 (1 mL or 2 mL H_2O_2 for 30 mL of sludge samples) it was improved more. That was an important point since, the AD uses the soluble COD to convert it into methane by methanogenesis. That means more COD solubilise, more efficient biogas production occurs (Wong et al., 2006).

In this study, persulfate oxidation did not performed good in the methane production. This situation showed us, an inhibition was occurred in persulfate treated reactors. When the contents of the biogas produced in reactors E and G analyzed, there was no oxygen observed (only nitrogen and carbon dioxide). That means the reactors worked in anaerobical conditions. Other inhibition sources can be VFA and ammonia. The VFA concentrations in the reactor were analyzed and seen that the reactors did not have very high concentration of VFAs, and concentrations were under the safe limits, for a satisfactory AD process, given in the literature. Similarly, the ammonia concentrations were in the safe limits. According to the literature, total ammonia nitrogen concentrations of 1.7 g/L or higher are known to inhibit methanogens (Franke-Whittle et al., 2014). In anaerobic digestion, ammonium-nitrogen concentration increases, and the methanogenic sludge can adapt to concentrations up to 1700 mg/L (Koster and Lettinga, 1984). Therefore, after the evaluation of all possible inhibition reasons, this situation was taught to be because of the inhibition effect of persulfates on the methanogens. The applied dosage of persulfate was probably excessive for methanogens. After the application of $S_2O_8^{2-}$ to the sludge samples, the pH of reactor contents decreased sharply to pH values of 3.4 - 5 and probably caused to the death of methanogenic bacteria. Therefore, the methanogenesis step of the AD process failed.

In the study of Sun et al. (2012), a conventional persulfate treatment with 0.1 g $K_2S_2O_8$/g SS was applied, and anaerobically digested for 20 days at 35^oC. The

VFA concentrations were between 210-580 mg/L during AD. At the end of the study, the cumulative gas production was increased by 44.9% (Sun et al., 2012). The persulfate dose used in their study was one tenth of that used in this study.

Isa et al. (1986) studied the effect of different levels of sulfate and sulfide on methane production relative to sulfate reduction in high-rate anaerobic digestion. Several mechanisms existed to show the inhibitory effect of the presence of sulfate on methanogenesis in natural ecosystems and in anaerobic digesters. The study showed that sulfate concentrations up to 5 g of sulfate S per liter did not significantly affect methanogens. However, higher concentrations of sulfate (more than 10 g of sulfate S per liter) created inhibition due to the salt toxicity. The inhibition of methanogens was also related to the levels of sulfide generation during the microbial reduction of sulfate. High concentrations of free H_2S had a slight effect on the sulfate-reducing bacteria (SRB). The sulfide ions and the free H_2S in the liquid phase affected the acetoclastic methane-producing bacteria (MPB) strongly. The acetoclastic MPB and also the hydrogenotrophic MPB were only inhibited at free H_2S concentrations of more than 1,000 mg/L. The presence of sulfate and sulfide provided the precipitation of nonalkali metals in the digester, which influenced the growth of the microorganisms that decreased the biogas production of the digester. The study indicated that the rate of biogas production reduced at a sulfate concentration of 10 g/L because of the salt toxicity resulted from the addition of Na_2SO_4 (Isa et al., 1986a) and (Isa et al., 1986b). In this study, the concentration of persulfate was 1g/g TS and that equalled to 13.6 g/L, which was higher than the concentrations that caused to the salt toxicity.

Another study showed the similar result that was investigated the effect of sulfate on methane production in Lake Mendota sediments and the mechanism of sulfate inhibition of methanogenesis. It was found that as little as 0.2 mM sulfate inhibited the methanogenesis (Winfrey and Zeikus, 1977).

Anaerobic digestion of organic wastes has four steps. In the hydrolysis step, insoluble organic compounds are converted into soluble monomers. Then in the acidogenesis step, acidification occurred with the acidifying bacteria by converting

water-soluble chemical substances to short-chain organic acids. In the acetogenesis step, the acetate bacteria convert the acid phase products into acetates and hydrogen which may be used by methanogenic bacteria. Lastly in the methanogenesis step, methane is produced by methanogenic bacteria. It is a fact that only a few bacteria are able to produce methane from acetic acid. The methanogenic bacteria can survive at pH from 6.8 to 7.2 (Shah et al., 2014).

There are various types of methanogenic bacteria. At low pH, aceticlastic and non-aceticlastic methanogenesis was seriously inhibited. The aceticlastic methanogenic process was performed by aceticlastic methanogens and Methanosarcina species especially with the consumption of VFAs. The non-aceticlastic methanogenesis process was performed by both hydrogenotrophic methanogens and Methanosarcina species with the consumption of acetate and H_2/H_2CO_3 (Qu et al., 2009).

To conclude, sulfate concentrations used in this study were thought be high for methanogenic activity, and that high sulfate dose created an inhibitory effect on methanogenesis by destroying especially the non-aceticlastic methanogens, since the persulfate pre-treatment resulted in lower rates of sCOD reduction. When looking at the reactors' initial results; after the application of $S_2O_8^{2-}$ pre-treatment (1 mg $S_2O_8^{2-}$/g TS) to the sludge samples, the pH in reactors E & G were decreased sharply to pH values of 3.4 and 5.0, and probably caused to the death of methanogenic bacteria and led to survival of acid formers only. The pH values in the reactors were then adjusted to about 7 to provide an effective AD process. However, it was understood that the sudden decrease of pHs in the reactors after the persulfate pre-treatments had already destroyed the methanogenic bacteria before starting the AD process. Therefore, no methane production occurred in the $S_2O_8^{2-}$ prtreated reactor (G) since, the methanogenesis step of the AD process failed. In combined MW/$S_2O_8^{2-}$ reactor (E), there was little amount of methane production but when the inoculum sludge contribution subtracted, it reached zero production. That little amount of generation might be sourced from the remedial effect of the microwave pre-treatment on the pH value in reactor.

5.1.2.3. Methane Yield. The methane yields in the reactors were presented in Figure 5.11 as mL methane/g VS_{added}. Pre-treatments increased both biogas and methane yields due to increase in solubilisation of organic matters. After the application of MW, combined MW/H_2O_2 and conventional H_2O_2 pre-treatments, methane yields were improved by the ratios of 65.5%, 40% and 20%. The highest yields were provided as 626 mL CH_4/g VS, 529 mL CH_4/g VS and 453 mL CH_4/g VS in reactors D, F and H, respectively.

In the study of Song et al. (2013), it was stated that the pre-treatment process of hydrogen peroxide (H_2O_2) improved the biogas yield and the biodegradation performance of rice straw. H_2O_2 pre-treatment of rice straw increased the methane yields during biogas production. 3% hydrogen peroxide concentration (w/w total solid) provided a methane yield of 290 mL/g VS, 88% higher than untreated rice straw (Song et al., 2013).

