

**METHANE PRODUCTION FROM ANAEROBIC CO-DIGESTION OF
WASTEWATER SLUDGE AND *SCENEDESMUS SP.***

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Presented to the

Faculty of

San Diego State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Civil Engineering

with a Concentration in

Environmental Engineering

by

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DEDICATION

I dedicate this writing to my friends, family, and colleagues who helped make the completion of this research possbile.

ABSTRACT OF THE THESIS

Methane Production from Anaerobic Co-Digestion of Wastewater
Sludge and *Scenedesmus sp.*

by
Don Dinh Nguyen
Master of Science in Civil Engineering with a Concentration in
Environmental Engineering
San Diego State University, 2014

The combination of rising energy consumption in the U.S. and sustained growth of developing countries has made clear the importance of developing an energy source that is renewable and minimizes greenhouse gas emissions. The use of algae as an energy source can satisfy both of these criteria, but the current focus on developing it as a biofuel requires a significant amount of energy input, making it not yet economically feasible.

This research combines a promising energy source with a decades-old wastewater treatment technology to generate biogas by combining the anaerobic digestion of algae and wastewater sludge. Bench-scale anaerobic digesters were setup with various proportions of the microalgae *Scenedesmus quadricuada* and thickened waste activated sludge (TWAS) and their biogas production was evaluated. In addition, the effects of operational parameters, such as temperature and alkalinity, on biogas production and residual characteristics were investigated.

Biogas production for the various algae and TWAS combinations ranged from 0.46 to 0.72 mL per mg of volatile solids (VS) digested, while VS and chemical oxygen demand (COD) were reduced on average, 47 and 50%, respectively, at 35°C. Total coliform (TC) and fecal coliform (FC) concentrations saw at least a one log reduction after digestion, allowing the digestant to meet the USEPA requirements for classification as a Class B biosolid and its use in certain land applications. The digestant had nitrogen and phosphorous levels in the range of 5 to 19% as N and 5 to 15% as P, respectively, putting it in the range of commercial fertilizer levels. It was also determined that decreasing digestion temperatures from 35°C produced significantly less biogas, while adjusting the amount of initial alkalinity in digesters did not have a significant effect on biogas production.

From these results, anaerobically digesting algae along with wastewater sludge can be utilized as a feasible method to harness the energy potential of algae. Although some of this potential remains locked up in the undigested portion, its synergy with wastewater treatment plants (WWTPs) cannot be overstated. Growing algae using existing waste streams at WWTPs such as CO₂ and effluent wastewater highlights this technology's ability to transform waste into a valuable commodity without enormous new infrastructure investment.

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CHAPTER 1

INTRODUCTION

From 1985 to 2010, U.S. total energy production has remained nearly flat, increasing from 68 to 75 quadrillion BTUs, just a little over a 10% increase. Compared to the nearly constant increase in total energy consumption for the same period of 76 to 98 quadrillion BTUs, almost a 30% increase, this means that the U.S. has been increasing its energy imports ever since to make up the deficit (U.S. Energy Information Administration 2011). To combat this and reduce its dependence on imported energy as well as reduce its emission of pollutants to the atmosphere, the U.S. has been trying to develop and increase production of renewable energy.

Solar, wind, and biofuels have been the main beneficiaries of this focus on renewable energy, with wind energy seeing the largest surge in growth and all three predicted to have large increases in use (U.S. Energy Information Administration 2012). However, each type of energy has had its share of drawbacks - solar and wind both require large swaths of land in the right locations and have been criticized for their adverse impacts on the environment. Biofuels, most of which is in the form of ethanol derived from corn (U.S. Energy Information Administration 2012), rely on the consumption of a food crop for energy, which has its own set of logistical and ethical problems.

Another viable source of biofuels is algae. There are multiple ways to extract energy from algae, but the focus of this research is on the potential generation of methane gas from the anaerobic digestion of algae, specifically the co-digestion of *Scenedesmus* algae with wastewater sludge.

1.1 ANAEROBIC DIGESTION

Anaerobic digestion (AD) is one of the oldest and most common processes used to stabilize biosolids (Tchobanoglous, Burton, and Stensel 2003; Ahring 2002). Its purpose in wastewater treatment is to convert bulky, odorous sludges into a more compact, inert material that has most of its pathogens destroyed. This is achieved by taking advantage of

naturally-occurring bacteria that break down organic and inorganic matter in the sludge in an anaerobic environment to produce biogas, which consists mainly of methane (CH_4) and carbon dioxide (CO_2).

AD consists of three basic steps: hydrolysis, fermentation (also referred to as acidogenesis), and methanogenesis. In hydrolysis, complex compounds such as lipids and proteins are broken down into their monomers. These monomers are then broken down into the three precursors of methane formation during fermentation: hydrogen, acetate, and CO_2 . In the final step of methanogenesis, two groups of obligate anaerobes are responsible for generating methane. One group splits acetate into CH_4 and CO_2 , whereas the other group reduces CO_2 with hydrogen to generate CH_4 . 72% of the CH_4 is generated by the acetate-splitting bacteria, the aceticlastic methanogens (Tchobanoglous, Burton, and Stensel 2003).

As methane is a principal component of natural gas, the methane generated by AD can be used as an energy source. At larger wastewater treatment plants (WWTPs) such as the Point Loma Wastewater Treatment Plant, AD produces not just enough CH_4 to meet the entire plant's power needs, but also enough to be able to sell excess to the local electricity grid (The City of San Diego 2013). About 25% of energy used in the U.S. came from natural gas in 2011, with 31% of it being used for electric power (U.S. Energy Information Administration 2012). Other common uses of natural gas are for home heating and cooking and in transportation where properly equipped buses and cars can use it.

1.2 ALGAE AND ANAEROBIC DIGESTION

Algae is a very popular source of biomass used for biofuels, and there are several characteristics of algae that make it a promising source of biofuel: high productivity rate, ability to tolerate a wide range of conditions, and lack of competition for land with food crops (Costa et al. 2012). Thus, research into the conversion of algae into biofuel is intense, with technologies such as pyrolysis and liquefaction having been developed (Demirbas 2010). However, the major problem with these thermochemical conversion techniques is that they require large inputs of energy to generate the high temperature or pressure needed for the conversion process. Further research needs to be conducted to make them into feasible commercial technologies.

