A FENTON REACTION IN THE RECIRCULATED BIOSOLIDS LINE OF AN ANAEROBIC DIGESTION SYSTEM

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ABSTRACT

Anaerobic digestion is a biological treatment method and a mature technology to recover energy in the form of methane (CH_4) from, for example, waste sludge. Due to the positive connection between pre-treatment and improved performance results, several biological, mechanical, thermal, chemical, thermochemical, and physico-chemical methods have been applied to enhance the anaerobic digestion of various wastewater streams. In this work, the fenton reaction is used to achieve this purpose. Two continuously stirred anaerobic digesters (CSADs), with an effective volume of 45 L each, were built and were operated in parallel at mesophilic conditions (32°C ± 1) during an operating period of 280 days. The only difference between the two digesters was hydrogen peroxide (H_2O_2) addition after day 180 to the recirculation line for one of them. Anaerobic settlers were built similar to the CSADs except that mixing and heating were omitted, and these anaerobic settlers were operated at room temperature (25°C ± 1). Each of these two anaerobic settlers was placed in series with one CSAD. The pilot-scale CSADs were operated similarly to a full-scale system at the Ithaca Area Wastewater Treatment Plant (IAWWTP). To mimic the conditions of the reactors at the wastewater treatment facility, the anaerobic digestion systems were maintained at mesophilic conditions (32°C) and were fed real, thickened, and combined primary, waste activated sludge, and tertiary sludge. The results indicated that H_2O_2 addition did not enhance the biogas production even though an increase in soluble chemical oxygen demand was observed. Total chemical oxygen removal

efficiencies were 63.75±2.9% and 62.1±3.2% for experimental and control CSADs, achieving a methane yield of 0.280 L $CH_4.g^{-1}$ and 0.279 L $CH_4.g^{-1}$, respectively. Large, but identical, variations in biogas production during the operating period were observed for in both systems. Such large variations could have been responsible for a false claim of H_2O_2 enhanced biogas production in a non-controlled study.

BIOGRAPHICAL SKETCH

Ahmet Erkan Uman was born and raised in Safranbolu, Karabuk in Turkey. He graduated from the Environmental Engineering Program at Cukurova University in 2010. He continued his master's studies in Cornell University, School of Biological and Environmental Engineering on anaerobic digestion process and received his M.S. degree in 2015.

Dedicated to my mother Yurdanur Uman, brother Kaan Uman, and fiancée Zeynep Ulupinar

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CHAPTER 1

INTRODUCTION

The need for finding new energy sources due to the limited reserves of fossil fuels and their pollution to the environment has become more important in recent years [1, 2]. This necessity has not only lead to new renewable energy technologies but also alternative fuels [3]. Biogas production from organic waste materials can be used as alternative energy source.

Biogas was first used to heat bath water in Assyria and Persia during the 10^{th} century BC and 16^{th} century, respectively [4]. An important discovery was made by Jan Baptist Van Helmont when he had discovered that flammable gases could be evolved from decaying organic matter. Similarly, in 1682, Robert Boyle suggested that by decaying animal and vegetable wastes, a gas could be produced after the process. In 1776, Count Alessandro Volta found that the measure of decaying organic matter was proportional to the volume of flammable gas produced [5].

The first anaerobic digestion facility was built in India in 1859. An anaerobic sewage treatment system was constructed in England in 1895, and biogas was produced and used to fuel street lamps in Exeter [6]. After the microbiological developments had been advanced, Buswell and others investigated the methanogenic

archaea and conditions that improve their activity [7].

Biological conversion of biomass to methane has shown promising results and has received increasing attention over the past years [8]. There are more than 13,000 operational biogas plants in both the US and Europe as of 2014 [9]. Due to its potential energy production in the form of methane while treating wastewater and minimizing waste, it is beneficial to enhance anaerobic digestion and produce more biogas for a sustainable future.

1.1 BACKGROUND

1.1.1 Fundamental Concepts of Anaerobic Digestion

Anaerobic microbial conversion of complex organic material consists of four major stages: hydrolysis, fermentation, acetic acid and hydrogen formation, and methane formation. Five distinct groups of microorganisms are involved in biotransformation of these complex organic materials to biogas. As shown in Fig. 1.1, proteins, fats, and carbohydrates are first hydrolyzed to fatty acids, amino acids, alcohol, carbon dioxide, hydrogen, acetate and sulfides by fermentative bacteria. Next, these products are digested into acetic acid, hydrogen, and carbon dioxide by obligatory hydrogen-producing acetogenic bacteria and this process is called acetogenesis. In this step, acetate production is also possible from hydrogen and carbon dioxide and *vice versa* by homoacetogenic bacteria. The last step is the

production of methane and carbon dioxide and there are two different archaea that can use different substrates to produce biogas: acetoclastic methanogens (convert acetate to biogas) and hydrogenotrophic methanogens (utilize hydrogen to produce biogas). It is also important that there is a syntrophic relation between hydrogen-producing acetogenic bacteria and hydrogenotrophic methanogens. Hydrogen concentration has to be low enough for hydrogen-producing acetogenic bacteria to be able to degrade fatty acids. If the hydrogen concentration is not low enough, substrate is converted to propionic acids, butyric acids, and ethanol instead of methane [10]. Syntrophy allows acetogenesis to become favorable by keeping the hydrogen partial pressure low enough [11].

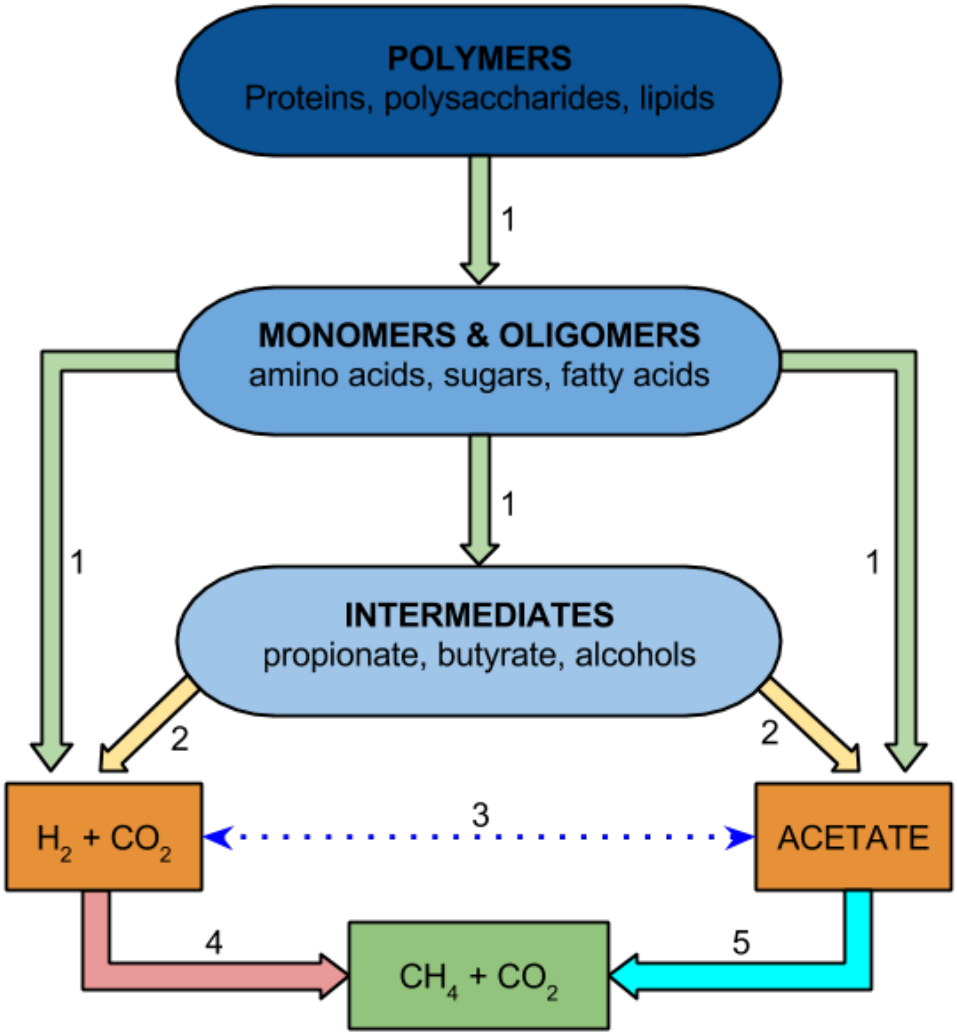


Fig. 1.1: Degradation of complex organic material into methane in anaerobic digestion [11].

1.1.2 Fenton Reaction

The fenton reaction was discovered by H.J.H. Fenton in 1894 when he tested the oxidation of tartaric acid along with other acids by hydrogen peroxide in the presence of ferrous iron ions [12]. After the reaction was described as an advanced oxidative process, it has become of great interest for breaking down industrial wastes and the treatment of hazardous wastes [13, 14]. It has been used to treat a variety of wastewater streams, including pesticides, surfactants, dyes, aromatic amines, and many others [15].

The fenton chemistry is still being studied and there are two types of pathways in the literature: the radical and non-radical pathways. The first mechanism is described as the production of hydroxyl radical (OH•) by one-electron reduction of hydrogen peroxide (H_2O_2) and this could allow OH• to obtain hydrogen from a carbon-hydrogen bond and initiate radical chain reactions. The interpretations of the fenton reaction *via* radical pathway evolved over time and consequently were modified to the following steps [15]:

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH\bullet \quad (1)$$

$$OH\bullet + H_2O_2 \rightarrow HO_2\bullet + H_2O \quad (2)$$

$$Fe^{+3} + HO_2\bullet \rightarrow Fe^{+2} + H^+ + O_2 \quad (3)$$

$$Fe^{+2} + HO_2\bullet \rightarrow Fe^{+3} + HO_2^- \quad (4)$$

$$Fe^{+2} + OH\bullet \rightarrow Fe^{+3} + OH^- \quad (5)$$

The step (1) initiates the chain reaction, steps (1), (2), and (3) cycle during the reaction, and step (4) and (5) terminate it. The hydroxyl radical is one of the most reactive chemical species known and it is the key parameter of using the fenton reaction for wastewater treatment.

Table 1.1 Relative Powers of Chemical Oxidants [16]

Compound	Oxidation Potential (volts)	Relative Oxidizing Power (Cl_2 = 1.0)
Hydroxyl Radical	2.8	2.1
Sulfate Radical	2.6	1.9
Ozone	2.1	1.5
Hydrogen Peroxide	1.8	1.3
Permanganate	1.7	1.2
Chlorine Dioxide	1.5	1.1
Chlorine	1.4	1.0
Oxygen	1.2	0.90
Bromine	1.1	0.80
Iodine	0.76	0.54

The second pathway is considered as the production of ferryl ion (FeO^{+2}) instead of step 1 in the first pathway:

$$Fe^{2+} + H_2O_2 \rightarrow FeO^{+2} + H_2O \quad (6)$$

$$FeO^{+2} + H_2O_2 \rightarrow Fe^{2+} + H_2O + O_2 \quad (7)$$

The radical pathway has been studied and questioned by many researchers. According to their results, ferryl species (Fe(IV)) could be produced in these reactions under different conditions. This controversy is still ongoing and it is difficult for me to come to a conclusion about which theory is true.

CHAPTER 2

Literature Review

The purpose of solid waste and wastewater treatment is to allow people to dispose of waste products without endangering their health and to prevent undesired potential damage to the environment. To improve the wastewater quality and minimize the contamination, treatment might consist of several steps such as biological, physical, and chemical processes. These steps are selected according to the wastewater quality and characteristics, as well as the treatment objectives [17].

During wastewater treatment, at the end of each step, the treated wastewater (supernatant) is being transferred to the next level of the process while solids, which are removed from raw wastewater in a primary settler and which are removed from the biological treatment processes (*e.g.,* waste activated sludge), are sent to additional operation units – the sludge treatment processes. The ultimate treated water can be discharged to receiving waters according to regulations [18]. Primary and secondary sludges, which are the byproducts of wastewater treatment, are typically first combined and sent to thickeners for minimizing the volume of the sludge by concentrating solids and removing water (Fig. 2.1). This volume reduction is useful to the subsequent treatments processes, such as dewatering, drying, combustion, and anaerobic digestion [19].