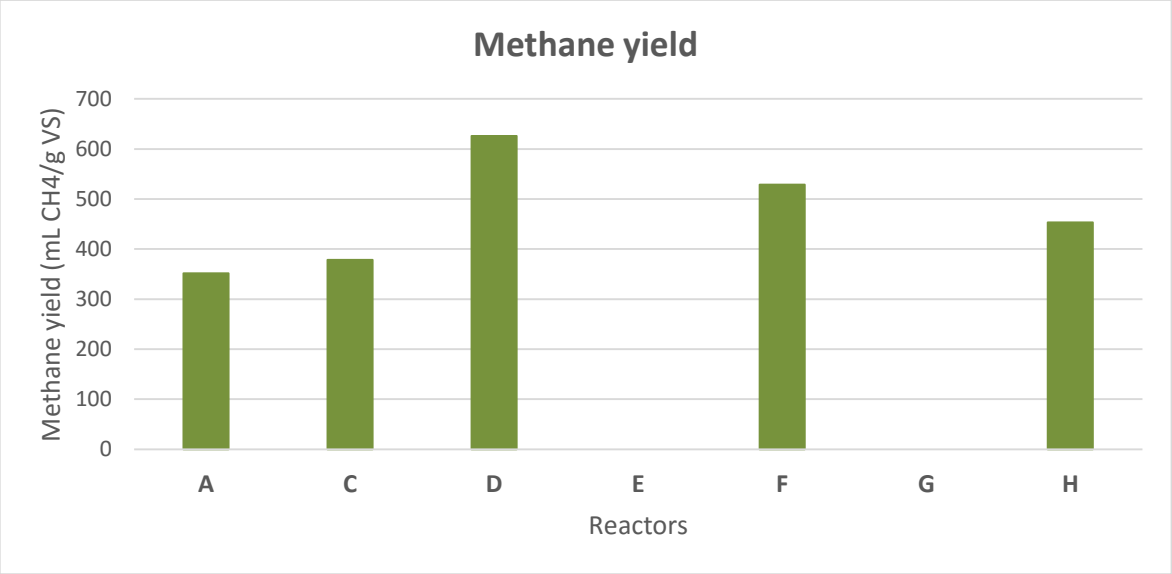

Figure 5.11. Methane yields in the reactors.

(A = SS, C = SS + Inoculum (control), D = MW, E = MW/$S_2O_8^{2-}$, F = MW/H_2O_2, G = $S_2O_8^{2-}$, H = H_2O_2.)

5.2. Results of Antibiotic Contaminated Anaerobic Reactors

Antibiotics are the contaminants existing in the waste waters and wastewater sludges that are arisen from the pharmaceutical industries and the wastes of the livestock farms.

In this part of the study, to see the effect of the antibiotic presence to sludge disintegration and the methane production, 1 mg CIP/g TS was synthetically added into the sewage sludge. MW pre-treatment was applied to both the raw and the contaminated sewage sludge. In batch reactors; untreated and antibiotically contaminated sewage sludges alone, their mixture with inoculum sludge (I:S = 1:1) and MW pretreated sewage sludges plus inoculum sludge were anaerobically digested for 40 days.

The results obtained from the antibiotic contaminated anaerobic reactors are presented in the following part.

5.2.1. Sludge Properties of Antibiotic Contaminated Anaerobic Reactors

One of the six parallels of the seven different reactor sets was opened weekly to take sludge samples for pH, alkalinity, TS, VS, COD, sCOD, NH_3-N and volatile fatty acids (VFA) analyses.

The reactor sets A and B, containing only sewage sludge and only inoculum sludge were set up as control reactors. The reactor set C contained inoculum and unpretreated sewage sludge samples, reactors D contained inoculum and MW pretreated sewage sludges. The reactor sets K, L and M were antibiotic contaminated version of the reactor A, C and D, respectively.

5.2.1.1. pH & Alkalinity. Table 5.4 presents the pH values in the reactors. As explained in previous section, the pH values of the reactor contents were adjusted to be in the favorable range of 7 - 7.2 for AD. pH values in the reactors showed an increasing trend throughout the AD process and varied in a range between 7 to 8.

The antibiotic CIP (1 mg/g TS) did not have an important effect on pH. The pH values in the antibiotic contaminated reactors were similar to the pH in the control reactor.

Table 5.4. pH values in the reactors.

	pH values						
	Days						
Reactors	**$0_{initial}$**	**$0_{adjusted}$**	**6**	**12**	**18**	**28**	**40**
A	6.65	7.1±0.1	7.14	7.25	7.55	7.60	7.84
B	7.60	7.1±0.1	7.51	7.82	7.68	7.99	8.01
C	7.48	7.1±0.1	7.10	7.28	7.38	7.56	8.10
D	7.50	7.1±0.1	7.15	7.46	7.45	7.59	7.56
K	6.67	7.1±0.1	7.22	7.39	7.49	7.58	7.70
L	7.33	7.1±0.1	7.16	7.50	7.31	7.47	7.67
M	7.50	7.1±0.1	7.25	7.44	7.45	7.64	7.88

(A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

The variations of alkalinity concentrations in the reactors are shown in Figure 5.12. The alkalinity of the reactors' contents showed a similar trend and the alkalinity concentrations stayed in the favorable range for AD. The inoculum sludge had a really high alkalinity concentration as expected. The alkalinity concentrations of the antibiotic contaminated reactors were slightly lower than the control reactor (no antibiotic containing reactor).

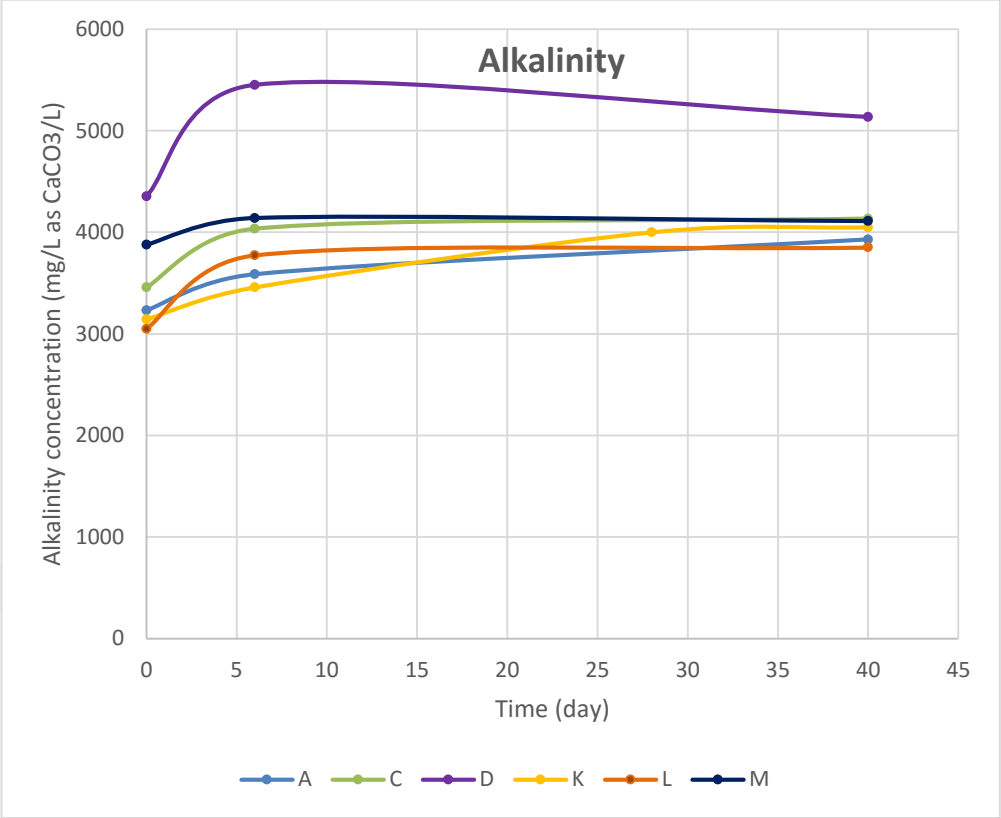

Figure 5.12. Variations of alkalinity concentrations in the reactors.

(A = SS (control), C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

5.2.1.2. Total Solids and Volatile Solids (TS & VS). The total and the volatile solids concentrations in the reactors were measured weekly. The variations in TS and VS concentrations are given in Figure 5.13 and Figure 5.14, respectively.