On the other hand, the AD of algae to produce methane is an algae-to-energy conversion technique that does not require large energy inputs. In this process, algae would be added to the anaerobic digesters of an existing wastewater treatment plant, and anaerobic bacteria would convert it to methane, which would be captured, scrubbed, and utilized on site or transported to a power plant.

The use of AD to digest algae and produce methane is an old technology that was first explored in the late 1950s but sidelined until relatively recently with the renewed interest in alternative energy production technologies. Golueke et al. were the first to explore the topic. They discovered that digesting *Scendesmus* spp. and *Chlorella* spp. compared to raw sewage sludge generated 0.17 to 0.32 L CH₄/gvs, but that the algal methane conversion efficiency was 20% lower than sewage sludge (Golueke, Oswald, and Gotaas 1957). Samson and Leduy determined a yield of 0.26-0.34 L CH₄/gvs from the digestion of blue-green algae *Spirulina maxima*, and that a two-fold increase in methane yield could be gained by adding sewage sludge in a 50% proportion (Samson and Leduy 1983, 1986). Yuan et al. also studied a blue algae but only got a yield of 0.19 CH₄/gvs and a methane concentration of 36.7% (Yuan et al. 2011). Sanchez and Travieso digested *Chlorella vulgaris* using 5-L batch reactors to get a methane yield of 0.31-0.35 L CH₄/gvs (Sánchez Hernández and Travieso Córdoba 1993). Then Cecchi et al. found that digesting the macroalgae *Ulva rigida* and *Gracilaria confervoides* from a lagoon with sewage sludge produced a maximum of 0.31 CH₄/gvs(Cecchi, Pavan, and MataAlvarez 1996). Ras et al. performed an experiment that coupled the algae production and digestion process using *C. vulgaris* and found a methane production of 0.24 L CH₄/gvs for a 28 day retention time (Ras et al. 2011).

In order to improve of the use of AD to digest algae, various techniques have been conducted to enhance the methane production rate. This is especially important considering that a big obstacle to AD of algae is the suspected recalcitrance of the algae cell wall to digestion and the resulting undigested algae (Golueke, Oswald, and Gotaas 1957; Sánchez Hernández and Travieso Córdoba 1993; Sialve, Bernet, and Bernard 2009; Gonzalez-Fernandez, Molinuevo-Salces, and Garcia-Gonzalez 2011; Tartakovsky et al. 2013). Chen and Oswald used heat treatment (100°C for 8h) to increase the methane production by 33%, but the increase in energy use from the additional heat was not offset by the increased methane yield (P. H. Chen and Oswald 1998) and Golueke et al. found a 5 to 10% increase in

algae biodegradability when increasing the AD temperature from 35 to 50°C (Golueke, Oswald, and Gotaas 1957). Nielsen and Heiske pretreated four species of macroalgae with maceration and found an improvement of up to 68% in methane yield (Nielsen and Heiske 2011). Mayo et al. found a gas production rate increase of up to three times that of untreated algae when using a pretreatment scheme of H₂O₂ and UV radiation (Mayo et al. 2011). Ehimen et al. (2013) used a filamentous algae, *Rhizoclonium*, with a digestion time of 28 days at 53°C in 0.5 L batch reactors. They produced significantly less methane than microalgae such as *Chlorella*, but they did increase the methane yield by greater than 20% using a combination of blending and enzymatic pretreatment.

Finally, the AD of algae may be enhanced by co-digesting it with other organic substrates. Samson and Leduy did this in 1983 with sewage sludge and got a two-fold increase in the methane yield (Samson and Leduy 1983), and Cecchi et al. got a yield of 0.31 CH₄/gvs when algae was combined with sewage sludge (Cecchi, Pavan, and MataAlvarez 1996). Yen and Brune added waste paper to algal sludge during AD in a 50/50 ratio, increasing the methane production rate two-fold compared to the digestion of just algae. Their results suggested an optimal C/N ratio between 20 and 25 (Yen and Brune 2007). Nielsen and Heiske codigested *Ulva lactuca* with cattle manure, and although they determined a decrease in the total methane yield, the reactor methane yield increased (Nielsen and Heiske 2011). Park and Li used fat, oil, and grease waste as codigestants for *Nannochloropsis salina* which resulted in a yield of 0.54 CH₄/gvs (S. Park and Li 2012).

In addition to the fact that algae is a good candidate for AD, the growth of algal biomass can be coupled with wastewater treatment, such as high-rate algal ponds (HRAPs), that grow algae in wastewater and treat it at the same time (J. B. K. Park, Craggs, and Shilton 2011). The ability of algae to grow in wastewater and remove pollutants from it is proven. Bich et al. were able to improve nitrogen removal in an HRAP by 23% as well as decrease suspended solids using *Chlorella vulgaris* and water hyacinth (Bich, Yaziz, and Bakti 1999). Ruiz-Marin et al. showed that *Scenedesmus obliquus* was able to remove 100% of N-NH₄ and 83% of P-PO₄ in wastewater after 48 hours (Ruiz-Marin, Mendoza-Espinosa, and Stephenson 2010). Kothari et al. (2012) were not only able to grow *Chlorella pyrenoidosa* in dairy wastewater successfully, but also achieved the removal of more than half of the sulfides and chlorides after 10 days. Furthermore, Wang et al. (2013) showed that the co-digested

sludge had increased dewaterability compared to digested sludge composed of just sludge or algae.

Not only can algae grow in regular wastewater, it can also grow in high-strength wastewater such as that from anaerobically-digested farm manure, which R. Chen et al. (2012) demonstrated Li et al. (2011) were able to grow a variety of algal strains in wastewater centrate (supernatant of centrifuged activated sludge) including *Scenedesmus*. Moreover, growing algae using wastewater may be necessary to create an algae AD system that is financially sustainable (Pittman, Dean, and Osundeko 2011).

For this research, *Scenedesmus sp.* was selected as the algal species to test. One study found that *Scenedesmus obliquus* grew better in municipal wastewater than *Chlorella vulgaris* (Ruiz-Marin, Mendoza-Espinosa, and Stephenson 2010), with both being the predominant species in oxidations ponds (Masseret, Bourduer, and Sargas 2000) and HRAPs (Canovas et al. 1996). *Scenedesmus* also had a high AD methane production compared to other algal species (Sialve, Bernet, and Bernard 2009; Frigon et al. 2013). Since a species of algae that could not only produce more methane (not to mention well known and studied), but also be able to grown easily at a wastewater treatment plant was desired, *Scenedesmus* was chosen.