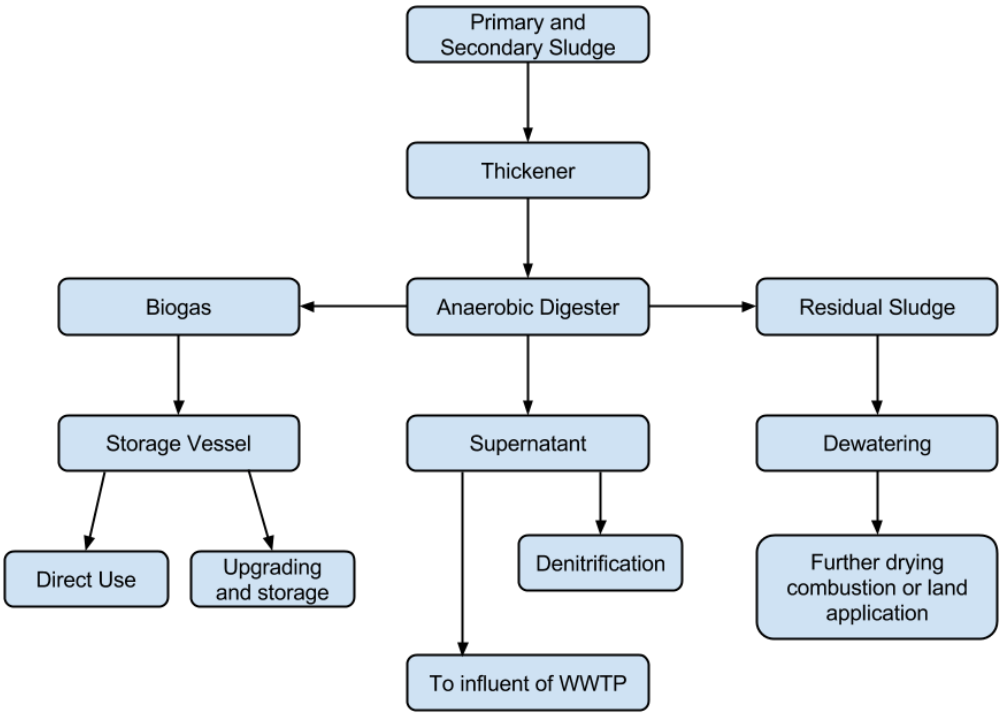


Fig. 2.1: Process diagram of the sludge processing [20].

Anaerobic digestion is the further degradation of complex organic material by a group of microorganisms in the absence of oxygen. The purpose of anaerobic digestion is to reduce the organic material and disease-causing microorganisms and to get a more stable, reduced-volume sludge for disposal. As a result of biological degradation of complex organic material, biogas will be produced in the process. Due to its potential for energy recovery and limited environmental impacts, anaerobic

digestion has been examined for development and improvement to enhance biogas production [20].

The rate-limiting step for anaerobical degradation of complex organic material is the hydrolysis step – *i.e.,* the breakdown of solids and lysis of microbial cells [21, 22]. The formation of undesired volatile fatty acids and toxic by-products further slows down hydrolysis [23]. To enhance the anaerobic digestion and biogas production, various pretreatment and co-treatment methods have been conducted on accelerating the hydrolysis step. These pretreatment methods may vary according to the sludge being treated and can be biological, mechanical, thermal, and/or, chemical. The purpose of the pretreatment step is to achieve lysis of microorganisms, which subsequently result in releasing the intracellular material into degradable forms [23-25].

Another possible way of improving hydrolysis is through co-digestion with other substrates, changing the physical or chemical properties of the substrate. This can lower the inert fraction of biosolids and increase the degradation rate [26]. It can also provide missing nutrients for anaerobic digestion [27]. As an overall benefit for the treatment plant, co-digestion increases biodegradability and biogas production of anaerobic digestion [28].

In Fig. 2.2, the location of co-treatment and pre-treatment locations for a typical wastewater treatment plant is shown. The application of co-substrates can be directly to the activated sludge unit (T1) or recycling line (T2). By increasing the degradation of organic material or sludge age, sludge formation can be reduced in these configurations. However, this might lead to increased carbon dioxide emissions [24].

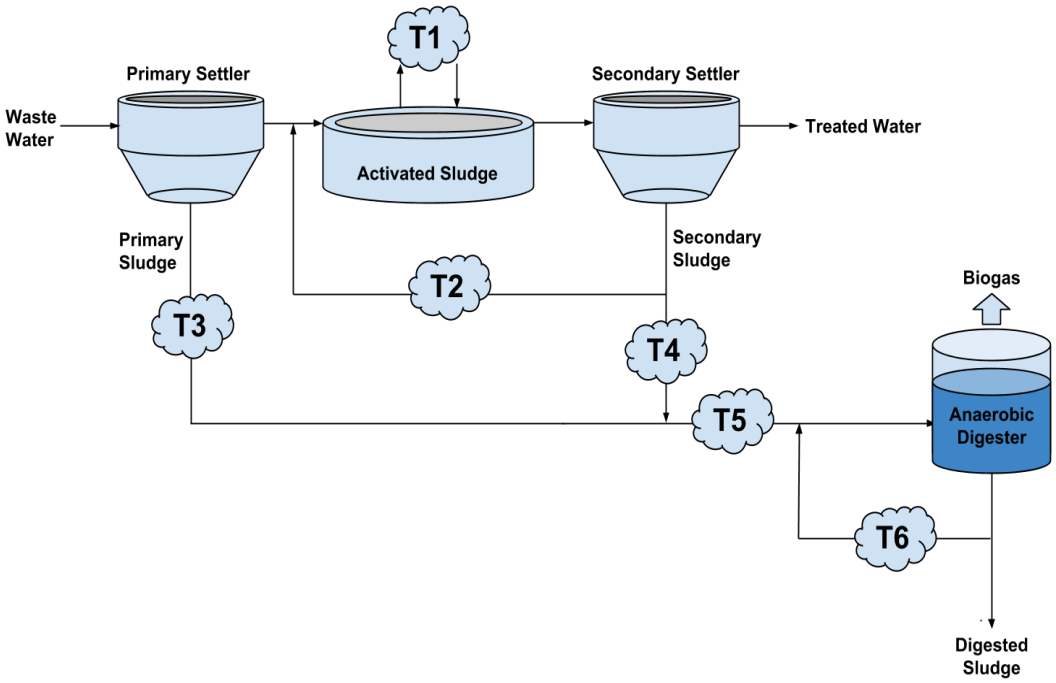


Fig. 2.2: Potential locations for sludge co-treatments and pretreatments for a typical wastewater treatment facility [24].

The pretreatment of primary sludge, secondary sludge, and the mixture of both can be applied at a number of places within the wastewater treatment plant (Fig. 2.2.). Since the degradation of primary sludge is rapid and its biodegradable content is higher compared to secondary sludge (T4) and recycling sludge (T6), pretreatment (T3) may not be effective [29]. Activated sludge consists of a mixture of substances such as polysaccharides, proteins, nucleic acids, lipids, humic substances, and uronic acids, amongst others. These substances are considered as recalcitrant to aerobic and anaerobic processes and reported to be 30-50% biodegradable [24].

2.1 Biological Pretreatment Methods

Biological pretreatment might consist of both anaerobic and aerobic processes. An aerobic pretreatment method prior to anaerobic digestion can be introduced, for example, by implementing microaeration [25, 30, 31]. The term microaeration was defined as the addition of oxygen into an anaerobic digestion process to enable both aerobic and anaerobic biochemical reactions in a single reactor [32]. Lim and Wang used a microaeration pretreatment method and showed that it can be used as an alternative pretreatment to enhance hydrolysis process [33]. According to their results, higher COD solubilization and greater VFA accumulation was observed along with 21% and 10% increases in methane yield when pretreatment (during 4 days with 37.5 mL O2/L-d) was applied to substrates with inoculum and without inoculum, respectively. Thermophilic conditions have also been investigated

for both aerobically and microaerobically pretreated sludge in anaerobic processes. A mixture of waste activated sludge (WAS) and horse manure was first preheated (70-80°C) and pretreated with a dissolved oxygen level of 1-3 and 0-0.1 mg/L for aerobic and microaerobic conditions, respectively. Then, this pretreated sludge was fed into the anaerobic mesophilic reactors (37°C, 120 ml) and an increase of 50% in biogas generation was found for the reactors pretreated with microaerobic conditions [34]. Similarly, a combination of a thermophilic aerobic reactor (65°C) and a mesophilic anaerobic digester (35°C) were operated for different hydraulic retention times (21 and 42 days) for 180 days with treating WAS and the results indicated that this combined process led to a higher chemical oxygen demand (COD) removal efficiency ranging from 20 to 40% compared to a single stage mesophilic reactor [35].

There are several configurations for using the anaerobic digestion process itself as a pretreatment method such as temperature-phased and double-phased (also called two-phased) anaerobic digesters [36-39]. The double-phased anaerobic digestion process includes the physical separation of the hydrolysis step from the methanogenesis step by two different reactors in series to improve the overall degradation. Along with its disadvantages, under stabilized operations, the double-phased anaerobic digestion process has been reported as resulting in enhancing overall degradation efficiency, producing higher biogas, and stimulating hydrolytic enzymes to participate in further degradation [36, 37, 40]. The temperature-phased anaerobic digestion is similar to the double-phased process except instead of

separating the hydrolysis and methanogenesis steps, two different anaerobic reactor setups are operated in series with the first stage being operated under thermophilic and the second stage under mesophilic conditions [41, 42]. It has shown enhanced biogas production and degradability along with pathogen removal [43, 44]. In a comparison of temperature-phased anaerobic digester process (55°C and 35°C) to a single-phased anaerobic digester (35°C), Han et al. concluded that volatile solids destruction was 18% higher and methane production was 16% higher for the temperature-phased anaerobic digester process [45]. Watts et al. also compared the temperature-phased anaerobic digestion process with three different thermophilic temperatures (47°C, 54°C, and 60°C, 2 days of HRT) treating WAS. They observed that volatile solids removal efficiency showed similar results with lower temperature-phased digesters (47°C, 54°C) compared to a single-phased digesters. However, when they operated it at 60°C, the performance improved as 35% in volatile solids destruction, which also resulted in higher biogas production [46]. Different hydraulic retention times for the first stage in a temperature-phased anaerobic digester process treating WAS have also been tested. Bolzonella et al. compared these retention times (1-5 days) and concluded that 30-50% of biogas increase was observed when the retention times were 2 or 3 days for the first thermophilic stage compared to the mesophilic and thermophilic single stage setups [40].

2.2 Mechanical Pretreatment

The purpose for using mechanical pretreatment is to disrupt the sludge particles and cell membranes. This disruption increases the surface area and releases the intracellular compounds into the liquid phase [47-49]. Increasing the surface area also allows better contact for anaerobic bacteria and substrate resulting in an improved digester process. It has been stated that chemical oxygen demand degradation and biogas production are inversely proportional to the particle size of the sludge for anaerobic digestion [50]. Mechanical pretreatment might consist of ultrasonication, high-pressure homogenization, collision, and grinding.

Ultrasonic pretreatment is achieved by the utilization of periodical sound pressure to disrupt the cell walls and floc matrix with sound waves at a variable frequency [51, 52]. The triggering mechanisms when ultrasonic pretreatment is applied to sludge are cavitation (low frequencies, 20-100 kHz) and radical chemical compounds formation (high frequencies, 100-1000 kHz), (OH•, HO_2, H•) [20, 24, 52]. The cavitation phenomenon is the generation of a combination of compressions, rarefactions, and bubble formations caused by the sound waves when in contact with the sludge. It is stated that during the application of waves, the bubbles generated by cavitation force collapse with a vigorous pressure, causing shock waves up to 5000°C temperature and 500 atmospheric pressure [53]. It was observed that to disrupt the cell membrane, high-energy input is required and this requirement ranges from 1 to

16 MJ kg^{-1} total solids and also depends on the sludge solids concentration [24]. Show et al. tested the total solids content with ultrasound pretreatment and they found that the content of TS is optimum between 2.3% and 3.2% [54]. Riau et al. implemented the ultrasound pretreatment prior to temperature-phased anaerobic digestion (55°C and 35°C) treating WAS and found 42% and 13% of increase on methane production and volatile solid removal in comparison to the control system, respectively [39].

High pressure homogenization is a process in which the sludge gets pressurized and goes through a narrow gap under strong depressurization. The sludge is subjected to high cavitation and shear stress, which causes cell rupture and wall breakage and eventually extraction of intracellular substances [49]. A high pressure homogenizer was implemented to a full-scale anaerobic digester plant (T6 in fig. 2.2) at a pressure of 150 bar. A 23% higher sludge reduction and 30% biogas enhancement has been reported in the anaerobic digestion process. Due to the recycled sludge, the chemical oxygen demand of digester effluent had not showed significant reduction and the highest decrease was during the summer period, which resulted in 30% of COD concentration reduction [55].