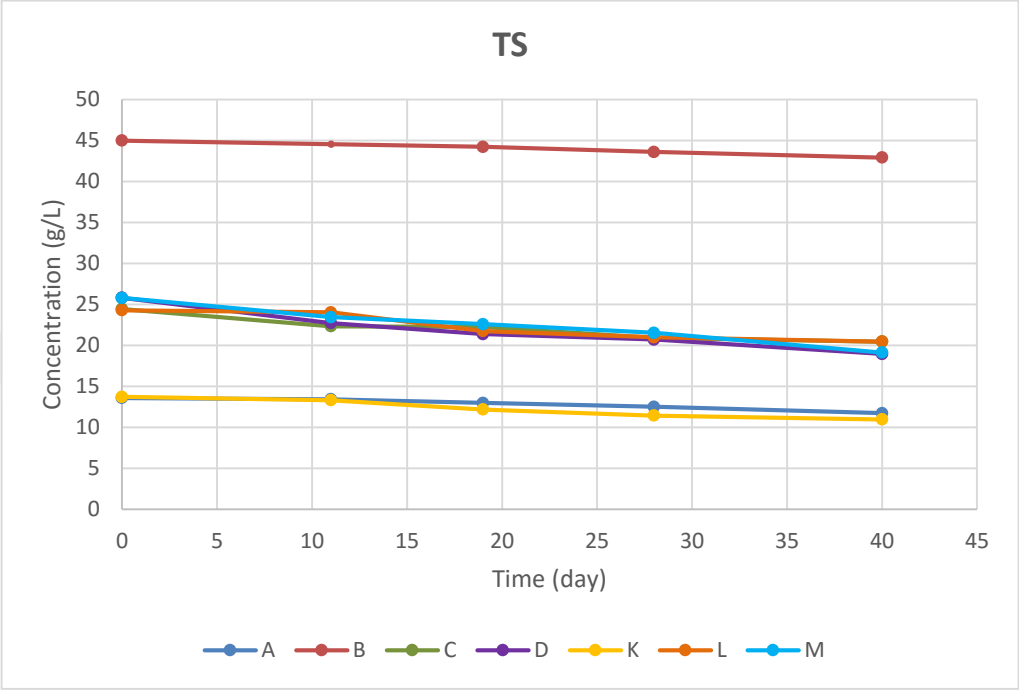

Figure 5.13. TS concentration variations in the reactors.

(A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

The initial TS concentrations of inoculum sludge in reactor B, sewage sludge in reactor A and antibiotic contaminated sewage sludge in reactor K were measured to be 45 g/L, 13.6 g/L and 13.74 g/L, respectively. Their mixture with inoculum sludge and the microwaved versions in reactors C, L, D and M had initial TS concentrations in the range of 24 - 26 g/L. The antibiotic presence in the sewage sludge caused a little increase in the initial TS concentrations as expected due to the addition of antibiotic CIP.

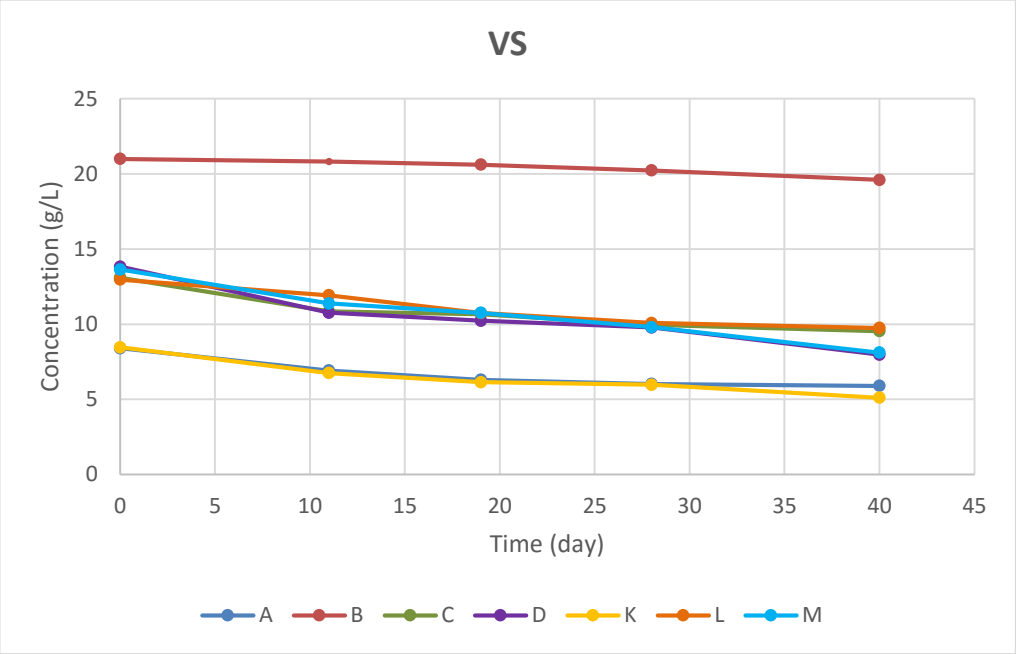

Figure 5.14. VS concentration variations in the reactors.

(A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

The initial VS concentrations of inoculum sludge in reactor B, sewage sludge in reactor A and antibiotic contaminated sewage sludge in reactor K were measured to be 21 g/L, 8.4 g/L and 8.46 g/L, respectively. Their mixture with inoculum sludge and the microwaved versions in reactors C, L, D and M had initial VS concentrations in the range of 12.9 - 14 g/L. The antibiotic contaminated sludge containing reactors showed slightly higher initial VS concentrations related to their increased initial TS concentrations.

At the end of the AD, MW pre-treatment increased both the TS and VS removal rates. The TS and VS removal rates were not affected negatively due to presence of antibiotics with the concentration of 1 mg CIP/g TS.

In the study of Masse et al. (2000), different types of antibiotics (named: Carbadox, Tylosin, Penicillin, Tetracycline, Sulphamethazine, Lyncomycin) with concentrations of 16 - 550 mg/kg dry matter were used and they found that the presence of antibiotics in the pig manure did not show a negative effect on the treatment efficiency. At the end of the study, high removals rates of TS and VS (65%

and 75%, respectively) were achieved (Masse et al., 2000). In the study of Arikan et al. (2006), it was shown that the presence of 9.8 mg/L OTC in the manure slurry did not have negative impacts on the AD process stability. There were not important changes in the removal rates of VS and soluble organic carbon, and also in methane content of the biogas produced (Arikan et al., 2006).

5.2.1.3. Chemical Oxygen Demand and Soluble Chemical Oxygen Demand (COD & sCOD). COD and sCOD concentrations in the reactors were analyzed weekly. The variations of the concentrations during the anaerobic digestion and the removal efficiencies are illustrated in Figure 5.15 and Figure 5.16, respectively.