1.3 RESEARCH OBJECTIVES

The main objective of this research is to investigate methane gas production during anaerobic co-digestion of wastewater sludge and the microalgae, *Scenedesmus sp.* The hypothesis of this research is that the anaerobic co-digestion of *Scenedesmus sp.* with wastewater sludge can enhance methane gas yield. To test this hypothesis, two major objectives were completed:

1. Investigate the increase in the production of methane gas during anaerobic co-digestion of wastewater sludge and *Scenedesmus sp.*
2. Evaluate the influence of operational parameters, such as wastewater sludge and algal biomass loading, detention time, temperature, and alkalinity on the quantity of methane gas generated.

1.4 RESEARCH OUTLINE

The remainder of the document describes the experimental approach, materials and analytical methods, followed by the experimental results and a discussion of the data with

caveats. The experimental approach and materials and analytical methods are described in Chapter 2. Chapter 3 presents the experimental results, and Chapter 4 offers a discussion of those results. Lastly, Chapter 5 summarizes the results and provides recommendations for future studies

CHAPTER 2

RESEARCH METHODOLOGY

In this chapter, the experimental approach, analytical methods, and materials used in the research are presented. 12 of the 24 Standard Operating Procedures developed and utilized for the research can be found in Appendix A.

2.1 EXPERIMENTAL APPROACH

Methane production was measured by adapting the Biochemical Methane Potential assay developed by Owens et al. (Owen et al. 1979) in 1978. The procedure was also guided by standard methods ASTM E2170-01 and ISO 11734:1995 as well as other published research (Moody 2009; Chynoweth et al. 1993; Hansen et al. 2004; Raposo et al. 2012; Shanmugam and Horan 2009).

To test methane production, lab-scale anaerobic digesters were set-up to mimic those at a wastewater treatment plant. 250 mL glass bottles were utilized with 3-port caps equipped with two sets of tubing and one-way polycarbonate luer stopcocks for biogas sampling, inoculum addition, and purging (see Figure 2.1). The tubing apparatus consisted of 1/8" outer diameter (OD) PTFE tubing inserted into 1/8" inner diameter (ID) Tygon® which was capped with a two way valve. The connection between the hard and flexible tubing was sealed with two plastic zip ties double wrapped around the tubes. The hard tubing was inserted into the cap port and sealed with a polypropylene fitting and Tefzel ferrule. Each tube apparatus was approximately 6-8 inches in length and terminated in the headspace of the digester.

After the digester bottles were washed with phosphate-free detergent and allowed to dry, a mixture of microalgae (*Scenedesmus quadricauda*), thickened waste activated sludge (TWAS), and DI water was added to each digester and the headspace was purged with compressed nitrogen gas (N₂) at an approximate rate of 2 liters/minute for 2 minutes in order to create an anaerobic environment. The seed bacteria (inoculum) were extracted from the



Figure 2.1. Lab-scale anaerobic digester.

collection container through a customized cap and tubing system to minimize oxygen interference (see Figure 2.2). The 3-port cap was customized so that 0.25" OD Tygon® tubing weighted with a magnetic stirrer was inserted into the inoculum through the cap while the other two ports were plugged. The inoculum bottle cap did not provide an air tight seal but it was meant to inhibit oxygen interference. A plastic syringe was used to extract the inoculum from the flexible tubing and introduce it to the digesters by fastening the syringe luer onto the valve connected to the shorter tube. After the addition of inoculum, the digesters were purged again with N₂ gas for 20 seconds.

The substrate (i.e. the *S. quadricauda*. and the TWAS) was added to the 250 mL glass digester bottles in a one-to-one ratio with the inoculum based on volatile solids (VS) for all tests, with the exception of a slight unintentional variation in Test 1. For quality control purposes, no less than 10 mL of TWAS was ever sampled. If a smaller sample size was needed, a diluted solution was prepared. The total volume of liquid plus solids for each digester in all tests was 200 mL.



Figure 2.2. Inoculum bottle with customized cap.

The lab-scale digesters were placed into an incubator/shaker at 150 rpm and biogas was sampled on a schedule determined by the amount of biogas that was being produced. The biogas was analyzed with a gas chromatography analytical instrument (Agilent 6890) to determine the chemical makeup.

The algae was grown in our lab using primary effluent as the nutrient and water source, 24 hour fluorescent lighting and 2.5% CO₂.

2.2 PRELIMINARY STUDIES

Preliminary studies were conducted to determine the ideal substrate, i.e. *S. quadricauda* and TWAS, loading to inoculum ratio such that the biogas production would not be limited by low substrate nor overloaded by inoculum. Three substrate loading to inoculum ratios were tested: 0.5, 1.0, and 1.5. Digesters were set-up in triplicate following the approach outlined in the previous section and were incubated at 35°C for 185 days. Results showed the best substrate to inoculums ratio to be 1.

Additionally, a second preliminary test was set-up to determine if a low VS loading was able to generate a measurable amount of biogas. Substrate to inoculum ratio was kept at 1 for both the low VS loading (400 mg/digester or 2 mg/L) and the high VS loading (1460 mg/digester or 7.3 mg/L) sets. Again, digesters were prepared in triplicate and incubated at 35°C for 7 days. It was observed that a low VS loading produced an easily measurable

amount of biogas, and therefore, it was used for the remainder of the tests. For graphs of preliminary results, see Appendix B, Figures B.1-B.3.

2.3 EXPERIMENTAL CONDITION

Several experiments were carried out to evaluate the influence of operational parameters, such as algal biomass loading, temperature, and alkalinity on the quantity of methane gas generated, see Tables 2.1 and 2.2.