Collision plate is a similar pretreatment method to high pressure homogenization except that the pressure is increased up to 30-50 bar and jetted and smashed to the collision plate [56, 57]. Choi et al. investigated this method with WAS

and varying pressure configurations in bench scale pretreatment setups. Because a measure of cell rupture due to the cytoplasm of the microorganisms is mainly composed of protein, they measured protein concentration and found a 86% increase at 50 bar pressure. They also reported that volatile solids removal of the digester was 13-50% with pretreated WAS and 2-35% without pretreatment at the same conditions [56]. A similar study has been conducted on a pilot scale anaerobic digestion process at a 30 bar pressure. It was stated that hydraulic retention time of anaerobic digester was decreased from 13 to 6 days, while the process performance and effluent quality showed no major changes [57].

The hydrolysis of biosolids can also be achieved by milling. It has been applied to WAS and 25% of increase in soluble chemical oxygen demand was observed after the milling process [58]. Similarly, in a comparison of ball and cutting mill pretreatment, Bairer et al. found that soluble chemical oxygen demand can be enhanced from 1-5% to 47% with ball milling of stabilized WAS [47]. Similar to the milling process, a lab-scale anaerobic digestion process operating for two different temperature conditions (35 and 55°C) has been studied for particle size effects. Decreasing the particle size from 2.2 to 1.1 mm by grinding had no effect on mesophilic digestion while biogas production was increased by 14% for thermophilic digestion [59].

Thermal Pretreatment

Heating the sludge for pretreatment purpose was extensively studied and has shown to be a successful method for enhancement of anaerobic digestion. In this method, the temperature of biosolids is increased in various ranges according to the method being used (50-250°C) to cause cell breakage and solubilization of the sludge prior to anaerobic digestion [25, 60, 61]. Besides anaerobic digestion enhancement, thermal pretreatment has been effectively implemented for dewatering and pathogen removal [62]. According to its temperature range, thermal pretreatment can be divided into two different groups as higher (above 100°C) and lower (below 100°C) temperature thermal pretreatment [61].

Due to the solubilization of the sludge and destruction of cell membranes, the chemical oxygen demand has been found linearly correlated to the temperature of pretreated sludge [63, 64]. The methane production, however, has not always shown direct correlation with pretreatment temperature. When comparing a thermal pretreatment of WAS with varying temperature (140 and 165°C), Dwyer et al. observed that solubilization was increased with higher temperature, but methane production did not change. They proposed that this was due to the formation of melanoidins in the pretreatment process, which was described as the accumulation of carbohydrates with amino acids as a result of chemical reactions at high temperatures [65]. This phenomenon is also called Maillard reactions and their biodegradability is

significantly difficult. Similar results were obtained by Carrere et al. Varying temperatures from 60 to 210°C were used to pretreat 6 different WAS samples. For all samples, COD solubilization was increased with increasing pretreatment temperature and it was concluded that the solubilization did not depend on the sludge sample. Although anaerobic digestion was also positively affected by increasing temperature, the biodegradability at 210°C was lower than at 190°C. They also concluded that the initial biodegradability of samples was inversely affected by thermal pretreatment [64]. These results were also in accordance with their first findings that for a WAS that was initially less biodegradable the biogas production improved by 75% whereas the one that seemed initially more biodegradable improved by only 24% [29].

Chemical Pretreatment

Organic waste compounds disintegration can be achieved by the addition of chemical substances such as acids, alkalis, and oxidants [66-69]. According to the different operating conditions and biosolids characteristics, effects of these substances may vary to the wastewater being treated and the anaerobic digestion process being used. Acid pretreatment is used for dewatering of WAS and lignocellulosic substrates. Due to the recalcitrant structure of lignocellulosic substances, its degradability in anaerobic digestion is limited and it can be improved by chemical pretreatment [70, 71].

Devlin et al. tested acid pretreatment and applied hydrochloric acid (HCl) to WAS for varying concentration (pH 1-6) and observed their effects in regards to anaerobic digestion and dewaterability. The most effective pretreatment have been observed when the pH was adjusted to 2. The methane yield was increased by 14.3% in semi continuous anaerobic digestion process (12 days of HRT, 35°C). They also concluded that polymer requirement for dewatering was decreased by 40% to achieve the same solid content [71].

Several alkali substances have been used for chemical pretreatment of WAS. These substances include $Ca(OH)_2$, NaOH, $Mg(OH)_2$, and KOH. The usages of these chemicals have been reported to have positive effects on anaerobic digestion such as volatile solids reduction increase, biogas production improvement, and more chemical oxygen demand solubilisation [67, 68, 72, 73]. When dosing the wastewater with these salts, it is also important to consider the toxicity that might be caused to anaerobic digestion. Na, K, Mg, and Ca could be inhibitory to some microorganisms in anaerobic digestion and reduce the biogas production. It has been reported that the optimum concentration of Ca is 200 and Mg is 720 mg L^{-1} for anaerobic digestion. K was found to be inhibiting when it reached 400 mg/L [24, 25].

As an oxidant, ozone (O_3) is used for disinfection of drinking water, pathogen removal, and WAS pretreatment. It can penetrate the cell membrane of microorganisms and disintegrate its structure to solubilize the intracellular

components [74-77]. It has been applied with various concentrations to both recycling sludge from anaerobic digester and WAS. Weemaes et al. ozonated the sewage sludge originating from primary and secondary treatment of a municipal wastewater treatment plant with the dosage of O_3 of 0.1 g O_3 g^{-1} COD and tested it on anaerobic digestion. They found 2.2 times more methane production than from untreated sludge. They also concluded that higher dosage of O_3 increased the overall process, but that was not significant [74]. Similarly, an optimum dosage of O_3 was found to be in the range of 0.1 – 0.2 g O_3 g^{-1} COD by several studies [75, 78].

Hydrogen peroxide (H_2O_2) is a powerful oxidant that has been used for various purposes in wastewater treatment such as hazardous organic waste removal, dewaterability enhancement, and bioremediation [79]. The oxidation power of H_2O_2 is not effective by itself and it is used with certain chemical substances to initiate its oxidative chain reactions. For this purpose, ozone (O_3), iron (Fe^{+2}), and UV-light can be used. As a result of these reactions, hydroxyl radical (OH•) is produced and it can participate in further degradation reactions [80].

Application of H_2O_2 to WAS pretreatment prior to and after anaerobic digestion has been reported to exhibit both positive and negative results. Rivero et al. studied the effects of H_2O_2 (2 g H_2O_2 g^{-1} VSS influent, approximately 60ml L^{-1} sludge) and thermal pretreatment (37 and 90°C) on WAS. They used 12 lab-scale mixed anaerobic digesters with different configurations (8 L active volume, 37°C,

Fig. 2.3). Among those, the combining effect of H_2O_2 and thermal pretreatment (90°C) has been shown as the most effective method (configuration 8, Fig. 2.3), which resulted in the range of 27.2 – 29% more volatile suspended solid removal. However, when only H_2O_2 was applied to the recycled biosolids and returned back to the digester, it did not result in more methane production; contrarily, a reduction was observed from 319.9 to 281.1 mL CH_4 g^{-1} COD removed. Only s positive result was observed with the solids removal efficiency and it was increased by 4.8% in this configuration (configuration 3, Fig. 2.3). In configuration 8, however, the methane production was increased significantly with oxidative and thermal pretreatments [81].

Another H_2O_2 addition study into the anaerobically digested recycling biosolids has been examined for lab- and full-scale processes. 0.03% H_2O_2 (v/v) was applied to the recycling biosolids for both operations. For the lab-scale process, a 10 L continuously stirred mesophilic anaerobic digester (32°C) was first fed for 90 days without H_2O_2 addition; then the same digester was again fed for 90 days with H_2O_2. The results indicate a 60% increase in biogas production. For the full-scale process, a 13% increase in biogas production and a 11.5% decrease in final residual solids have been reported [82].

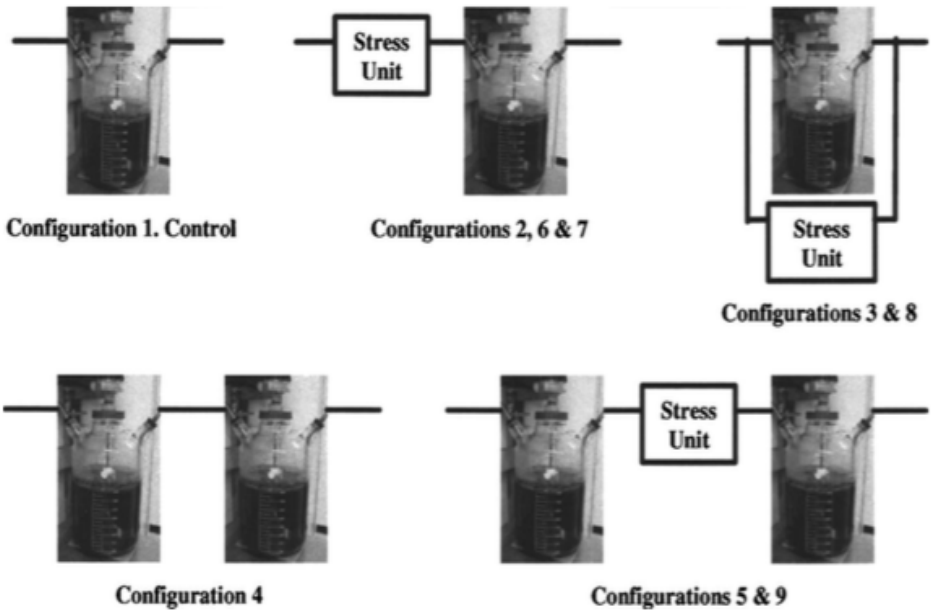


Fig. 2.3: Reactor configuration: Configuration 1 and 4 used as control. For configuration 6 the sludge was only preheated at 90°C. For configuration 2, 3, and 5, 2g H_2O_2 per g VSS was applied at 37°C. For configuration 7, 8, and 9, 2g H_2O_2 per g VSS was applied at 90°C (in the stress unit, hydrogen peroxide and temperature was applied to the sludge according to the configuration used, the HRT was 24h) [79].

Dewil et al. tested varying amounts of H_2O_2 (5, 25, and 50 g H_2O_2 kg^{-1} DS) pretreatment with WAS prior to anaerobic digestion in laboratory scale batch reactors (1 L, 37°C, 200 hours of total HRT). A maximum increase of 75% in biogas production found when WAS was pretreated with 50 g H_2O_2 kg^{-1} DS. They concluded that the biogas production was slightly increased with increasing

amounts of H_2O_2 [83]. Similary, Erden and Filibeli pretreated the WAS with 50 g H_2O_2 kg^{-1} DS and compared its digestibility in single-stage thermophilic anaerobic digestion (13.5L, 5 days of HRT, and 55°C) and two-stage anaerobic digestion (8.5 L, 5 days of HRT, and 37°C) for 30 days. They reported that the maximum sludge solubiliziation was achieved with single stage thermophilic anaerobic digestion and a 26.8% of VS reduction was observed. 21.5% VS reduction was observed in their previous study with a mesophilic anaerobic digester treating WAS, at a 5 day HRT during an operating period of 30 days [84]. For both experimental digesters, 1.3 times higher total methane production was observed [85].

CHAPTER 3

A Fenton reaction in the recirculated biosolids line of an anaerobic digestion system at a municipal wastewater treatment facility did not increase biogas production

3.1 Abstract

A previous study with a full-scale, 2-stage anaerobic digestion system treating primary sludge, waste activated sludge, and tertiary sludge with iron had shown promising results after addition of hydrogen peroxide to anaerobically treated biosolids to initiate a Fenton reaction. This study had found an increased energy cogeneration output of 13%; however, these results were obtained with one anaerobic digestion system with variable biosolids loading rates during its operating period and a further control study was needed to evaluate this claim [82]. Here, we tested the hypothesis that a Fenton reaction in the recycled biosolids line would improve biogas production. Two identical systems each consisting of a 45-L continuously stirred anaerobic digester (CSAD) followed by a 45-L anaerobic settler were operated in parallel during an operating period of 280 days with the only change being H_2O_2 addition after day 180 for one of the systems. To mimic the conditions of the reactors at the wastewater treatment facility, the anaerobic digestion systems were maintained at mesophilic conditions (32°C) and were fed real, thickened, and combined primary,

waste activated sludge, and tertiary sludge. The results indicated that H_2O_2 addition did not enhance the biogas production even though an increase in soluble chemical oxygen demand was observed. Total chemical oxygen removal efficiencies were 63.75±2.9% and 62.1±3.2% for experimental and control CSADs, achieving a methane yield of 0.280 L $CH_4.g^{-1}$ and 0.279 L $CH_4.g^{-1}$, respectively. Large, but identical, variations in biogas production during the operating period were observed for in both systems. Such large variations could have been responsible for a false claim of H_2O_2 enhanced biogas production in a non-controlled study.