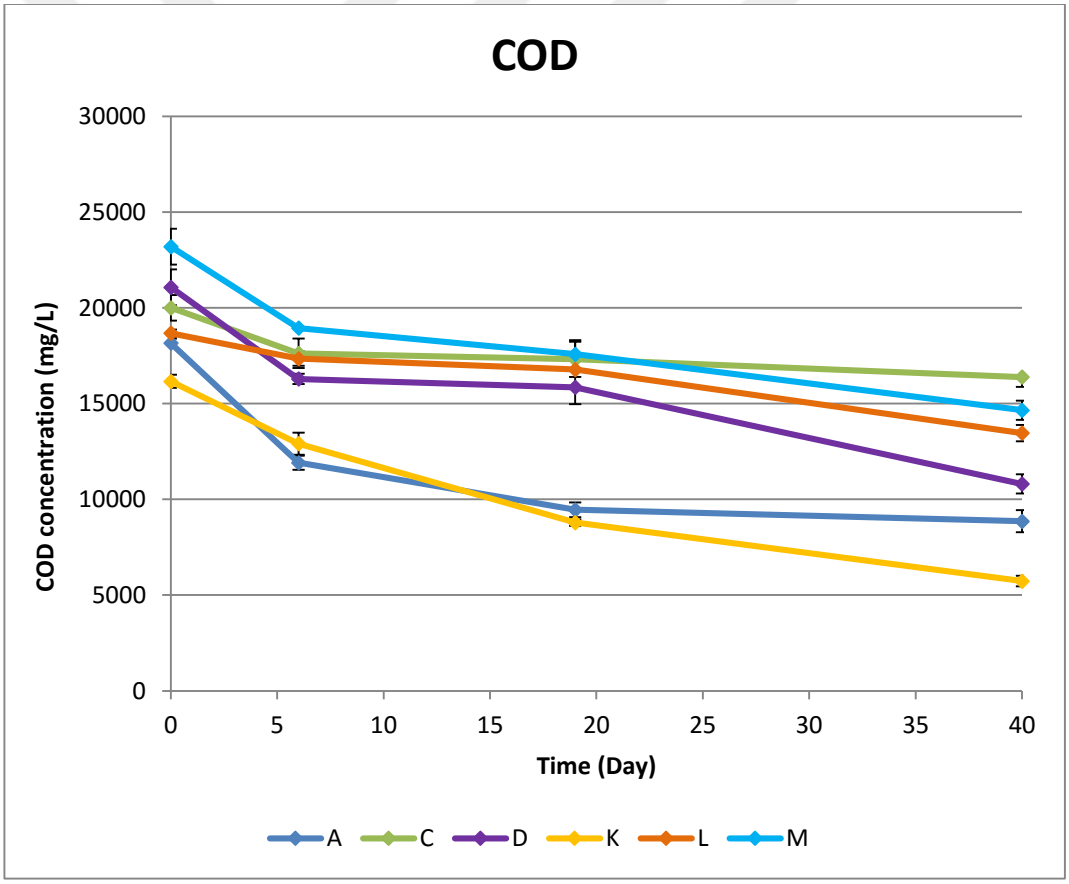

Figure 5.15. Variations of COD concentrations in the reactors.

(A = SS (control), C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

The initial COD concentrations of inoculum sludge in reactor B, sewage sludge in reactor A and antibiotic contaminated sewage sludge in reactor K were measured

to be 40743 mg/L, 18159 mg/L and 16166 mg/L, respectively. The antibiotic presence in the sewage sludge decreased the COD concentrations in the reactors since the presence of the antibiotics slowed down and decreased the microbial activity in sludge.

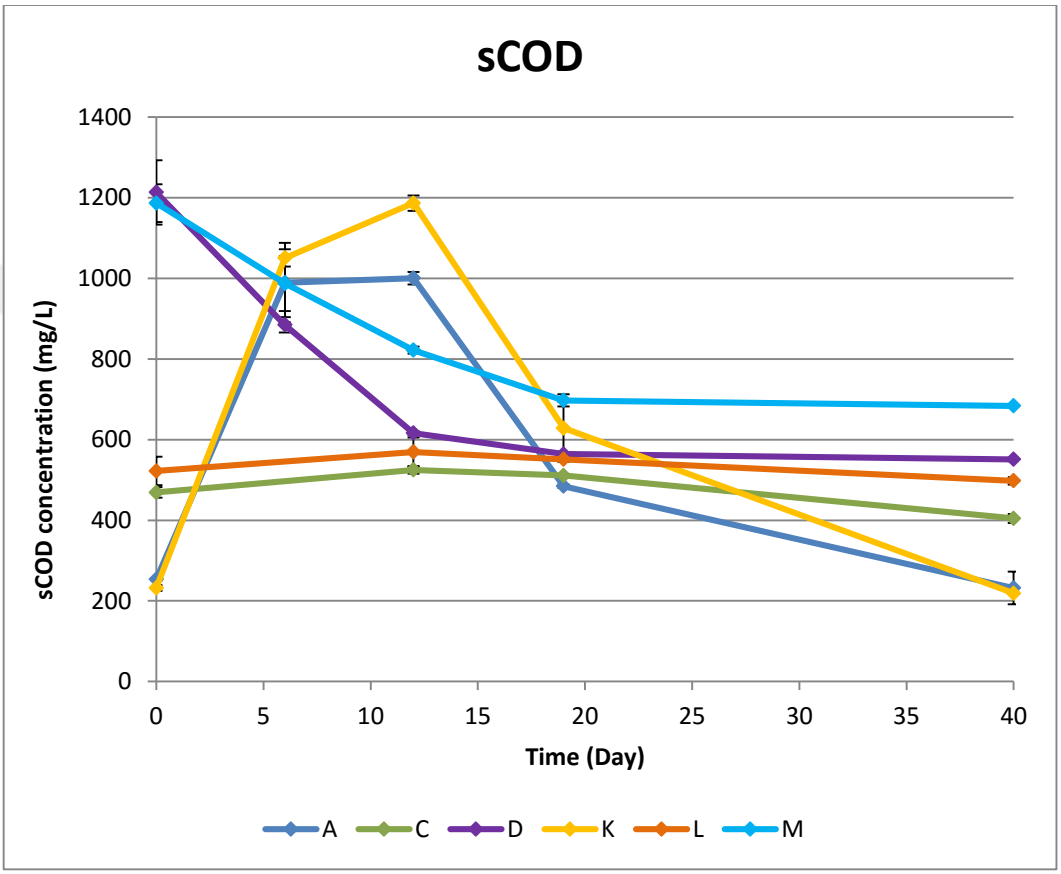

Figure 5.16. Variations of sCOD concentrations in the reactors.

(A = SS (control), C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

The initial sCOD concentrations in the reactors were changing between 253.3 and 1213.2 mg/L. The initial COD concentrations of inoculum sludge in reactor B, sewage sludge in reactor A and antibiotic contaminated sewage sludge in reactor K were measured to be 1040.4 mg/L, 253.3 mg/L and 232mg/L, respectively. The antibiotic presence in the sewage sludge also decreased the sCOD concentrations in the reactors.

At the end of the AD, MW pre-treatment increased both the COD and sCOD removal rates. The presence of antibiotics (1 mg CIP/ g TS) did not affect negatively the removal rates of COD and sCOD during the AD process.

Akyol et al. (2016) investigated the behaviour of the bacterial and archaeal communities during single-stage and two-stage AD of OTC existent cattle manure (82.5 mg OTC/kg dry manure). Their study showed that the OTC-medicated methanogenic digester had lower concentrations of sCOD than the non-medicated methanogenic digesters (Akyol et al., 2016). With another study, Akyol et al. (2014) investigated the acidogenic phase of OTC-medicated and non-medicated cattle manures in two-phase AD. At the end of the study, with the OTC concentration of 82.5 mg/kg dry manure, it was found that OTC-medicated manure slurries had lower concentrations of sCOD than the non-medicated ones (Akyol et al., 2014).

However, Masse et al. (2000) found that the presence of Carbadox, Tylosin, Penicillin, Tetracycline, Sulphamethazine, Lyncomycin antibiotics with the concentrations between 16 - 550 mg/kg dry matter in the pig manure did not have a negative effect on the treatment efficiency. At the end of the study, high removal rates of COD and sCOD (62% and 76%, respectively) were achieved (Masse et al., 2000).