Table 2.1. Experimental Set-up Parameters

Sample ID	Test ID	Days	Temperature (°C)	Algae:Sludge Ratio	Alkalinity (mg/L as CaCO ₃)
T1-0	1	60	35	0:100	70
T1-25	1	60	35	25:75	70
T1-49	1	60	35	49:51	70
T1-76	1	60	35	76:24	70
T1-100	1	60	35	100:0	70
T2-20	2	30	20	50:50	70
T2-10	2	30	10	50:50	70
T3-N	3	30	35	50:50	70
T3-I	3	30	35	50:50	1640
T3-H	3	30	35	50:50	3210

The first set of experiments, T1, was designed to evaluate the potential of the microalgae, *S. quadricuada*, as a supplementary feedstock. In these experiments, the contribution of the VS from *S. quadricuada* was 0, 25, 49, 76, and 100% of the total substrate VS. Experimental Set 1 was conducted in triplicate and incubated, along with a control run, at the mesophilic temperature of 35°C for 60 days.

The second set of experiments, T2, was conducted to determine the effect of colder temperatures on waste mineralization and biogas production. For these experiments, 50% of the substrate VS was from *S. quadricuada*. Experimental Set 2 was incubated in triplicate at 20°C and at 10°C for 30 days, along with controls for each temperature.

The effect of alkalinity was investigated in the third and final set of experiments, T3. Alkalinity was adjusted using sodium bicarbonate (NaHCO₃) and three alkalinity levels were tested: 70 (alkalinity level without addition of NaHCO₃), 1640, and 3210 mg/L as CaCO₃. Quadruplicate digesters, in addition to a triplicate control of DI and inoculum, were set up for

Table 2.2. Digester Contents by Volume and Weight of Volatile Solids

Sample ID	mL Algae	VS from Algae (mg)	mL TWAS *	VS from TWA S (mg)	mL Inoculum	VS from Inoculum (mg)	mL DI Water	Substrate: Inoculum Ratio
T1-0	0.0	0.0	2.9	143.3	14.0	148.4	137.7	0.97
T1-25	45.1	36.1	2.2	108.7	14.0	148.4	104.7	0.98
T1-49	90.3	72.2	1.5	74.1	14.0	148.4	71.8	0.99
T1-76	135.4	108.3	0.7	34.6	14.0	148.4	38.8	0.96
T1-100	180.6	144.5	0.0	0.0	14.0	148.4	5.8	0.97
T1-CTRL	0.0	0.0	0.0	0.0	14.0	148.4	236.0	0.00
T2-10	12.0	73.8	1.7	72.2	17.0	147.9	162.3	0.99
T2-20	12.0	73.8	1.7	72.2	17.0	147.9	162.3	0.99
T2-CTRL	0.0	0.0	0.0	0.0	17.0	147.9	183.0	0.00
T3-N	18.2	109.7	2.7	110.9	19.5	220.4	148.9	1.00
T3-I	18.2	109.7	2.7	110.9	19.5	220.4	148.9	1.00
T3-H	18.2	109.7	2.7	110.9	19.5	220.4	148.9	1.00
T3-CTRL	0.0	0.0	0.0	0.0	19.5	220.4	180.5	0.00

* TWAS was diluted before being added. For Test 1: 12.2-48.7 mL of 16.7x diluted TWAS was added. Test 2: 8.7 mL of 5x diluted TWAS was added. Test 3: 13.4 mL of 5x diluted TWAS.

each alkalinity level, and the digesters were incubated at 35°C for 30 days. After 15 days, one digester of each alkalinity level was sacrificed and pH and alkalinity were measured. On the final day, pH and alkalinity of the remaining digesters were measured.

At the beginning and end of each experiment, various tests were completed to determine the chemical oxygen demand (COD), total solids (TS), volatile solids (VS), total and fecal coliform populations, and nutrient levels. Calibration curves were created for COD, total phosphorous, total nitrogen, ammonia, nitrate, nitrite, orthophosphate, methane, and carbon dioxide.

2.4 ANALYTICAL METHODS

Biogas volume was measured using a clean, dry 20 mL Perfektum Micro-Mate glass syringe purchased from Fisher Scientific. The syringe was equipped with a luer lock that could fasten onto the stopcock valves at the ends of the tubing of the lab-scale digesters. The valve was opened and the glass plunger was allowed to be expelled.

The amount of methane (CH_4) and carbon dioxide (CO_2) in the biogas was determined using a Agilent 6890 gas chromatography (GC) machine equipped with thermal conductivity detector (TCD) and sample injection valve. The column utilized was an All Tech Chromosorb 106 80/100 (6'x1/8" x .085"). A computer with Agilent ChemStation software was connected to the GC and used to view and export results.

A biogas sample of approximately 18 mL was taken from a digester with a glass syringe. The sample was manually injected through a sampling valve at a constant speed, and the cycle was initiated. Sample valve temperature was kept at $70^\circ\text{C} \pm 2^\circ$. The gas sample was routed through an All Tech Chromosorb Column and detected with a thermal conductivity detector (TCD). The GC oven was held at 40°C for four minutes, then ramped up by 50°C each minute for three minutes, and held at 190°C for two minutes before being allowed to cool back down to 40°C where it was held for one minute before another sample could be injected. The TCD was set at 250°C , the nitrogen gas reference flow at 25 mL per min, and the nitrogen gas make-up flow at 5 mL per min.

The GC was calibrated with 99.8% bone dry carbon dioxide (CO_2) gas and 99.0% C.P. grade methane (CH_4) gas. A 30:70 ($\text{CO}_2:\text{CH}_4$) pre-made standard was analyzed and results were compared to the prepared 30:70 standard to ensure accuracy. A quality control chart was created from seven runs of the 30:70 pre-made standard and calibration checks were run each new day of testing. For each GC run, peak area was converted to moles of gas using a calibration curve, which was converted to percent CH_4 or CO_2 assuming the contribution from other gases was negligible.

COD was determined using Hach Method 8000 in the range of 20-1500 mg/L, based on the *Standard Methods for Water and Wastewater*, Method 5220-D (Clescerl, Greenberg, and Eaton 1998) which is USEPA approved. 2 mL of sample, standard, or diluted sample were pipetted into a Hach high range COD reagent vial, inverted, and digested for 2 hours at 150°C . Once the temperature had cooled to 120°C , the vials were removed from the digester, inverted one time, and allowed to cool to room temperature. Optical density readings were measured with a Thermo Spectronic Genesys 20 spectrophotometer at 620 nm.