3.2 Introduction

Anaerobic digestion (AD) is a mature technology to recover energy from wastewater sludges. By converting the complex organic materials into biogas (55-70% CH_4), it has the capability of producing a valuable energy source [20]. This production of energy results in AD being considered to be the most energy efficient way for degrading waste sludge by reducing the cost of treatment [86]. There are four stages of converting this complex organic material to methane (CH_4) anaerobically along with five different microbial communities: (i) hydrolysis of complex organic polymers into their monomers such as fatty acids, amino acids, and sugars; (ii) fermentation of these monomers into alcohols and organic acids; (iii) conversion of these fermentation products to acetic acid and hydrogen; (iv) conversion of hydrogen and acetate to methane (CH_4) [11].

Hydrolysis has been observed as the rate-limiting step in anaerobic digestion of many sludges [24, 87]. To accelerate the hydrolysis of the sludge and eventually to enhance the biogas production and solids removal efficiency, several methods such as biological (*e.g.*, microaeration, temperature-phased anaerobic digestion, two-phased anerobic digestion) [25, 30, 31] mechanical (*e.g.,* ultrasonication, high pressure homogenization, collision plate, and grinding) [57, 88]; thermal (higher and lower temperature ranges) [60-62]; and chemical (ozone and alkali treatments) [87, 89, 90] have been developed and applied to the primary, secondary (also called waste activated sludge), and tertiary sludges. The main purpose for integrating a pretreatment step into the anaerobic digestion process is to make intracellular material accessible for further biodegradation by decomposing sludge and causing cell membrane breakage.

As an alkali pretreatment, several chemical substances have been used for pretreating the sludge such as $Ca(OH)_2$, NaOH, $Mg(OH)_2$, and KOH [67, 68]. Hydrogen peroxide (H_2O_2) is also a chemical, which has been used for hazardous organic waste removal, dewaterability enhancement, and bioremediation [79]. The application of H_2O_2 itself is not effective and its oxidative power has to be triggered by the presence of other substances. For this purpose, ozone (O_3), iron (ferric and ferrous), and UV-light can be used [80]. As a result of these reactions, the hydroxyl radical (OH•), which is one of the most reactive chemical species known [91], is produced and it can participate in further reactive processes.

The combination of (H_2O_2) and Fe (II), which initiates a Fenton reaction, has been mostly applied to WAS as a pretreatment method and is reported to have positive effect on anaerobic digestion [79, 83, 92]. However, its application to recycled biosolids has not been studied in a well-controlled study. In this study, our objective was to compare the effect of the Fenton reaction on the recycled biosolids with pilot-scale continuously stirred anaerobic digesters (CSADs) and anaerobic settlers in terms of biogas production. We used two parallel systems: one with and one without a Fenton reaction in the recycled biosolids line.

3.3 Materials and Methods

3.3.1 System set-up and Inoculum

Two continuously stirred anaerobic digesters (CSADs) (Fig. 3.1) were operated at mesophilic conditions (32°C ± 1) with an effective volume of 45 L each. For heating, stainless steel-heating coils were inserted to recirculate hot water from a custom-made heated water circulator. Mixing was achieved by circulating 1.04 $L.min^{-1}$ of the head space biogas from top to the bottom of the CSADs by using peristaltic pumps (Cole-Parmer; Vernon Hills, IL). Anaerobic settlers were built similar to the CSADs except that mixing and heating were omitted, and these anaerobic settlers were operated at room temperature (25°C ± 1). Each of these two anaerobic settlers was placed in series with one CSAD. The CSADs and anaerobic

settlers were inoculated by filing them with an active biomass of 9.57±0.1 $g.L^{-1}$ volatile solids concentration from a full-scale CSAD at the same municipal wastewater treatment facility, where our system was operated (Ithaca Area Wastewater Treatment Plant, Ithaca, NY). The pilot- and full-scale CSADs were operated similarly. Since our system was operated at the same facility, the inoculum was directly transferred into the CSADs and anaerobic settlers through a hose without exposing it to the atmosphere.

3.3.2 Influent characteristic and experimental conditions

To mimic the conditions of the anaerobic digestion system at the wastewater treatment facility, freshly thickened primary and waste activated sludge from the thickening unit of the wastewater treatment facility and recycling biosolids from the pilot-scale anaerobic settlers were used as influent at 1:1 (v/v). Thickened sludge was directly taken from the effluent port of the thickeners at the wastewater treatment facility, while recycled biosolids were taken from the bottom of the anaerobic thickeners and the mix of sludge and biosolids was immediately fed into the pilot-scale CSADs. We fed a total volume of 6 L of thickened sludge and recycled biosloids (1:1 v/v) every other day to each of the two CSADs, resulting in a hydraulic retention time (HRT) of 30 days for the CSAD and an HRT of 60 days for the total system volume of a CSAD and anaerobic settler. Both experimental and control system were operated under the same operating conditions during Phase I (Days 0-

179). During Phase II (Days 180-220) with a duration of one HRT period (30 days), 2.2 ml of 50(%) H_2O_2 was added into the recycled biosolids of the anaerobic settler to obtain 0.03% (v/v) before feeding into the CSAD. During Phase III (Days 220-280), with a duration of two HRT periods (60 days) the H_2O_2 amount was increased 10 times to 22.2 ml (0.3% v/v).

3.3.3 Monitoring parameters and analytical methods

For every alternate-day feeding cycle, biogas production (Actaris Meterfabriek, Delft, The Netherlands), CSAD temperature, effluent pH (AB15+ pH, mV, REL mV meter, Fisher-Scientific), and ambient pressure and temperature were monitored. Biogas production was corrected for ambient temperature and pressure to 25°C and 30 inches Hg. Alkalinity, ammonium (Ammonia Electrode, Model Orion 95-12; Thermo-Scientific), biochemical oxygen demand (BOD_5), soluble chemical oxygen demand (SCOD), total chemical oxygen demand (TCOD), total volatile fatty acids (TVFAs), total solids (TS), volatile solids (VS) analyses for the influent and effluent of CSADs and anaerobic settlers was measured according to *Standard Methods for the Examination of Water and Wastewater (Alkalinity: 2320, Ammonia: 4500, BOD_5: 5210, COD: 5220, VFA: 5560, Solids: 2540)* (22^{nd}) [93]. Effluent samples were first centrifuged at 3500 rotation per minute (RPM) (1850g) for 15 min for TVFA and filtered through a cellulose membrane with a pore size of 0.45 μm for SCOD and individual volatile fatty acids (iVFAs). Biogas composition was measured

using a gas chromatograph (SRI Instruments 8610C, Lehigh Valley, PA), which was equipped with a thermal conductivity detector (TCD) and a packed column (0.3-m HaySep-D packed Teflon; Restek, Bellefonte, PA). iVFAs were measured using a gas chromatograph (HP Hewlett Packard 5890 Series II, Palo Alto, CA), which was equipped with a flame ionization detector and a capillary column (NUKOL, Fused Silica Capillary Column, 15m X 0.53 mm X 0.50 μm film thickness; Supelco Inc., Bellefonte, PA).

3.3.4 Biochemical methane potential analysis

The method for measuring the methane capacity of the substrates used in this study was according to Owen et al. [94]. Effluent of the pilot-scale CSAD was used for inoculation of the 250-mL media bottles. For substrate preparation, first, varying percentages (*i.e.,* 0, 0.037, 0.19, 0.37, 0.55, 0.74, and 1.85%) of hydrogen peroxide (H_2O_2) was added into the recycled biosolids of the control anaerobic settler. Due to the possibility that high concentration of H_2O_2 could damage the methanogens in the inoculum, the mixture of H_2O_2 and recycled biosolids were left for 2 hours before mixing with the inoculum. After this period, they were mixed with the thickened sludge from the thickeners at the Ithaca area wastewater treatment facility at 1:1 (v/v) to mimic the same conditions with pilot-scale anaerobic digestion system. The mixture of these recycled biosolids with H_2O_2 and thickened sludge from WWTP were first added to the empty media bottles. Then the nutrient solution, which

included required trace elements and nutrients, was added into the media bottles. And finally, the inoculum added to the media bottles. The inoculum:substrate ratio was 2:1 (VS basis). Control bottles only included the nutrient solution and the inoculum. All bottles were flushed with nitrogen, sealed, and stored at 32°C for 30 days. Biogas content and volume measurement were made periodically using a wet-syringe method and a gas chromatograph (HP Hewlett Packard 5890 Series II, Palo Alto, CA).

3.4 Results and discussion

Pilot-scale CSADs and anaerobic settlers showed equal performance under similar operating conditions

The two identical systems, consisting of a CSAD followed by an anaerobic settler, under similar operating condition and feeding strategies achieved similar biogas production, chemical oxygen demand removals, solids removals, and ammonia concentration. Both reactors showed equally variable performance parameters based on the temporal changing biosolids characteristics (Fig. 3.2). The high variability of the substrate characteristics was due to the ever-changing dynamics of the thickened sludge at the wastewater treatment facility. The sludge of the thickeners at the WWTP consists of three components: primary settling unit sludge, waste activated sludge, and tertiary sludge. The fact that the sludge characteristics from each of these three units are also variable, affecting the quality and quantity of the biosolids in the

thickeners, resulting in influent TCOD concentrations between 11 – 38 $g.L^{-1}$ for thickened sludge from the IWWTP. This was 28.7 – 68 $g.L^{-1}$ for the total influent, which included thickening sludge and recycled biosolids (Fig. 3.2B). Therefore, the biogas productions in the CSADs were fluctuating during the entire operating period (Fig. 3.2A). As a result of these fluctuations, SCOD, ammonia, and solids concentration of the influent were also varying. Noteworthy is that these variations were very similar in both system, showing that from an experimental point of view, these systems were equal before the experimental change was made. In accordance, the TCOD concentrations in the effluent of the CSADs were consistent with each other during the entire operating period. During Phase I, the TCOD concentration of the effluents of experimental and control CSADs were 17.93±0.6 $g.L^{-1}$ and 17.18±0.2 $g.L^{-1}$, respectively.

The inconsistency in the biogas production during the first HRT (Days 1-30) was due a problem with our gas meters. The average biogas production of the experimental CSAD in Phase I –first 30 days excluded (*i.e.* 25-180 days) was 11.99 $L.d^{-1}$, while the control CSAD was 11.92 $L.d^{-1}$ (Table 1) (t-test, confidence level %95, for Phase 1 p-value 0.9091) (Fig. 3.2A). This indicates that both experimental and control CSADs were at the same condition before H_2O_2 was added in the recycling biosolids.

It has been stated that both ammonia and volatile fatty acids (VFAs) could be inhibitory to anaerobic digestion if their concentrations are higher than 4000 mg $N.L^{-1}$ for total ammonia [95] and 1000 $mg.L^{-1}$ for VFAs [96]. For this reason, we monitored the ammonia and TVFA concentrations, which were in the recommended range for anaerobic digestion and did not exceed these limits (Fig. 3.2D) (Table 3.1). Alkalinity was measured as calcium carbonate ($CaCO_3$) and used to observe the stability of the CSADs. An alkalinity level of 2527-5887 mg $CaCO_3$ L^{-1} was observed during the operating period for CSADs. The results of IVFA indicated that there was no accumulation of fatty acids (*i.e.,* C1-C8) in the reactors.

Table 3.1 Summary of average performance data of CSADs effluent for the entire operating period

Parameter	Form	Units	Experimental			Control		
			Phase I	Phase II	Phase III	Phase I	Phase II	Phase III
Biogas	-	$L.d^{-1}$	11.99*±5.9 (n=90)	11.87±5.4 (n=14)	10.21±3 (n=31)	11.92±6 (n=90)	11.59±5.5 (n=14)	10.36±3 (n=31)
COD	TCOD	$g.L^{-1}$	17.93±0.6 (n=20)	18.75±0.5 (n=4)	20.04±0.9 (n=4)	17.18±0.62 (n=20)	18.77±0.71 (n=4)	19.82±0.41 (n=4)
	SCOD	$mg.L^{-1}$	272.4±13.7 (n=21)	256.9±10.3 (n=5)	287.1±12.2 (n=5)	269.9±10.6 (n=21)	262.3±9.8 (n=5)	257.9±14.1 (n=5)
COD removal efficiency	TCOD	%	58.9±7.7 (n=20)	63.75±2.9 (n=4)	68.10±11.2 (n=4)	62.63±8.7 (n=20)	62.12±3.2 (n=4)	60.81±16.7 (n=4)
	SCOD	%	79.60±9.5 (n=21)	86.80±8.2 (n=4)	91.75±1.2 (n=4)	79.70±9.8 (n=21)	84.64±8.5 (n=4)	91.02±1.5 (n=4)
TVFA	-	$mg.L^{-1}$	55.2	70.6	51.2	45.2	54.05	55.2
Ammonia	N	$mg.L^{-1}$	728.4	945.4	778.4	726.8	910.7	745.8
Solids	TS	$g.L^{-1}$	18.42±0.11 (n=10)	21.25±0.14 (n=4)	18.23±0.37 (n=5)	18.89±0.69 (n=10)	22.03±0.09 (n=4)	17.86±0.11 (n=5)
	VS	$g.L^{-1}$	10.68±0.12 (n=10)	11.69±0.12 (n=4)	9.68±0.18 (n=5)	11.10±0.62 (n=10)	12.26±0.05 (n=4)	9.57±0.09 (n=5)
Solids removal efficiency	TS	%	56.53±5.4 (n=10)	57.54±2.0 (n=4)	58.52±6.4 (n=5)	59.12±5.3 (n=10)	55.51±2.3 (n=4)	53.23±13.8 (n=5)
	VS	%	61.05±4.9 (n=10)	63.98±3.0 (n=4)	61.84±7.0 (n=5)	62.74±4.8 (n=10)	60.52±2.1 (n=4)	56.13±15.7 (n=5)
Alkalinity	$CaCO_3$	$mg.L^{-1}$	3752	3680	3643	3690	3704	3617
pH	-	-	7.09±0.21 (n=90)	6.91±0.12 (n=14)	6.92±0.16 (n=31)	7.11±0.17 (n=90)	6.93±0.14 (n=14)	6.94±0.15 (n=31)

*First 30 days excluded for both CSADs, also a correction factor of 0.704 $L.d^{-1}$ was subtracted from all the data through days 30-180 for only the experimental CSAD due to an experimental error in biogas production measurement. Error bars were calculated as standard deviations.