5.2.1.4. Total Kjeldahl Nitrogen (TKN). TKN concentrations in the reactors were analyzed and the results are given in Table 5.5.

The antibiotic presence in sludge samples increased the initial TKN concentrations in the reactors as shown in Table 5.5.

Table 5.5. TKN concentrations in the reactors.

Reactors	TKN (mg/L)
A	735
B	1808
C	1043
D	1290
K	760
L	1590
M	1500

(A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

5.2.1.5. Ammonia Nitrogen (NH_3-N). Ammonia concentrations showed mostly an increasing trend during AD, as expected. Ammonia concentrations can create an inhibitory effect for methanogens, if the concentrations of ammonia increase up to a range of between 4000 and 6000 mg/L (Fricke et al., 2007; Chen et al., 2008). In this study, ammonia concentrations in the reactors were in the safe range; so the level of ammonia concentrations didn't create an inhibitory effect on methanogens.

The study of Akyol et al. (2014), investigating the acidogenic phase of OTC-medicated and non-medicated cattle manures in two-phase AD, showed that OTC-medicated manure slurries (82.5 mg OTC/kg dry manure) had slightly higher concentrations of NH_3−N than the non-medicated ones (Akyol et al., 2014). In this study, it was observed that the NH_3−N concentrations were showed a little increase in the antibiotic existent reactors initially.

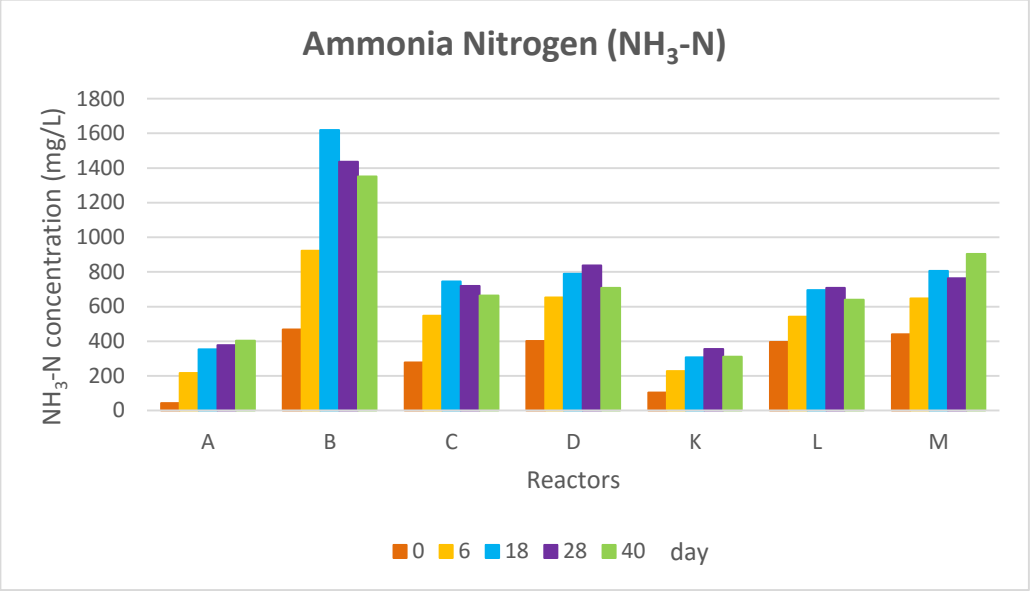

Figure 5.17. Variations of ammonia nitrogen (NH_3-N) concentrations in the reactors. (A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

5.2.1.6. Total Phosphorus. Phosphorus concentrations of the sludge samples in reactors were analyzed and the results are given in Table 5.6.

As shown in Table 5.6; the initial ortho-phosphate concentrations of the sludge samples in the reactors were increased with the MW pre-treatment. However, the antibiotic presence slowed down the solubilisations of phosphorus in the reactors.

Table 5.6. Phosphorus concentrations in the reactors.

	Phosphorus (mg/L)		
Reactors	**PO_4^{3-}**	**P_2O_5**	**P**
A	910	680	300
B	2130	1590	700
C	1310	980	430
D	1520	1140	500
K	850	630	280
L	1080	810	350
M	1740	1300	570

(A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

5.2.1.7. Total Organic Carbon (TOC). TOC concentrations of the sludge samples in the reactors were analyzed initially before and after the AD process and the results are given in Figure 5.18.

The presence of 1 mg/g CIP antibiotic harmed the bacterial community, and so carbon fractions in the antibiotic containing reactors decreased the during the AD process. MW pre-treatment increased the reduction rates, as expected. The highest TOC removal rates were obtained in reactors D and M as 25.1% and 18.1%.

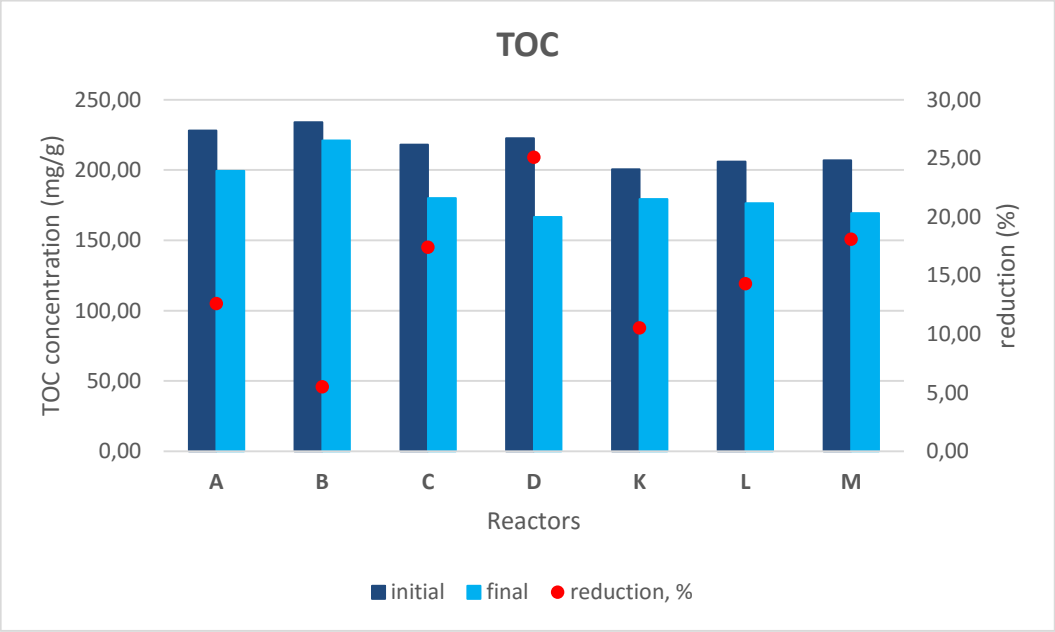

Figure 5.18. Initial and final TOC concentrations in the reactors and the reduction rates.

(A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

5.2.1.8. Volatile Fatty Acids (VFA). The volatile fatty acids (VFA) concentrations in the reactors were analyzed weekly during the anaerobic digestion. The variations in VFA concentrations are shown in Figure 5.19.

Nine types of VFAs were analyzed with the GC. In this study, all the measured types of VFAs were converted into the acetic acid and represented in Figure 5.19 below. Initially, acid concentrations in the reactors were lower, than the concentrations increased especially in the only sewage sludge containing reactors

(A & K) up to 400 – 550 mg/L as acetic acid. Towards the end of the anaerobic digestion, acid concentrations decreased due to the degradation of the acids into methane and carbon dioxide. The changes of volatile fatty acids concentration during the anaerobic digestion can indicate the performance of the digestion. The VFA concentrations in the remaining reactor were really low as 0 – 15 mg/L as acetic acid during the AD process. Therefore, no VFA accumulation was observed in all reactors at the end.