Total and volatile solids were determined based on *Standard Methods for Water and Wastewater*, Methods 2540-B, 2540-E and 2540-G (Clescerl, Greenberg, and Eaton 1998). Evaporating dishes were first cleaned by heating in a high temperature box furnace

(Lindberg/Blue M BF 51766A-1, Asheville, NC) at 550°C for one hour, after which they were allowed to cool to room temperature and weighed. 10 mL of sample was transferred to the dishes, and the dishes were weighed and placed into a drying oven (Yamato DX-400, Santa Clara, CA) at 105°C for at least two hours. After drying, the dishes were removed and cooled in a dessicator until they reached room temperature. They were weighed to determine the TS weight and placed into the 550°C oven for 45 minutes. The dishes were taken out to partially cool in the open air for five minutes and then placed into a dessicator to cool to room temperature. Once at room temperature, they were weighed to determine the VS weight.

Alkalinity was determined using the titration method outlined in *Standard Methods for Water and Wastewater*, Method 2320-B (Clescerl, Greenberg, and Eaton 1998). Hydrochloric acid in the strengths of 0.02 N and 0.1 N were used to titrate a known volume of sample to a pH of 4.5 as measured by an Oakton pH 500 series pH meter with Accumet probe.

Total nitrogen was measured using the Hach Persulfate Digestion Method 10072, which is similar to method 4500-N C of *Standard Methods for Water and Wastewater* (Clescerl, Greenberg, and Eaton 1998). A Total Nitrogen Persulfate Powder Pillow was added to each HR Total Nitrogen Hydroxide Digestion Reagent vial, followed by 0.5 mL of sample. The vials were then capped, shaken vigorously for 30 seconds, inserted into a DRB 200 digester for exactly 30 minutes at 105°C, and removed to cool to room temperature. Next, one Total Nitrogen Reagent A Powder Pillow was added to each vial, which were capped, shaken for 15 seconds, and allowed to sit for a three-minute reaction period. Then the same sequence was repeated for Total Nitrogen Reagent Powder Pillow B with a two-minute reaction period. Lastly, 2 mL of digested sample was transferred into a Total Nitrogen Reagent C vial, which was then capped, inverted slowly 10 times, and allowed to sit for a five-minute reaction period. After the digestion period, the vials were read in a spectrophotometer at 410 nm. This reading was compared to a NO₃⁻-N calibration curve to find the total nitrogen concentration.

Total phosphorous was measured using the Hach Molybdoavanadate Method with Acid Persulfate Digestion, Method 10127, which is adapted from method 4500-B C of *Standard Methods for Water and Wastewater* (Clescerl, Greenberg, and Eaton 1998). First, 5

mL of sample was added to a Total Phosphorous TNT vial, followed by the addition of a Potassium Persulfate Powder Pillow. The vial was then capped and shaken to dissolve the powder, inserted into a DRB 200 digester for 30 minutes at 150°C, and removed to cool to room temperature. Next, 2 mL of 1.54 N sodium hydroxide was added to each vial, which was then capped and inverted to mix. Immediately afterwards, the same sequence was performed for 0.5 mL of molybdoavanadate reagent. The vial was then allowed to sit for a seven-minute reaction period. Lastly, the vials were read in a spectrophotometer at 420 nm. This reading was compared to a PO₄³⁻ calibration curve to find the total phosphorous concentration.

Orthophosphate was measured using the Hach Ascorbic Acid Method 8048, adapted from *Standard Methods for the Examination of Water and Wastewater* (Clescerl, Greenberg, and Eaton 1998). First, a vial was filled with 10 mL of sample, followed by the addition of a PhosVer 3 Powder Pillow. Then the vial was capped, shaken for 15 seconds, and allowed to sit for a two-minute reaction period. Lastly, the vials were read in a spectrophotometer at 880 nm. This reading was compared to a PO₄³⁻ calibration curve to find the orthophosphate concentration.

All glassware used for nutrient testing was acid washed using a 1% hydrochloric acid solution. Glassware acid washed for the first time was acid washed overnight for at least 12 hours and subsequent washes were done for at least 10 minutes. Glassware was then rinsed in DI water at least five times.

Total and fecal coliform counts were determined using the IDEXX Colisure® method, which is approved by the EPA for coliform quantification in drinking water (Olstadt et al. 2007). All required supplies coming in contact with the digestant and not already sterile were autoclaved or sprayed down with 70% isopropyl/ethyl alcohol, and the entire procedure was performed in a Jouan MSC 12 Class II Type A Biosafety Cabinet.

Depending on the anticipated concentration of coliforms in the digestate, it was diluted as appropriate in a final volume of 100 mL. Triplicate bottles were prepared and one pack of Colisure® was added to each bottle. The bottles were swirled until no large pieces of solid remained. The contents of each bottle were then poured into a Quanti-Tray/2000, and each tray was sealed in a Quanti-Tray sealer. The trays were incubated for 24 hours in a 35°C incubator.

After 24 hours, the trays were read for total coliforms, with red or magenta wells counted as positive and yellow ones negative. If there were orange or pink wells, the trays were incubated for another 24 hours, up to 48 hours. At this point, any wells still orange or pink were counted as negative, and red or magenta were counted as positive. To count fecal coliforms, the trays were subject to a portable UV lamp in the dark, and fluoresced cells were counted as positive. The cell counts were converted to MPN (CFU) by using the IDEXX Quanti-Tray/2000 MPN table.

The initial coliform concentrations in each digester were determined by a weighted average of the CFU measurements of the individual digester components.

2.5 MATERIALS

The TWAS and inoculum were collected from the San Elijo Water Reclamation Facility on the morning of use. TWAS was scooped up and poured into a wide-mouthed plastic container. Inoculum was collected from the inside of an operational anaerobic digester using a spigot and a glass bottle. The bottle was filled to the top with inoculum to minimize oxygen interference. Inoculum was stored at room temperature or in an incubator at the test temperature while preparing the experiment. TWAS was stored in the cold room at 4°C.