Even though the settlers were not mixed and not fed by fresh inoculum, they still produced some biogas but at much lower rates than in the CSADs. While the experimental settler produced 0.25±0.3 $L.d^{-1}$, the control settler produced 0.24±0.5

$L.d^{-1}$ and their production rates were very stable during the entire operating period. The reason why the TCOD concentration of the control settler was higher than the experimental settler while their SCOD concentration were approximately equal is that the control settler was settling its solids better (Fig. 2s). Therefore, the solids concentration in the control settler was also slightly higher than the experimental settler (Table 2) during Phase I.

Table 3.2. Summary of average performance data of settlers effluent for the entire operating period*

Parameter	Form	Units	Experimental			Control		
			Phase I	Phase II	Phase III	Phase I	Phase II	Phase III
COD	TCOD SCOD	$g.L^{-1}$ $mg.L^{-1}$	19.68±0.95 (n=20) 276.3±13.8 (n=20)	25.42±1.27 (n=4) 270.3±3.2 (n=5)	25.01±0.71 (n=4) 270.1±13 (n=5)	22.40±1.36 (n=20) 255.8±12 (n=20)	27±0.98 (n=4) 270.3±4.5 (n=5)	22.49±1.47 (n=4) 286.2±12.5 (n=5)
TVFA		$mg.L^{-1}$	61.7	47.8	86.1	46	52.5	47.3
Ammonia	NH_3-N	$mg.L^{-1}$	708.8	983.9	879.3	709.5	938.5	802.5
Solids	TS VS	$g.L^{-1}$ $g.L^{-1}$	20.06±0.25 (n=10) 11.56±0.18 (n=10)	31.39±0.15 (n=4) 17.20±0.13 (n=4)	23.59±0.17 (n=5) 12.71±0.18 (n=5)	22.95±0.16 (n=10) 13.17±0.10 (n=10)	32.04±0.10 (n=4) 17.62±0.10 (n=5)	21.52±0.13 (n=5) 11.88±0.13 (n=5)
Alkalinity		$mg.L^{-1}$	4188	4319	4224	3832	4257	4162
pH		-	7.09±0.21 (n=90)	6.91±0.12 (n=14)	6.92±0.16 (n=31)	7.11±0.17 (n=90)	6.93±0.14 (n=14)	6.92±0.15 (n=31)

*Error bars were calculated as standard deviations.

Even though an increase in soluble chemical oxygen demand was observed after H_2O_2 addition, it did not affect the biogas production

CSADs consistency between experimental and control setups was also observed during Phases II and III and, the TCOD concentration was 18.75±0.5 and 20.04±0.9 $g.L^{-1}$, respectively, for the experimental CSAD, 18.77±0.71 and 19.82±0.41 $g.L^{-1}$, respectively, for the control CSAD (Fig. 3.2B). SCOD concentrations of the effluents from CSADs were rather stable and showed no change in Phase II or III between control and experimental CSADs (Fig. 3.2C). While the average biogas production rate of the experimental CSAD was 11.87 and 10.21 $L.d^{-1}$ during Phases II and III respectively; for the control CSAD, it was 11.594 and 10.366 $L.d^{-1}$ (Fig. 3.2), (Table 3.1), (for the entire operating period p-value 0.9331). The use of H_2O_2 into the recycled biosolids did not affect the biogas production during either Phase. Statistical analysis based on the biogas production also showed that the difference between the control CSAD and experimental CSAD were not significant for (Phase 2 p-value 0.9068, for Phase 3 p-value 0.8229, for Phase 3-4 p-value 0.9644). The methane contents of the biogas during the oxidative treatment Phases indistinguishable for both systems. These findings were also in accordance with a previous study in which addition of 2 $g.L^{-1}$ H_2O_2 per VSS influent (60 ml) to recycled biosolids [which was 20% of the volume of the reactor sludge] to a one-stage mesophilic anaerobic digester (37°C, 8 L of active volume) did not enhance biogas production; conversely, a decrease from 319.9 to 281.1 mL CH_4 g^{-1} COD removed was observed after the addition of H_2O_2. While the biogas production was not enhanced, a 4.8% of solid removal efficiency increase was stated. However, in the same configuration, when they pretreated the recycled biosolids with heat at 90°C and

2 $g.L^{-1}$ H_2O_2 per VSS influent, a significant increase in biogas production was reported [81].

Comparing the recycled biosolids with H_2O_2 and without H_2O_2, several parameters were found to be different (Fig. 3.3), even though the biogas production of CSADs were not enhanced. H_2O_2 addition increased the solubilization of anaerobically treated biosolids. The increase for SCOD concentration was approximately 1.5 times that of untreated biosolids during Phase II (Fig. 3.3A). This increase was also consistent with the increasing concentration of H_2O_2 in Phase III and the SCOD concentration was doubled. The TCOD concentration, however, showed a slight increase compared to SCOD concentration. In Phase II and III, the TCOD concentration increase was 13.38% and 33.46%, respectively. The TCOD increase in H_2O_2 added sludge was probably due to the increasing settleability of the solids in the samples and it can be also stated that increasing amount of H_2O_2 caused more sludge to settle. The reason that the overall change in COD concentration did not cause any increase in biogas production could be that the increased amount was insignificant when it was compared to the influent. It is also possible that the same amount of solubilization could be achieved in the CSAD. The H_2O_2 addition did not appreciably increase the volatile solids (VS) concentration even though a COD increase was observed (Fig. 3.3C). As stated in a thermal pretreatment study earlier [65], H_2O_2 addition could have caused formation of recalcitrant products in the sludge and this could be the source of higher COD in the pretreated recycled biosolids. In

another pretreatment study, the application of H_2O_2 did not result in an increase in SCOD concentration. In this study, to evaluate SCOD concentration increases and eventually methane enhancement in anaerobic digestion, ultrasonication and H_2O_2 addition to excess wastewater sludge was examined. According to their results, the sonication by itself increased the SCOD concentration and improved biogas production. When H_2O_2 and sonication were applied together, the process did not result in any additional increase in either SCOD concentration or biogas production. The same effect was observed when only H_2O_2 was applied [97]. The reason for this discrepancy result in SCOD concentration could be due to the sludge characteristics. It is observed that if the sludge is readily biodegradable, the oxidative pretreatment might not be effective [29]. In our study, the recycled sludge was already digested and H_2O_2 was added to this recycled biosolids while their sludge was an excess sludge from a treatment plant.

Similarly, BOD_5 concentrations were also higher in pretreated biosolids (Fig. 3.3C,D). The average changes for BOD_5 during Phase II and III were 41% and 32% for soluble BOD_5 concentration, 10% and 15% for total BOD_5 concentration, respectively. A similar result was reported in a previous study. H_2O_2 were applied to recycled biosolids (0.03% v/v) and its effects were observed for biogas production and solids removal efficiency in both lab- and full-scale anaerobic digesters. BOD_5 change was found to be 60% for soluble, and 48% for total in pretreated biosolids [82]. According to their results from bench-scale experiment (10 L, 30 days of HRT,

32°C), the average biogas production was 60% higher. However, these results were obtained with a single reactor setup without a control. Basically, the reactor was fed for 90 days with biosolids from anaerobic digesters and another 90 days by pretreating these biosolids with H_2O_2. For full-scale experiment, 13% more biogas and 11.5% more reduction in biosolids were reported. To obtain this data, a full-scale anaerobic digester with settler had been operated in the same condition compared to the bench-scale setup. Again, H_2O_2 (0.037% v/v) was added to the recycled biosolids, and compared with the annual result of the year before when H_2O_2 had not been added (*i.e.,* 2005-2007 *vs.* 2008). The reason why this biogas increase was not observed in our setup could be explained by two reasons: 1) biosolids recycling was only performed after H_2O_2 addition to the full-scale setup; 2) temporal fluctuations of the biosolids in the thickeners at the IAWWTP are so high that changes in gas production could have been due to the temporal variation in substrate changes, and thus changes in performance data. In the original configuration, the sludge HRT was 30 days for their CSAD. However, when a recycling unit is applied to the system, the sludge retention time is increased, which means the microorganisms would obtain more contact time with substrates and eventually more biogas would be produced in the digesters. Another reason could be the variability of the thickened sludge. The influent of wastewater treatment plants varies from day to day and as a result of this variability, the solids content and quality of settlers and thickeners fluctuate with potential changes of biogas production in the anaerobic digesters. This was particularly observed in our study (Fig. 3.1A). Therefore, the biogas production

essentially depends on the influent characteristic at a treatment plant. The observed results on biogas production in the study could be explained only due to these fluctuations. Possibly, both reasons can explain the 13% increase in biogas production. Our study shows that it is important to include a control system.

The solid removal efficiency of the experimental CSAD was slightly higher the than control CSAD during Phase II and III (Table 3.1). Similar result was also reported and a 4.8% increase was found in solids removal efficiency when H_2O_2 was applied to recycled biosolids [81]. This was possibly due to the solids increase in recycled biosolids after the addition of H_2O_2 (Fig. 3.3). The solids concentration in the CSADs were stable around 10 $g.L^{-1}$ and solids removal efficiency was affected by an increase in influent solids concentration. Similar results were also noticed in the settlers (Table 3.2). The total solids concentration of experimental settler was 8.7% higher than control settler in Phase III. H_2O_2 is reported to increase the dewaterability and settleablity, which in this circumstance, could be the reason for the increase in solids concentration in experimental settler [98].

The BMP test also showed no significant difference when H_2O_2 concentration was increased (Fig. 3.4). At the end of 30 days incubation, the total methane production was 15.7±3.4 ml, 14.8±2.2 ml, 18.9±2.3 ml, 15.9±2 ml, 16.9±0.8 ml, 16.7±1.3 (error bars were calculated as standard deviations and n = 2, 3, 3, 3, 3, 3, respectively) ml for 0, 0.037%, 0.19%, 0.37%, 0.55%, 0.74%, 1.86% samples,

respectively. Comparing these results statistically also indicates that the difference between the varying H_2O_2 samples is insignificant (t-test, confidence level %95, p-value for 0.037%, 0.19%, 0.37%, 0.55%, 0.74%, 1.86% samples, respectively: 0.9962, 0.4453, 0.7628, 0.5748, 0.7015, 0.5932). At the end of the first day, however, all samples produced around 3.7ml methane while the control bottle only produced 1.7 ml of methane. This result shows that H_2O_2 is capable of accelerating the hydrolysis, but eventually this advantage disappears in a 30-day HRT configuration.

Table 3.3 Summary of average performance data of the effluent of experimental settler before and after the H_2O_2 addition during Phase II and III*

Parameter	**Form**	**Units**	**Recycled Solids**		**Recycled Solids with H_2O_2**	
			Phase II	Phase III	Phase II	Phase III
COD	TCOD	$g.L^{-1}$	25.42±1.27 (n=4)	25.01±0.71 (n=5)	28.82±1.47 (n=4)	33.38±1.08 (n=5)
	SCOD	$mg.L^{-1}$	270.3±3.2 (n=4)	270.1±13 (n=5)	443.1±15.4 (n=4)	613.2±19.3 (n=5)
BOD_5	Soluble	$mg.L^{-1}$	24.05±6.6 (n=4)	27.1±2.4 (n=5)	41.25±7.5 (n=4)	39.97±8.8 (n=5)
	Total	$mg.L^{-1}$	1890±319 (n=4)	1599±155 (n=5)	2109±399 (n=4)	1894±349 (n=5)
TVFA		$mg.L^{-1}$	47.8	86.1	68.6	160.9
Ammonia	NH_3-N	$mg.L^{-1}$	983.9	879.3	901.4	873.4
Solids	VS	$g.L^{-1}$	17.20±0.13 (n=4)	12.71±0.18 (n=5)	17.78±0.12 (n=4)	14.44±0.35 (n=5)
pH		-	6.91±0.12	6.92±0.16	7.17±0.18	7.19±0.20

*Error bars were calculated as standard deviations.