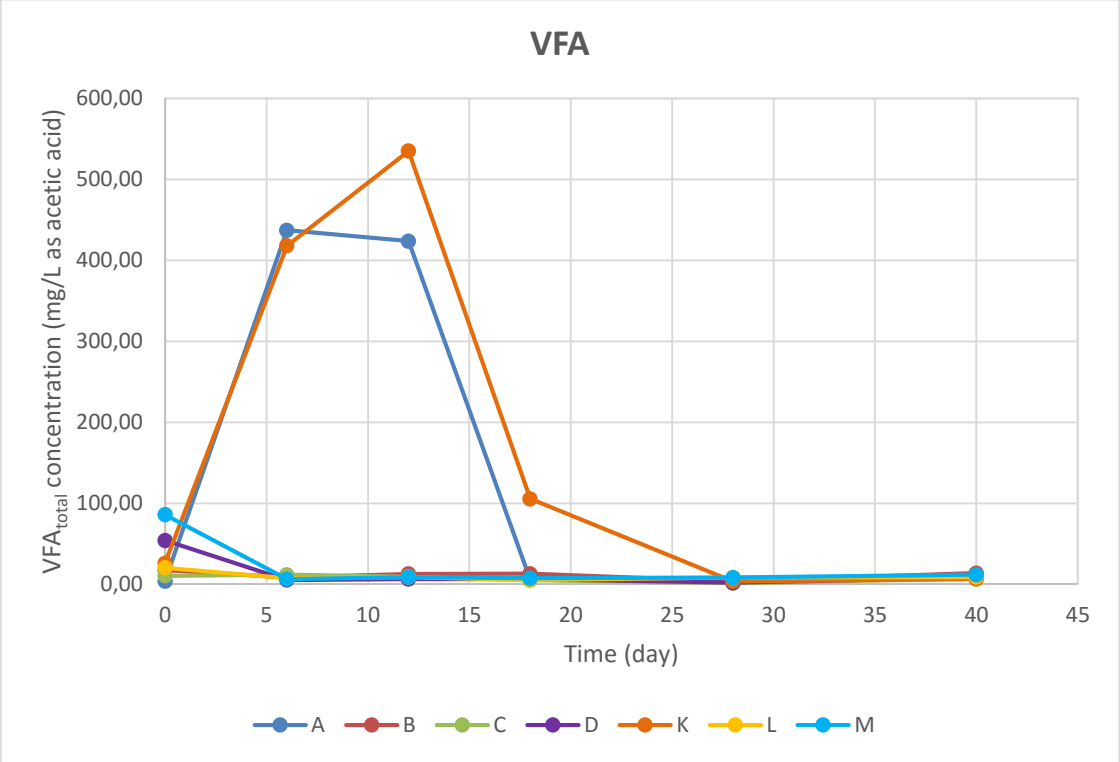

Figure 5.19. Variations of Volatile Fatty Acids (VFA) concentrations in the reactors. (A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

5.2.2. Biochemical Methane Production (BMP) of Antibiotic Contaminated Anaerobic Reactors

In this part of the study, the effect of antibiotic contamination on the biochemical methane production of sludge samples during the anaerobic digestion in batch reactors were investigated at 37°C for 40 days. The BMP tests in the study were conducted according to the procedure that described by Owen et al. (1979) in serum bottles (Owen et al., 1979).

Biogas and methane amounts produced in the anaerobic reactors were measured periodically, and results are given in the Figure 5.20, Figure 5.21 and Figure 5.22 as the cumulative biogas and methane production in the reactors, and the methane yields in terms of organic removal, respectively.

5.2.2.1. Biogas Production. Total gas productions in the anaerobic reactors were measured daily, and represented the biogas productions in the anaerobic reactors. The amount of cumulative biogas productions in the anaerobic reactors were given in Figure 5.20. All results were presented as the average values obtained from the parallel reactors.

The methanogens showed an exponential growth, and so biogas productions increased exponentially. At the end of AD, biogas productions were slower. This slowness was related to specific growth of methanogenic bacteria that was linked to biogas production rate of batch reactors (Nopharatana et al., 2007).

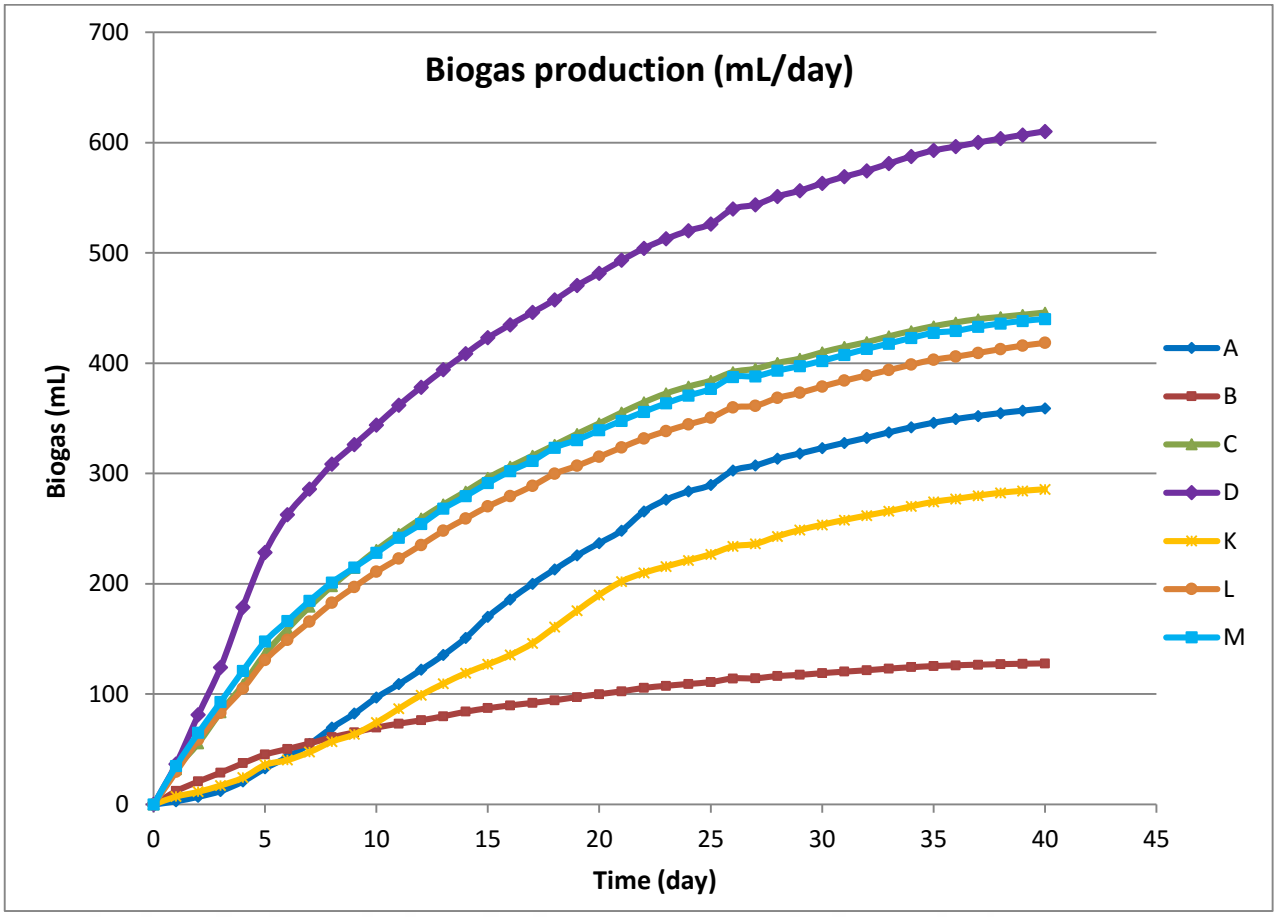

Figure 5.20. Cumulative biogas production in the reactors.