The *S. quadricauda* culture was purchased from Carolina Biological (152069 Burlington, NC). The alga was cultured in 10 liter glass flasks/bottles using primary effluent from San Elijo Water Reclamation facility as the source of nutrients and water. The culture was mixed by a magnetic stirrer on a stir plate (Fisher Scientific 11-600-100SH, Bubuque, Iowa). A Lithonia fluorescent lighting fixture (Lithonia Lighting 1233, Conyers, GA) and two F40T 12/CW 40 watt bulbs (Philips 410894, Amsterdam, Netherlands) were purchased from Home Depot. The carbon dioxide supplied to the algae was 99.5% industrial grade (Praxair Inc., Danbury, CT) and was diluted with air from San Diego State University's air compression system. Primary effluent collected from the San Elijo Wastewater Treatment plant provided the water and nutrients for algae growth.

Lab-scale anaerobic digesters were set-up using 250 mL glass media bottles (Fisher FB-800-250 Pittsburgh, PA), GL45 3-ported caps with 1/4-28 PTFE insert and polyethylene collar (Vaplock BC-322N, Lake Elsinore, CA) purchased from Western Analytical, EPDM (ethylene propylene diene monomer) gaskets (Western Analytical GS-017, Lake Elsinore,

CA), polycarbonate luer one-way stopcocks (Cole-Parmer EW-30600-06, Vernon Hills, IL), 1/16"ID x 1/8"OD PTFE tubing (Cole-Parmer EW-06605-27, Vernon Hills, IL), and 1/8" ID Tygon® R-3603 tubing (Saint Gobain Performance Plastics 14-169-1E, Garden Grove, CA). Eppendorf and Thermo Scientific pipettes (Eppendorf Research 100-1000 μ L, Eppendorf Research Plus 1-10 mL, and Thermo Scientific Finnpipette 1-10 mL) and pipette tips (Eppendorf epTIPS pipette tips, 50 to 1000 μ L and 10 mL, Hauppauge, NY) were used to measure and transfer media and, in some cases, plastic syringes with a luer-lok (Becton Dickinson 309653, Franklin Lakes, NJ) were used to transfer TWAS and inoculum. Perfektum Micro-Mate glass syringes (Popper & Sons 5037, New Hyde Park, NY) purchased from Fisher Scientific with polycarbonate luer large-bore three-way stopcocks (Cole-Parmer EW-30600-23, Vernon Hills, IL) were used for biogas sampling. Digesters were incubated in one of two incubators: New Brunswick Innova 42R Incubator Shaker (New Brunswick Scientific I42R, Edison, NJ) or *VWR 1575R* Incubator Shaker (*Sheldon Manufacturing, Inc.* 1575R, Cornelius, OR).

The amount of methane (CH_4) and carbon dioxide (CO_2) in the biogas was determined using a gas chromatography (GC) machine (Agilent 6890, Santa Clara, CA) equipped with thermal conductivity detector (TCD) and sample injection valve. The column utilized was an All tech Chromosorb 106 80/100 (6'x1/8" x .085" SS, Part # C-5000, Bath Serial # 156-705100057, MAOT 275°C, Deerfield, Illinois). The nitrogen gas used as make-up flow was 5.0 grade compressed nitrogen gas (Praxair, Danbury, CT). A computer with Agilent ChemStation software Rev. B.01.03[204] was connected to the GC and used to view and export results.

Six liter summa canisters (Restek TO-Can® 24174, Bellefonte, PA) were used to create calibration standards. GC calibration gases used were 99.8 % CO_2 , bone dry (Air Liquid America 23402, Los Angeles, CA) purchased from Supelco, 99.0 % C.P. grade CH_4 (Air Liquid America T109-14, Los Angeles, CA) purchased from Supelco, and a 30.04% CO_2 69.96% (balance) CH_4 mix (Praxair ME-CD30P-A3, Danbury, CT). Refrigerated liquid nitrogen gas (Praxair, Danbury, CT) was used for cleaning and purging the summa canisters. An Edwards high vacuum pump (Edwards E2M8, Crawley, Sussex, England) and a Fisher Scientific Traceable Manometer (Fisher Scientific 06-664-21, Bubuque, Iowa) were used to fill and vacuum the summa canisters.

COD, total nitrogen, and total phosphorous samples were digested in a Hach DRB 200 reactor (Hach DRB200-03, Loveland, CO). High range (20-1500 mg/L) COD vials were used to determine initial and final COD (Hach 2125915, Loveland, CO). Total phosphorus was determined using the Hach TNT Reagent Set, High Range Molybdoavanadate (Hach 2767245, Loveland, CO). Total nitrogen was determined using the Hach TNT Reagent Set, High Range (Hach 2714100, Loveland, CO). Optical density readings were measured with a Thermo Spectronic Genesys 20 spectrophotometer (Thermo Electron Corp. 4001/4, Waltham, MA).

Distilled water was purified with Milli-Q Advantage 10 (Millipore, Molsheim, France) water purification system and was used for dilutions, coliform testing, preparing lab-scale digesters, and final rinsing of glassware.

Alkalinity was measured with hydrochloric acid, 0.02N (Hach 2330353) and 0.1N (Hach 1481253, Loveland, CO).

Coliform testing was done inside a Jouan MSC 12 Class II Type A Biosafety Cabinet (52020000, Jouan Laboratory Equipment, Winchester, VA). The safety cabinet was sterilized with 70% isopropyl alcohol or 70% ethanol in addition to the UV light built into the cabinet. Glassware, pipette tips, and DI water were sterilized in one of two autoclaves (Consolidated SSR-3A-PB, Boston, MA) (Consolidated ADV-PB, Boston, MA). Total and fecal coliforms were measured using Colisure® reagent with Quanti-tray 2000s (IDEXX 99-27076, Westbrook, ME). Quantri-tray 2000s were sealed with the IDEXX Quanti-tray sealer (WQTS2X-115). A portable UV lamp (IDEXX WL200, Westbrook, ME) was used to determine fecal coliforms.

Total and volatile solids were measured using CoorsTek porcelain evaporating dishes (70 mL) (CoorsTek 60197, Golden, CO), a drying oven (Yamato DX-400, Santa Clara, CA), a high-temperature box furnace (Lindberg/Blue M BF 51766A-1, Asheville, NC), a dessicator, and a precision scale (Mettler-Toledo AE200, Columbus, OH) calibrated with a 100 gram calibration weight (Mettler-Toledo ME-216504, Columbus, OH).

CHAPTER 3

RESULTS

In this chapter, the experimental results of the anaerobic co-digestion of wastewater sludge with *S. quadricauda* and influencing parameters are presented.