3.5 Conclusions

- Anaerobic digestion is a highly stable and predictable system once it reaches the pseudo steady-state conditions and exhibits similar performance under similar operating condition.
- The application of H_2O_2 when it was used by itself on recycling biosolids, it has the potential of accelerating the hydrolysis but had no effect on biogas production. Due to this fact, H_2O_2 should not be supplemented into the recycled biosolids of the full-scale plant.

3.6 Acknowledgement

We would like to thank to Turkish Ministry of Education Scholarship program for financial support in completing this study. We would also acknowledge the workers at the Ithaca Area Wastewater Treatment Plant for supplying inoculum and substrate and for letting us use a work area at the plant.

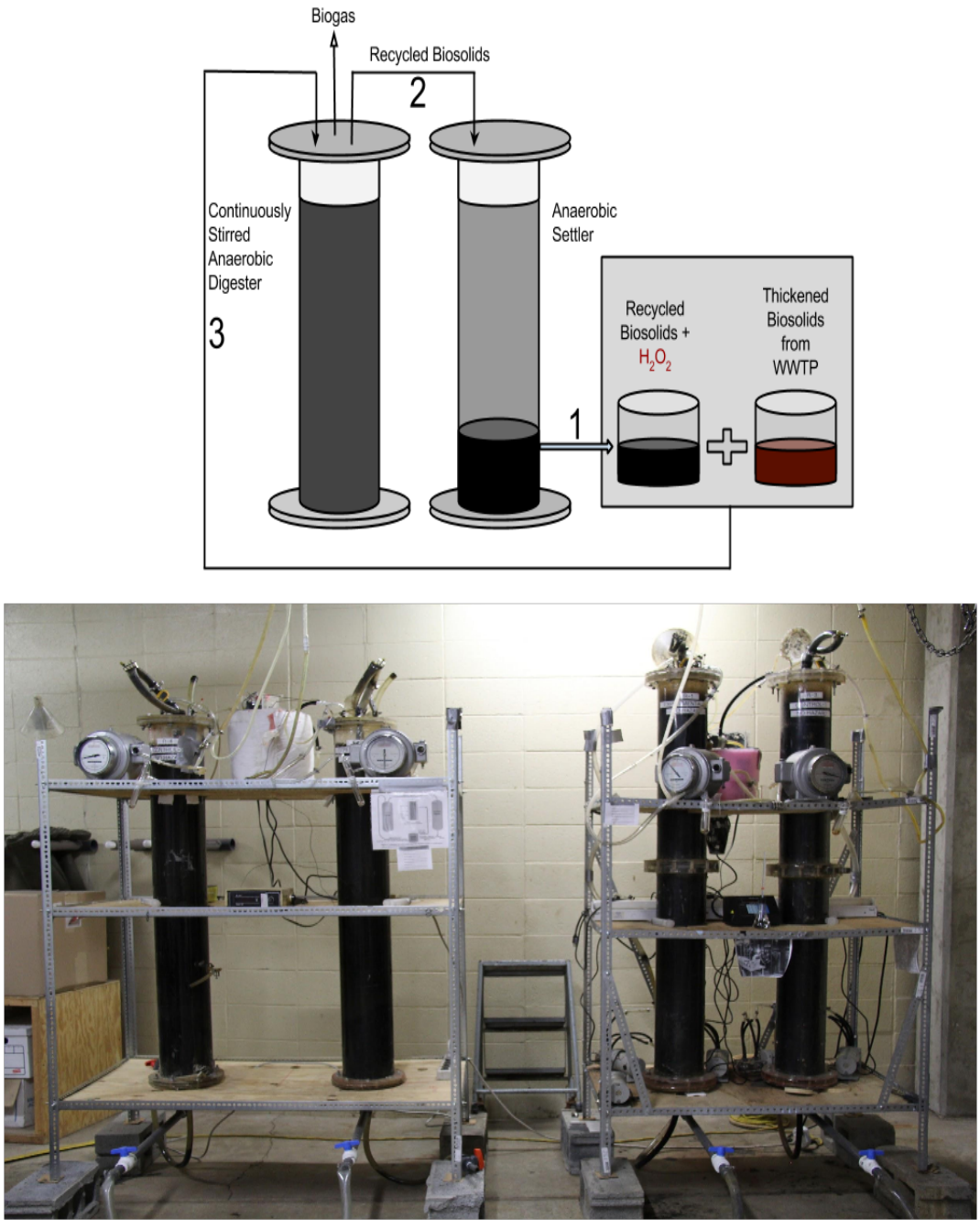


Fig. 3.1 Setup of the 45-L continuously stirred anaerobic digesters (CSADs) and anaerobic settlers: a) schematic of the pilot-scale CSADs; b) picture of pilot-scale CSADs on the right and anaerobic settlers on the left at the IAWWTP

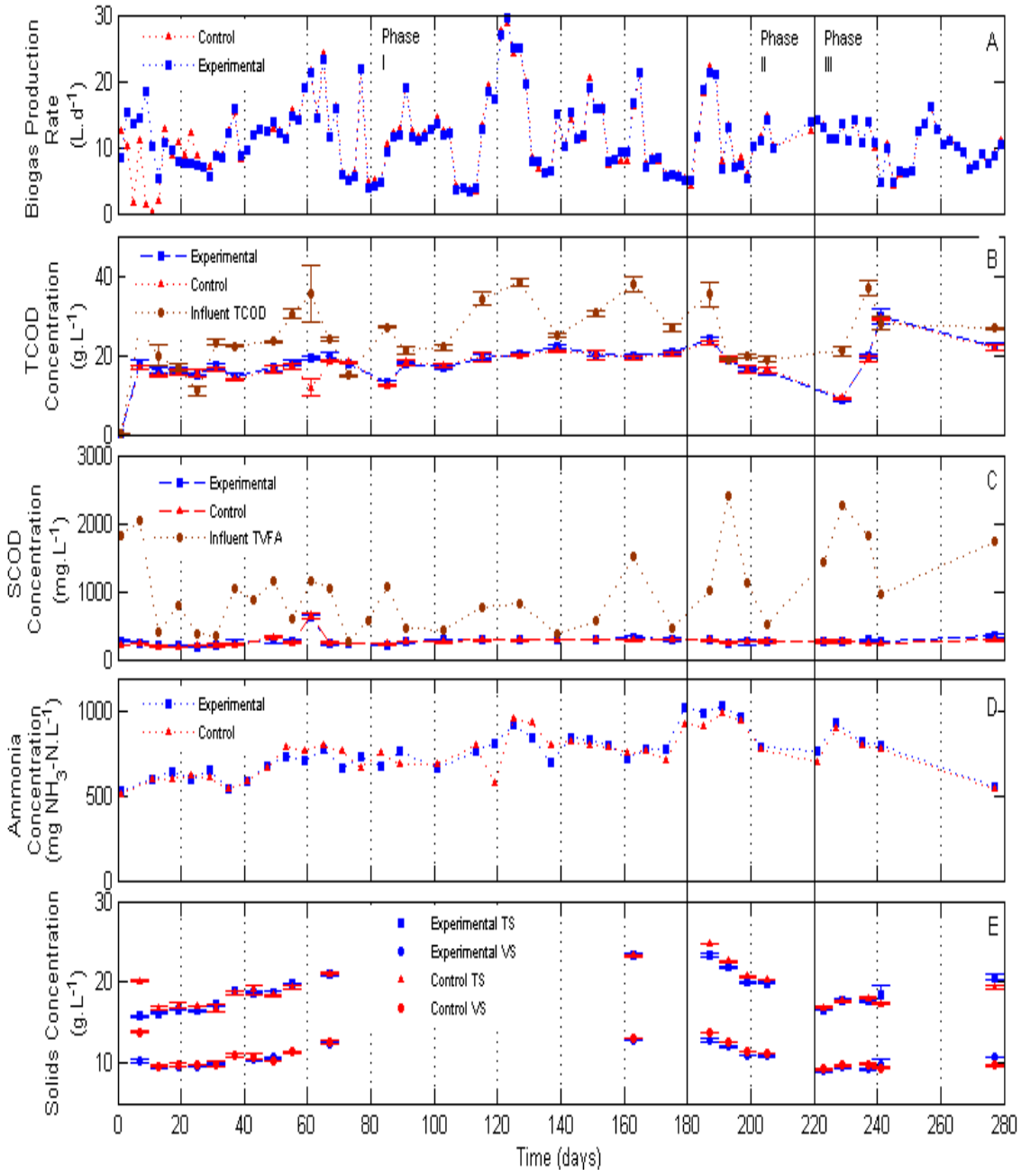


Fig. 3.2 Performance data during the operation period for the CSADs: a) biogas production rate; b) TCOD concentration; c) SCOD concentration *vs.* influent TVFA concentration; d) ammonia concentration; e) solids concentration. Phase I) no hydrogen peroxide (H_2O_2) added; phase II) 0.037% (v/v) H_2O_2 added into the recycled biosolids of the experimental system; phase III) 0.37% (v/v) H_2O_2 added into the recycled biosolids of the experimental system

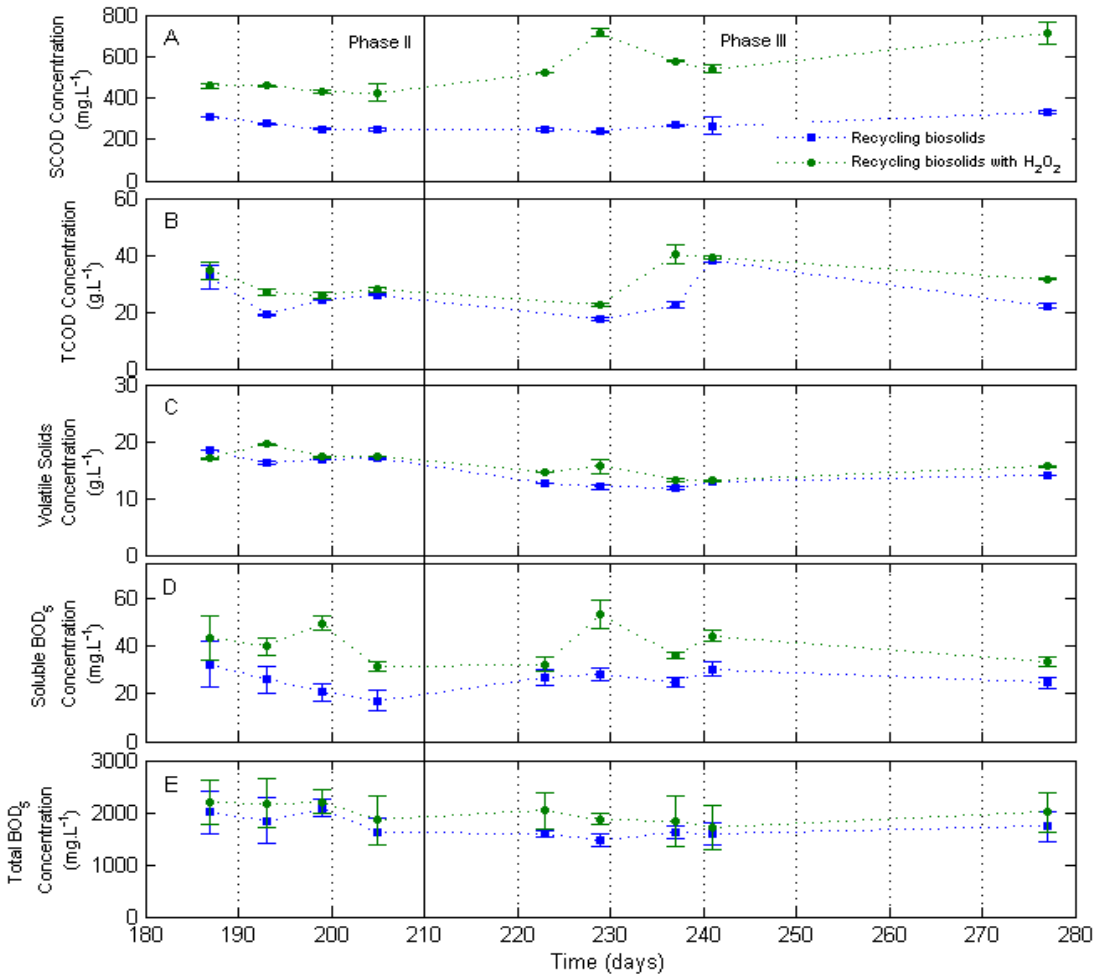


Fig. 3.3 Effluent characteristics of the recycled sludge with and without H_2O_2 during the operating period for recycled biosolids: a) SCOD concentration; b) TCOD concentration; c) volatile solids concentration; d) soluble BOD_5 concentration; e) total BOD_5 concentration. Phase II) 0.037% (v/v) H_2O_2 added into the recycled biosolids of the experimental system; phase III) 0.37% (v/v) H_2O_2 added into the recycled biosolids of the experimental system