(A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

As the Figure 5.20 indicates, in reactor D, the highest amount of biogas produced as 610 mL/d. The reactors C, M and L were followed with the biogas productions of 446 mL/d, 440 mL/d and 418 mL/d, respectively. MW pre-treatment provided an important improvement in the biogas production of the sludge samples. However, the antibiotic presence affected the biogas productions negatively, especially in MW pre-treatment applied reactors. The contribution of the inoculum sludge was subtracted from each reactor, and it was found that the cumulative biogas productions were increased by 51.7% and 7.4% in reactors D and M, as compared to control reactors C & L.

The presence of antibiotic decreased the biogas production rates of sludge samples. The amount of biogas productions in antibiotic contaminated reactors M and K were lower than D and A with the ratios of 35.3% and 20.5%, respectively.

Sanz et al. (1996) stated that the antibiotics can disturb the production of biogas during AD. They studied with a wide group of antibiotics (Rifampicin, Lactamics, Tetracyclines, Macrolides and Chloramphenicol) to understand the effect of them on the different bacterial populations. Their results showed that some antibiotics had partial inhibitory effects on AD and decrease methane production by reducing the activity of acetoclastic methanogens; being active only on the acetogenic bacteria (which convert propionate and butyrate into acetate) and, methanogenic archaea (which convert acetate into methane). As a result, it was understood that antibiotics cause serious problems in methane production (Sanz et al., 1996).

The study of Akyol et al. (2016), investigating the behaviour of the bacterial and archaeal communities during AD of OTC existent cattle manure (treated with an OTC concentration of 82.5 mg/kg dry manure) proved that OTC had inhibitory effects on the acetoclastic and syntrophic methanogenesis pathways of AD process. Bacteria were found as more sensitive than Archaea. The study resulted that the syntrophic gram-negative bacteria highly affected the methane production and also VFA consumption in AD process (Akyol et al., 2016). Coban et al. (2016), studying the effects of veterinary antibiotics on biogas producing microbial communities, proved that OTC presence in reactors decreased the biogas production with the accumulation of VFAs. OTC and VFA concentrations had a negative impact on the growth of Methanobacteriales and Methanosarcinales genes which were in correlation with biogas production. 50, 100 and 200 mg/L concentrations of OTC containing reactors decreased the biogas production rates in reactors as 41, 57 and 61 %, respectively, over the control (Coban et al., 2016).

The study of Ince et al. (2013) showed that 1-3.3 mg/L concentrations of OTC caused 50-60 % inhibition in biogas production in which the methane percentages of the biogas stayed stable (Ince et al., 2013). In another study; Turker et al. (2013) stated that OTC medication of manure created an inhibition in biogas production. OTC concentrations of 1.1-3.4mg/L resulted in 14-24% lower biogas production during the AD of cattle manure in batch reactors (Turker et al., 2013).

5.2.2.2. Cumulative Methane Production. The methane contents of the biogas produced in the reactors during AD were analyzed every week. The amount of cumulative methane productions in the anaerobic reactors were given in Figure 5.21. All results were presented as the average values obtained from the parallel reactors.

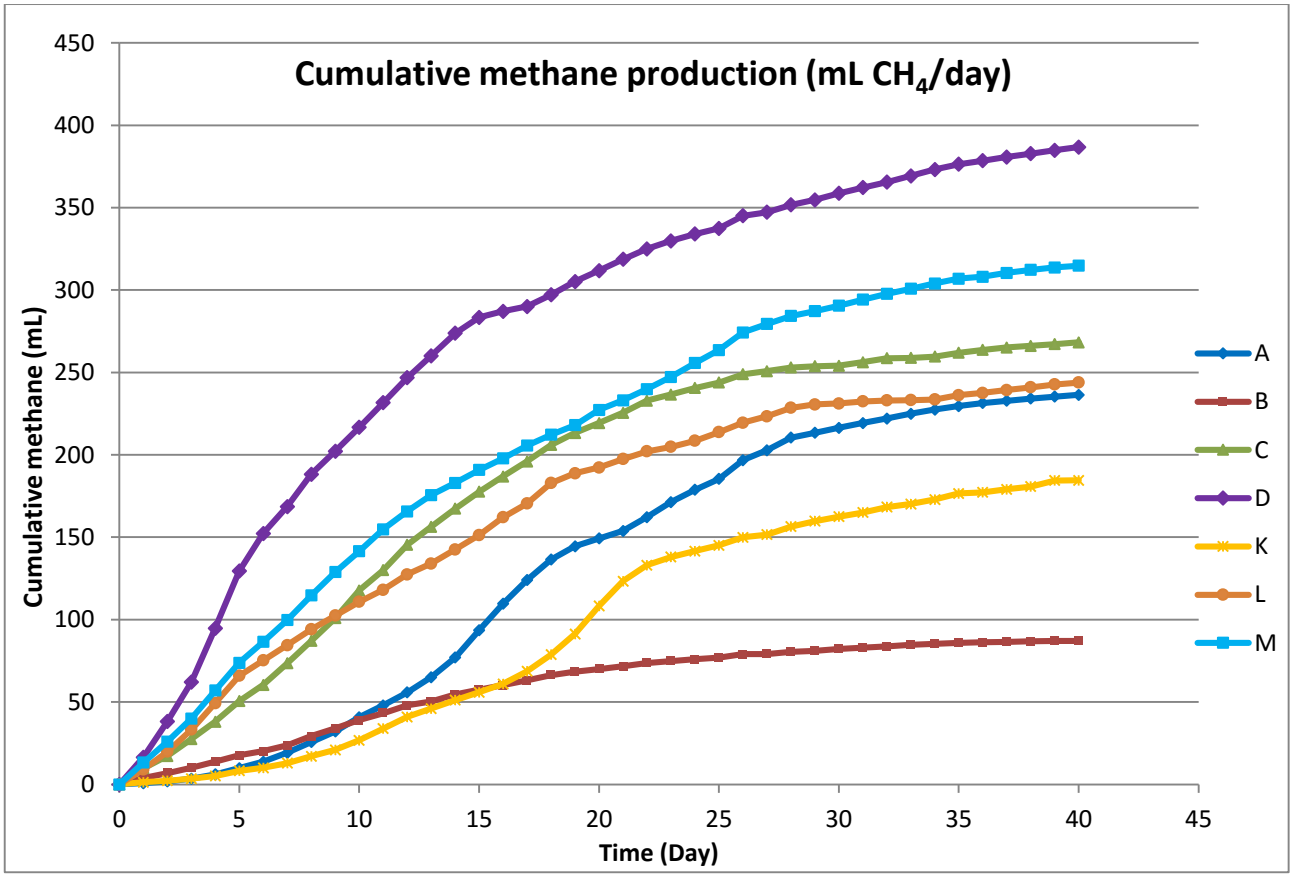

Figure 5.21. Cumulative methane production in the reactors.

(A = SS (control), B = Inoculum, C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

As the Figure 5.21 indicates, in reactor D, the highest amount of methane produced as 387 mL/d. The reactors M, C and L were followed with the methane productions of 315 mL/d, 268 mL/d and 244 mL/d, respectively. MW pre-treatment provided a significant improvement in the methane production of the sludge samples. The contribution of the inoculum sludge was subtracted from each reactor, and it was found that the cumulative methane productions were increased by 65.5% and 45.2% in reactors D and M, as compared to control reactors C & L.