Preliminary tests show the best substrate to inoculum ratio to be 1:1. It was also discovered that a low VS loading (400 mg of initial VS) produced a volume of biogas that was reasonably easy to measure and so was set as the target VS to start the digestion with. For graphs of preliminary test results, see Appendix B.

3.1 BIOGAS AND METHANE PRODUCTION

Tables 3.1 and 3.2 show the beginning and ending parameters for each of the three tests, with a specific focus on VS and COD. Figures 3.1 thru 3.3 display the volume of biogas produced over the course of the digestion period for each of the three tests, with non-normalized (actual reading) and normalized (volumes from controls subtracted) results. The change in VS and COD between the start and end of digestion is shown in Figure 3.4.

Table 3.3 presents the total volume of biogas produced along with the biogas normalized against various parameters such as initial and digested VS, and Figure 3.5 displays the same information graphically.

Finally, Table 3.4 displays the volume of methane produced based on a gas chromatographer biogas analysis average of 72% methane in the biogas. In addition, Table 3.4 and Figure 3.6 both show the % Yield of methane, which is calculated by comparing the actual methane production to the maximum theoretical methane production based on either initial or digested COD. This is done by multiplying the theoretical yield of 0.395 mL CH₄/mg COD times the amount of COD. For 20°C and 10°C, the factors of 0.376 and 0.363 mL CH₄/mg COD are used, respectively.

Table 3.1. Starting Parameters for All Experimental Sets

Sample ID	Test ID	Digester Contents	Varied Condition	Initial TS (mg)	Initial VS (mg)	Initial COD (mg)
T1-0	1	100% TWAS	--	391	292	400
T1-25	1	25% Algae, 75% TWAS	--	432	293	420
T1-49	1	49% Algae, 51% TWAS	--	474	295	440
T1-76	1	76% Algae, 24% TWAS	--	509	291	454
T1-100	1	100% Algae	--	550	293	474
T2-10	2	50% TWAS: 50% Algae	20C	396	294	483
T2-20	2	50% TWAS: 50% Algae	10C	396	294	483
T3-N	3	50% TWAS: 50% Algae	No Alk Addition	614	441	728
T3-I	3	50% TWAS: 50% Algae	Increased Alk	614	441	728
T3-H	3	50% TWAS: 50% Algae	Highest Alk	614	441	728

Table 3.2. Ending Parameters for All Experimental Sets

Sample ID	Test Duration (Days)	Remaining VS (mg)	Remaining COD (mg)	VS digested (mg)	COD digested (mg)	% VS digested	% COD digested
T1-0	67	160	218	132	183	45%	46%
T1-25	67	140	203	153	217	52%	52%
T1-49	67	140	206	155	234	53%	53%
T1-76	67	175	232	116	222	40%	49%
T1-100	67	170	253	123	221	42%	47%
T2-10	30	317	420	-23	63	-8%	13%
T2-20	30	307	372	-13	111	-4%	23%
T3-N	30	297	470	144	258	33%	35%
T3-I	30	347	486	94	242	21%	33%
T3-H	30	317	499	124	229	28%	31%