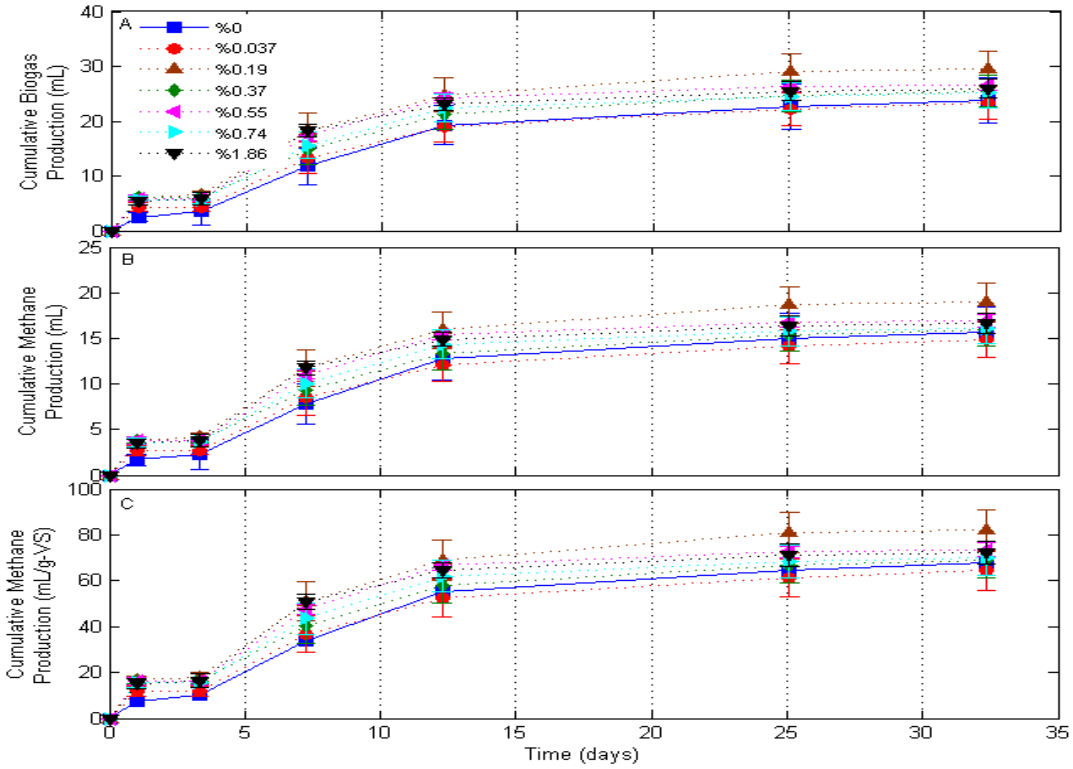


Fig. 3.4 Biological methane potential (BMP) of varying H_2O_2 samples: a) cumulative biogas production; b) cumulative methane production; c) cumulative methane production mL/g-VS.

APPENDIX

Appendix 1: Performance data during the operation period for anaerobic settlers

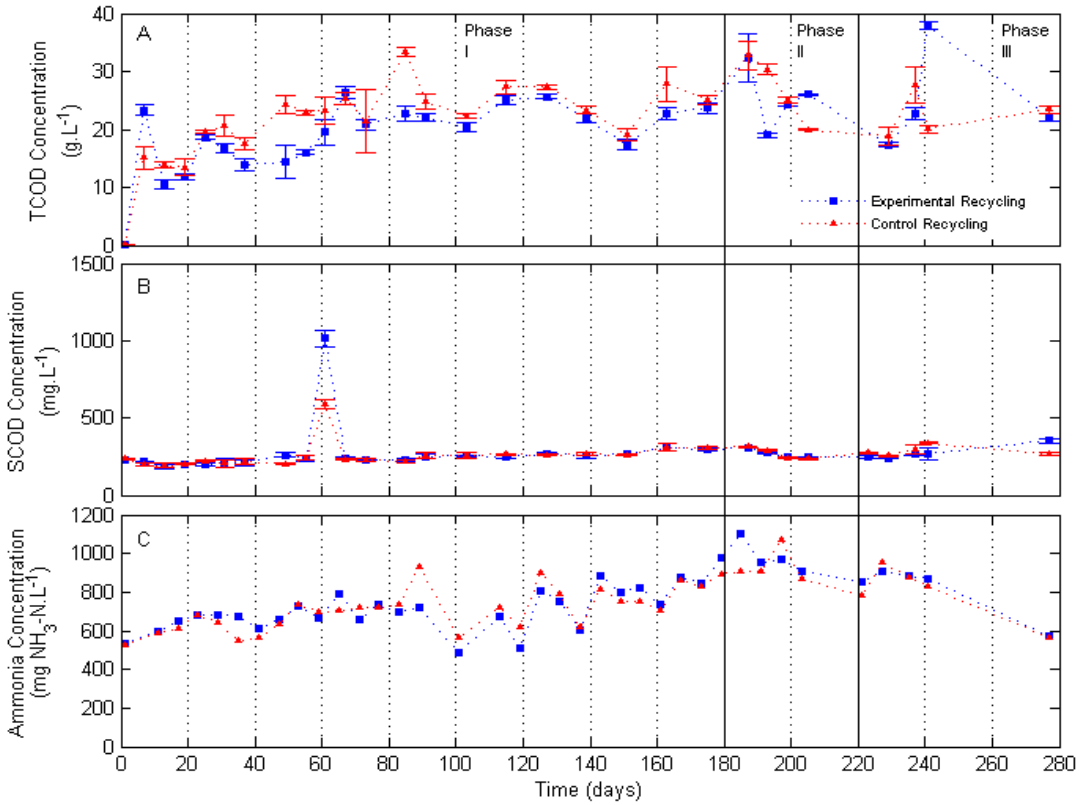


Fig A1 Performance data during the operation period for anaerobic settlers: a) TCOD concentration; b) SCOD concentration; c) ammonia concentration. Phase I) no hydrogen peroxide (H_2O_2) added; phase II) 0.037% (v/v) H_2O_2 added into the recycled biosolids of the experimental system; phase III) 0.37% (v/v) H_2O_2 added into the recycled biosolids of the experimental system

Appendix 2: T-test graphs for CSADs

2-Sample t: E2T, C2T

2/13/15, 4:26 AM

Method

μ_1: mean of E2T
μ_2: mean of C2T
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
E2T	114	11.6732	5.4511	0.5105
C2T	114	11.6119	5.5768	0.5223

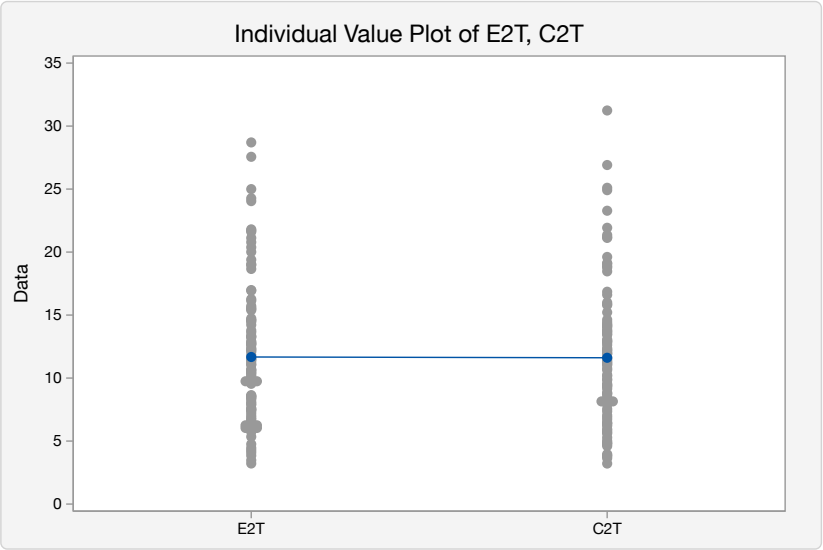
Estimation for Difference

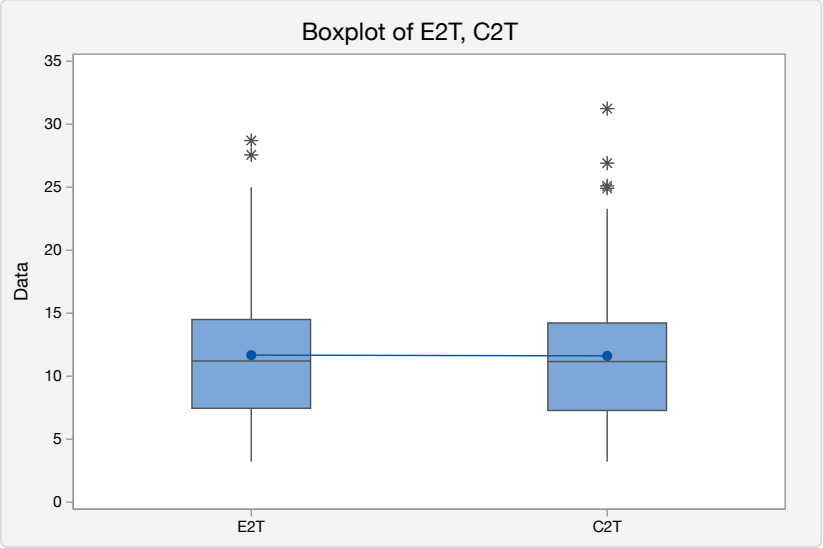
Difference	95% CI for Difference
0.0614	(-1.3779, 1.5007)

Test

Null hypothesis	$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis	$H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
0.08	225	0.9331


Individual Value Plot of E2T, C2T
Data
35
30
25
20
15
10
5
0
E2T
C2T


Boxplot of E2T, C2T
Data
35
30
25
20
15
10
5
0
E2T
C2T

2-Sample t: E2T-Phase1, C2T-Phase1

2/13/15, 4:32 AM

Method

μ_1: mean of E2T-Phase1
μ_2: mean of C2T-Phase1
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
E2T-Phase1	78	12.1706	5.9250	0.6709
C2T-Phase1	78	12.0607	6.0846	0.6889

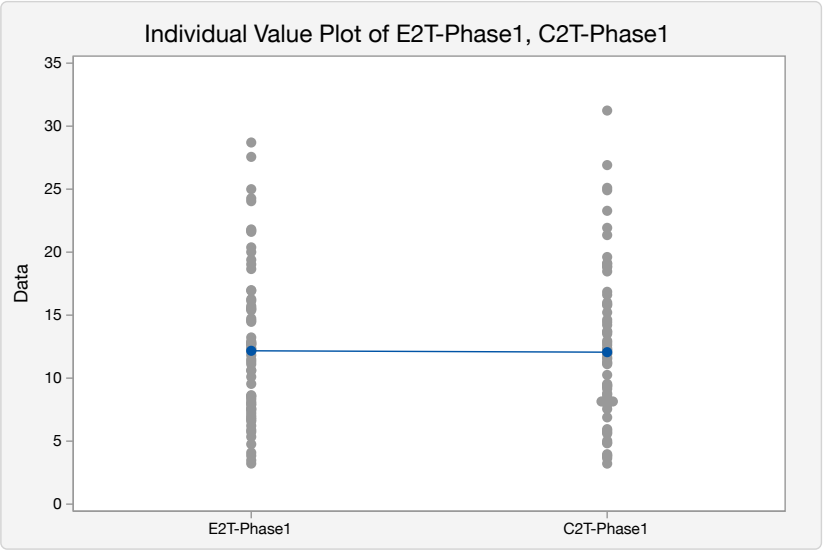
Estimation for Difference

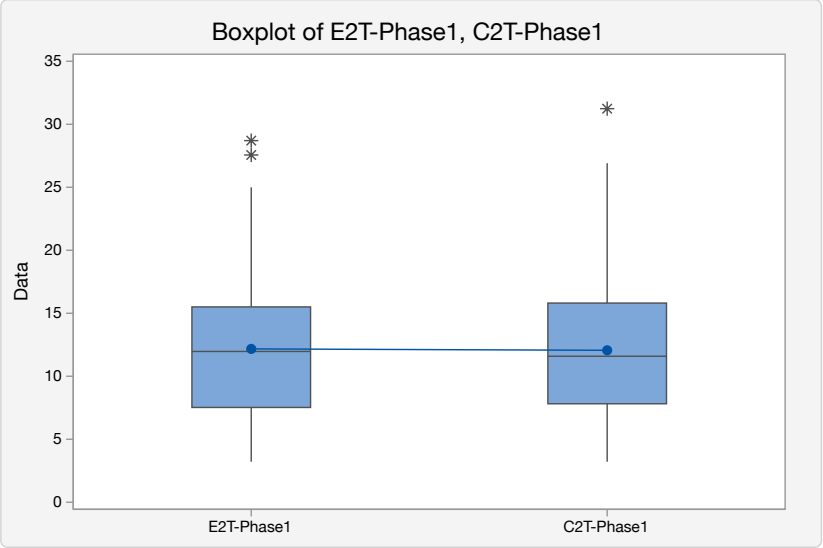
Difference	95% CI for Difference
0.1100	(-1.7898, 2.0097)