The amount of methane productions in antibiotic contaminated reactors M and K were lower than D and A with the ratios of 24% and 22%, respectively. The presence of antibiotics decreased the methane production rates of sludge samples, since the antibiotics harmed the bacterial community. Bacteria were mostly sensitive to the antibiotics. With respect to the literature, antibiotic presence created inhibition effects in the acetogenesis and methanogenesis steps of AD process, and had an inhibitory effect on the types of methanogenic bacteria.

Lallai et al. (2002) studied the effect of the antibiotics on the anaerobic process. They used 60 & 120 mg/L amoxicillin concentrations, and found that the methane concentrations in biogas were 75% and 68%, respectively. The methane production rate decreased as the amoxicillin concentration increased (Lallai et al., 2002). The study of Masse et al. (2000) showed that the presence of penicillin and tetracycline (16 & 550 mg/kg dry matter, respectively) in manure slurries decreased the methane production with the rates of 35% and 25%, respectively, over the control (Masse et al., 2000). The study of Arikan et al. (2006), investigating the effect of OTC during AD of manure from medicated calves, found that 3.1 mg/L OTC concentration resulted 27% reduction in methane production in anaerobic batch reactors. The methane yields obtained for non-medicated and medicated reactors recorded as 256 L/kg VS and 184 L/kg VS, respectively (Arikan et al., 2006). The study showed that the antibiotic presence caused a decrease in methane yields.

5.2.2.3. Methane Yield. The methane yields in the reactors were presented in Figure 5.22 as mL methane/g VS_{added}. MW pre-treatment increased both biogas and methane yields due to increase in solubilisation of organic matters. After the application of MW pre-treatment, methane yields were improved by the rates of 65.5% and 45.2% in reactors D and M, as compared to control reactors C & L.

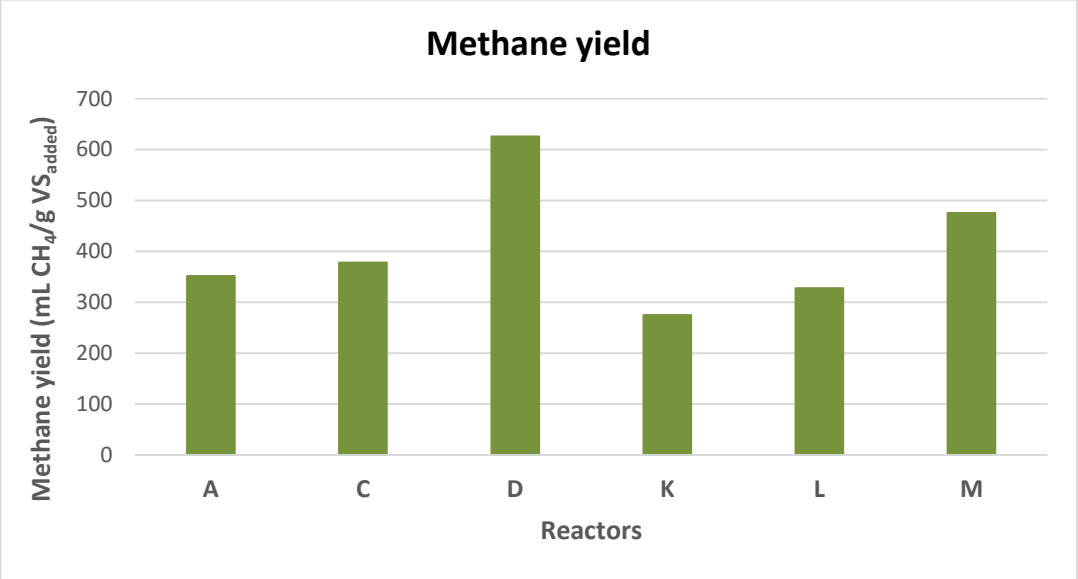

Figure 5.22. Methane yields in the reactors.

(A = SS (control), C = SS + Inoculum (control), D = MW (control), K = SS + Antibiotic, L = SS + Antibiotic + Inoculum, M = Antibiotic MW.)

The presence of antibiotics decreased the methane yields of sludge samples, since the antibiotics harmed the bacterial community. The CIP probably had inhibitory effects on the acetogenesis and methanogenesis steps of AD process. The methane yields provided in reactor M (476 mL CH_4/g VS) was lower than the yields provided in D (626 mL CH_4/g VS) with a ratio of 24%. Similarly, the yields provided in reactor K (275 mL CH_4/g VS) was lower than in A (352 mL CH_4/g VS) with a ratio of 22%.

Akyol et al. (2016) studied with the OTC medicated and non-medicated cattle manures in single-stage and two-stage digesters. Their study showed that the OTC concentration of 82.5 mg/kg dry manure resulted in a reduction rate of 43% on methane yield in medicated two-stage digesters during the digestion period. And the OTC concentration of 81 mg/kg dry manure resulted in a reduction rate of 52% on methane yield in medicated single-stage digesters (Akyol et al., 2016).

6. CONCLUSIONS

This study investigated the effects of MW, H_2O_2, $S_2O_8^{2}$, combined MW/H_2O_2 and MW/$S_2O_8^{2-}$ sludge pre-treatments and also the presence of antibiotics on anaerobic stabilization and biochemical methane production potential of sewage sludges.

The pre-treatments applied to the sludge samples prior to anaerobic digestion speeded up the hydrolysis step and improved the biodegradability of the organics in sludge by increasing their solubility.

Application of MW, H_2O_2 and combined MW/H_2O_2 (1 g H_2O_2/g TS) pre-treatments increased the methane yields by 65.5%, 20% and 40%, providing 626 mL CH_4/g VS, 453 mL CH_4/g VS and 529 mL CH_4/g VS methane yields, respectively. However, persulfate pre-treatment (1 g $S_2O_8^{2-}$/g TS) decreased the biogas production and eliminated the methane production due to the inhibiting effect of the $S_2O_8^{2-}$ dose on the survival of the methanogenic bacteria.

The presence of 1 mg/g TS CIP antibiotic in sewage sludge samples decreased the cumulative biogas and the methane productions in the anaerobic digestion process. The methane yield of 275 mL CH_4/g VS obtained from 1 mg CIP/g TS contaminated sludge (reactor K) was 22% lower than the yield obtained in control reactor A, containing uncontaminated sludge (352 mL CH_4/g VS). Similarly, the methane yield was 24% lower in MW pretreated antibiotic contaminated sludge containing reactor M (476 mL CH_4/g VS) as compared to the MW pretreated control reactor D (626 mL CH_4/g VS). Although, MW pre-treatment improved the methane productions from the antibiotic contaminated sludge samples, the improved yields were not as high as the yields obtained from the MW pretreated uncontaminated sludge samples.

The presence of antibiotic (1 mg CIP/g TS) in sewage sludge slowed down the acetogenesis and methanogenesis steps of the AD by decreasing the activity of

methanogenic bacteria, and so decreased the amount of methane production. It did not have negative effect on the sludge stabilization.

7. RECOMMENDATIONS AND FUTURE WORKS

For the future works; H_2O_2 and $S_2O_8^{2-}$ pre-treatments can be applied to sludge samples at different concentrations. Especially, $S_2O_8^{2-}$ pre-treatment should be studied at concentrations lower than 1 g $S_2O_8^{2-}$/g TS to eliminate its inhibition effect. Moreover, the effect of antibiotics on anaerobic digestion and biogas production can be studied by using sludge samples having different doses of antibiotic concentrations.

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