Test

Null hypothesis	$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis	$H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
0.11	153	0.9091


Individual Value Plot of E2T-Phase1, C2T-Phase1
Data
35
30
25
20
15
10
5
0
E2T-Phase1
C2T-Phase1


Boxplot of E2T-Phase1, C2T-Phase1
Data
35
30
25
20
15
10
5
0
E2T-Phase1
C2T-Phase1

2-Sample t: E2T-Phase2, C2T-Phase2

2/13/15, 4:33 AM

Method

μ_1: mean of E2T-Phase2
μ_2: mean of C2T-Phase2
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
E2T-Phase2	14	12.458	4.817	1.287
C2T-Phase2	14	12.235	5.190	1.387

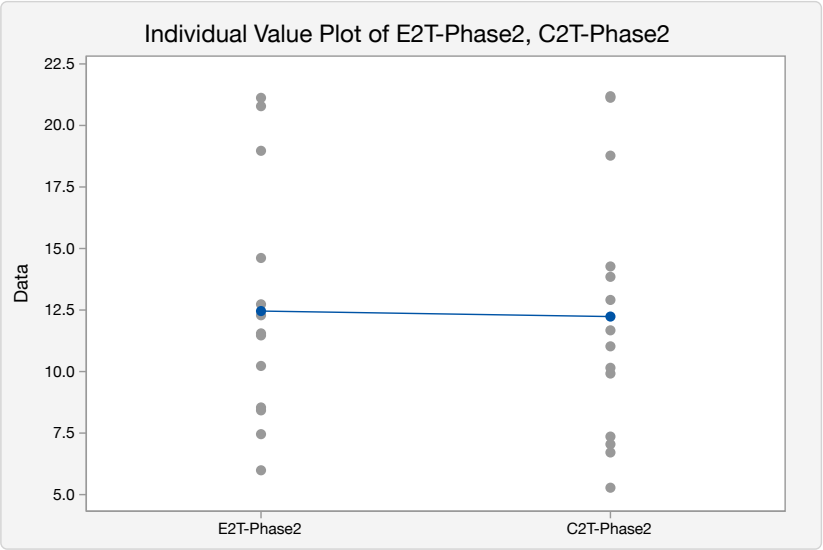
Estimation for Difference

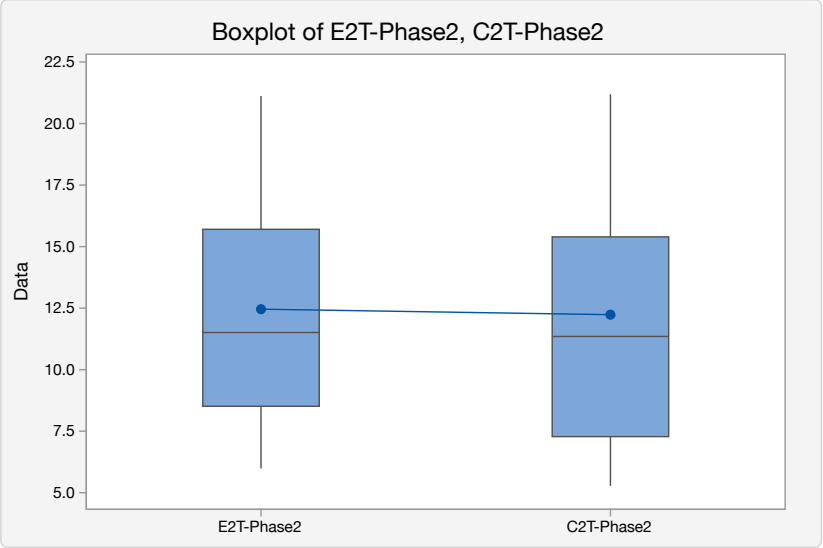
Difference	95% CI for Difference
0.224	(-3.674, 4.121)

Test

Null hypothesis	$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis	$H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
0.12	25	0.9068


Individual Value Plot of E2T-Phase2, C2T-Phase2
Data
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
E2T-Phase2
C2T-Phase2


Boxplot of E2T-Phase2, C2T-Phase2
Data
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
E2T-Phase2
C2T-Phase2

2-Sample t: E2T-Phase3, C2T-Phase3

2/13/15, 4:34 AM

Method

μ_1: mean of E2T-Phase3
μ_2: mean of C2T-Phase3
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
E2T-Phase3	22	9.4101	3.1795	0.6779
C2T-Phase3	22	9.6243	3.1270	0.6667

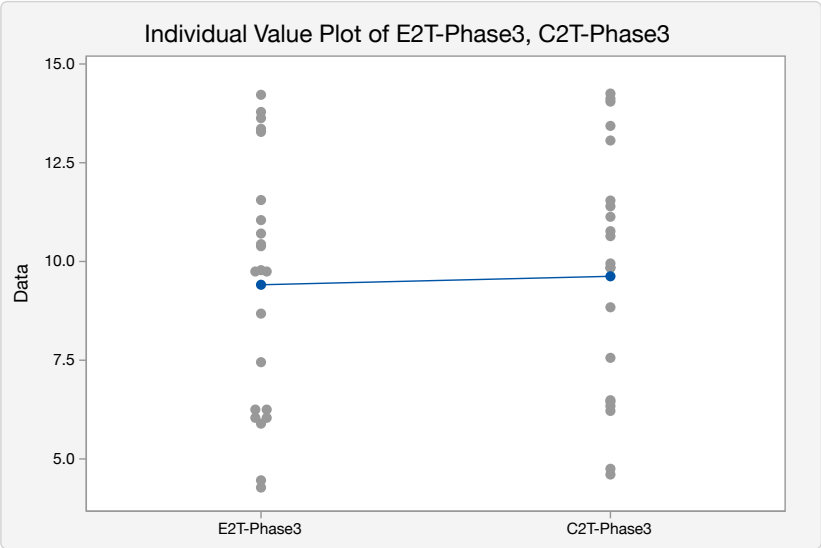
Estimation for Difference

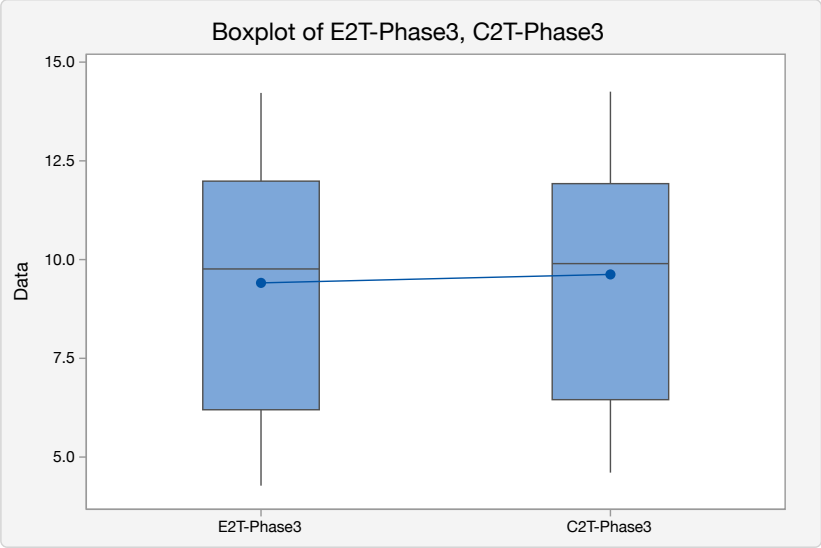
Difference	95% CI for Difference
-0.2141	(-2.1343, 1.7060)

Test

Null hypothesis	H_0: $\mu_1 - \mu_2 = 0$
Alternative hypothesis	H_1: $\mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-0.23	41	0.8229


Individual Value Plot of E2T-Phase3, C2T-Phase3
15.0
12.5
10.0
7.5
5.0
Data
E2T-Phase3
C2T-Phase3


Boxplot of E2T-Phase3, C2T-Phase3
15.0
12.5
10.0
7.5
5.0
Data
E2T-Phase3
C2T-Phase3

2-Sample t: E2T-Phase2&3, C2T-Phase2&3

2/13/15, 4:36 AM

Method

μ_1: mean of E2T-Phase2&3
μ_2: mean of C2T-Phase2&3
Difference: $\mu_1 - \mu_2$

Equal variances are not assumed for this analysis.

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
E2T-Phase2&3	36	10.5955	4.1178	0.6863
C2T-Phase2&3	36	10.6394	4.1879	0.6980

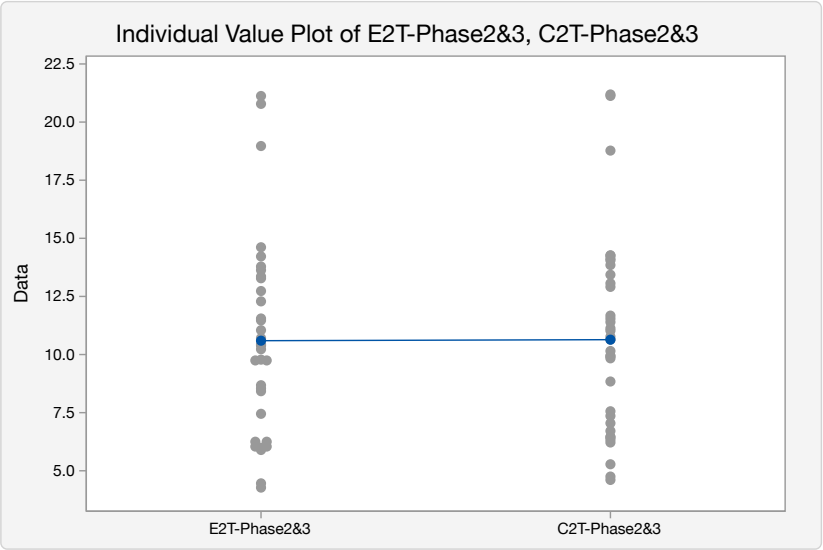
Estimation for Difference

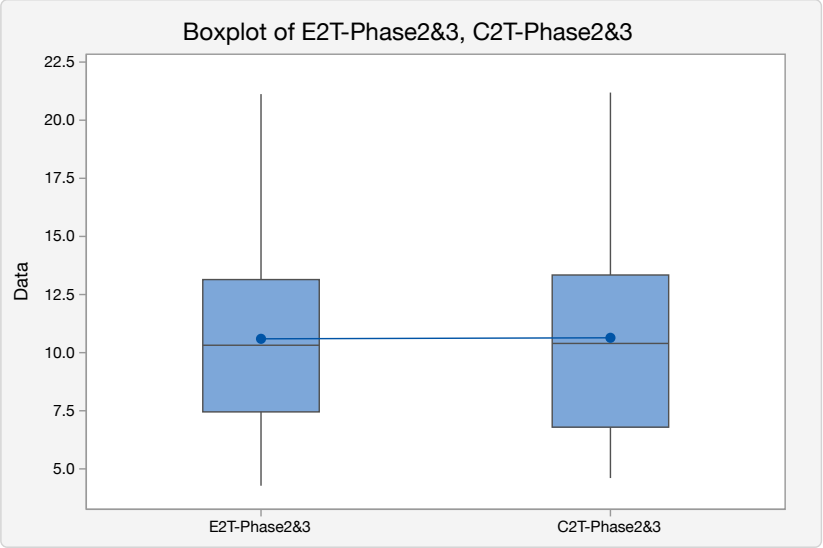
Difference	95% CI for Difference
-0.0439	(-1.9966, 1.9089)

Test

Null hypothesis	$H_0: \mu_1 - \mu_2 = 0$
Alternative hypothesis	$H_1: \mu_1 - \mu_2 \neq 0$

T-Value	DF	P-Value
-0.04	69	0.9644


Individual Value Plot of E2T-Phase2&3, C2T-Phase2&3
Data
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
E2T-Phase2&3
C2T-Phase2&3


Boxplot of E2T-Phase2&3, C2T-Phase2&3
Data
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
E2T-Phase2&3
C2T-Phase2&3

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