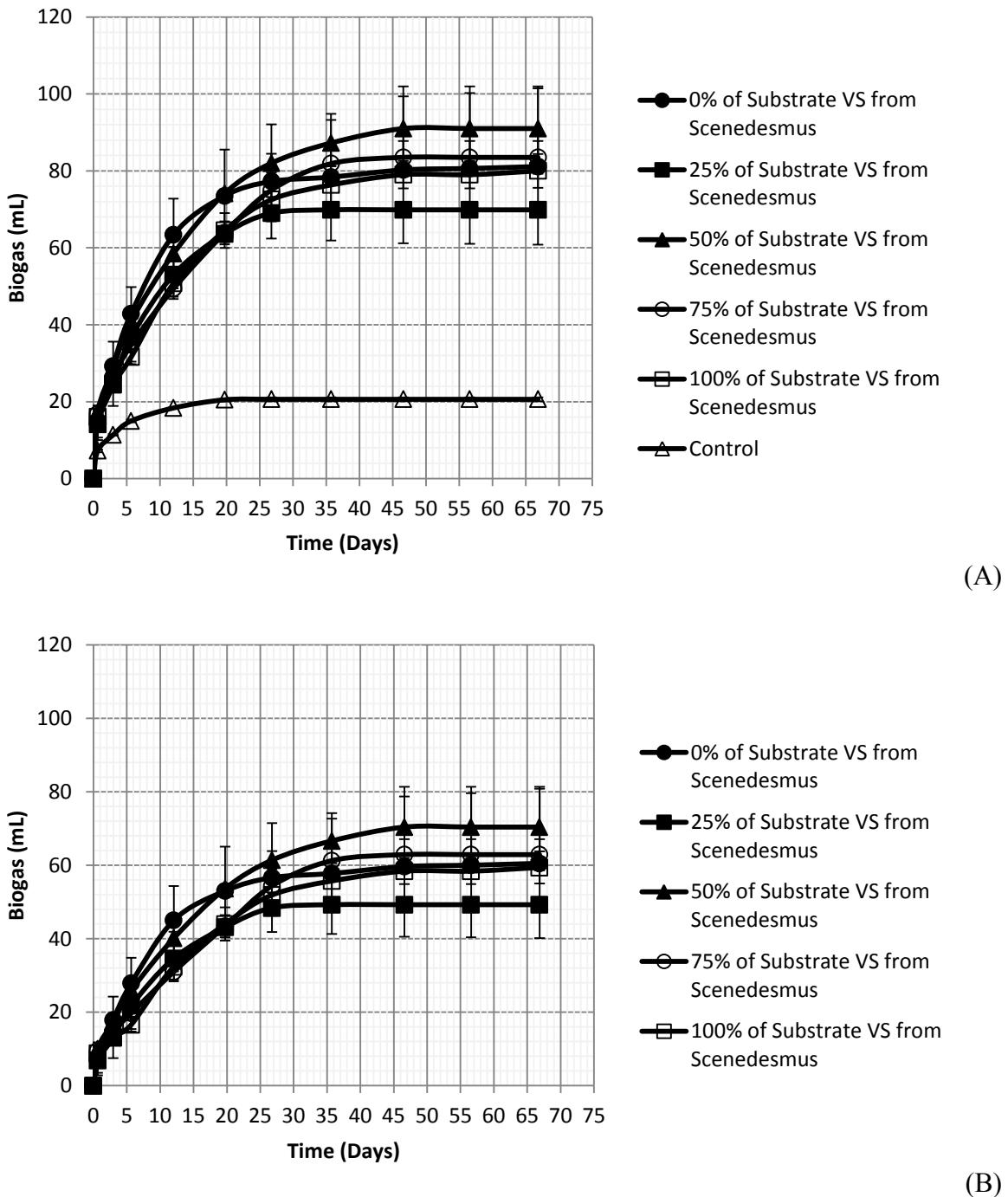
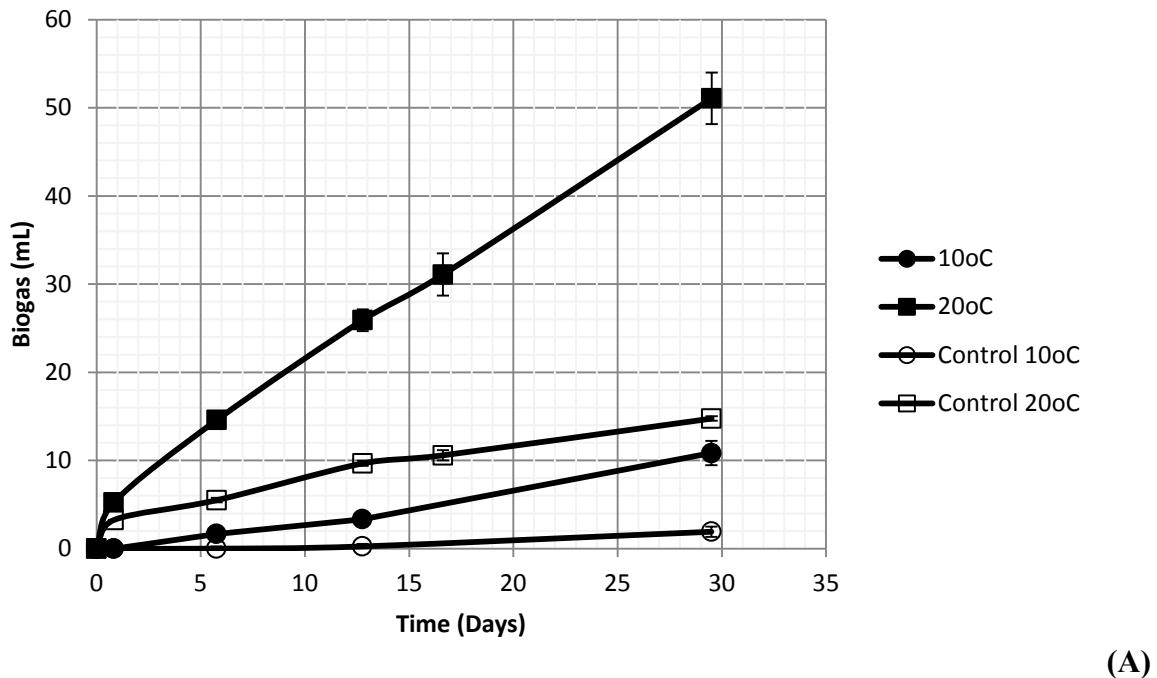
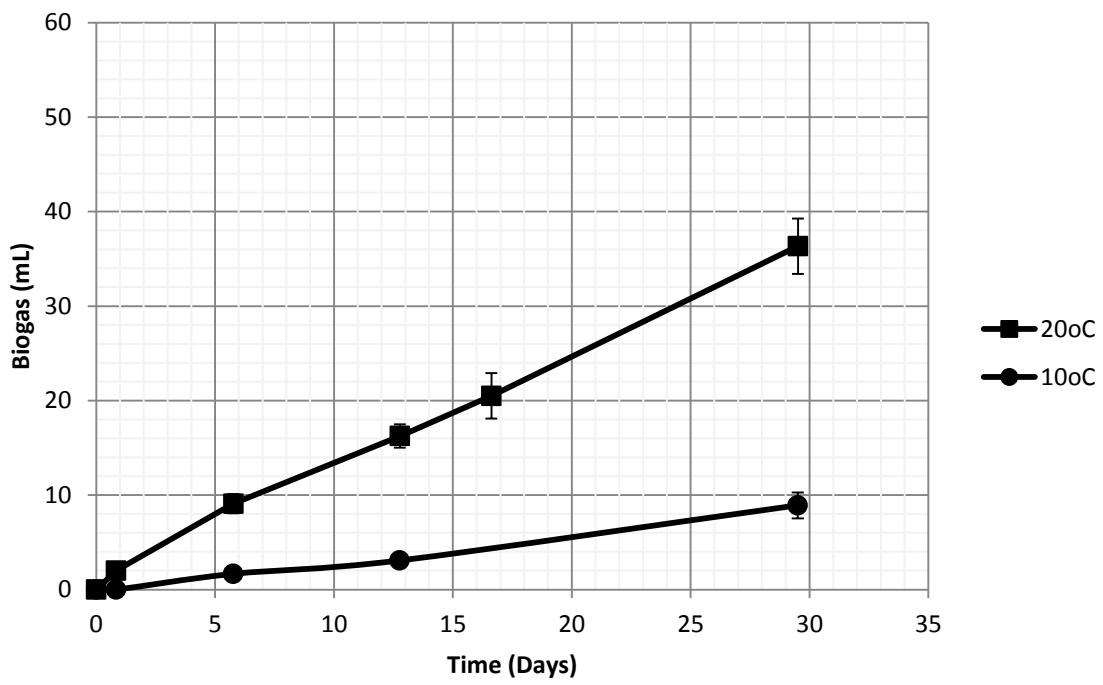


Figure 3.1. (a) Test 1 cumulative biogas production for varying proportions of TWAS and *Scenedesmus* (b) Test 1 normalized cumulative biogas production for varying proportions of TWAS and *Scenedesmus*.



(A)



(B)

Figure 3.2. (a) Test 2 cumulative biogas production for varying temperatures (b) Test 2 normalized cumulative biogas production for varying temperatures.

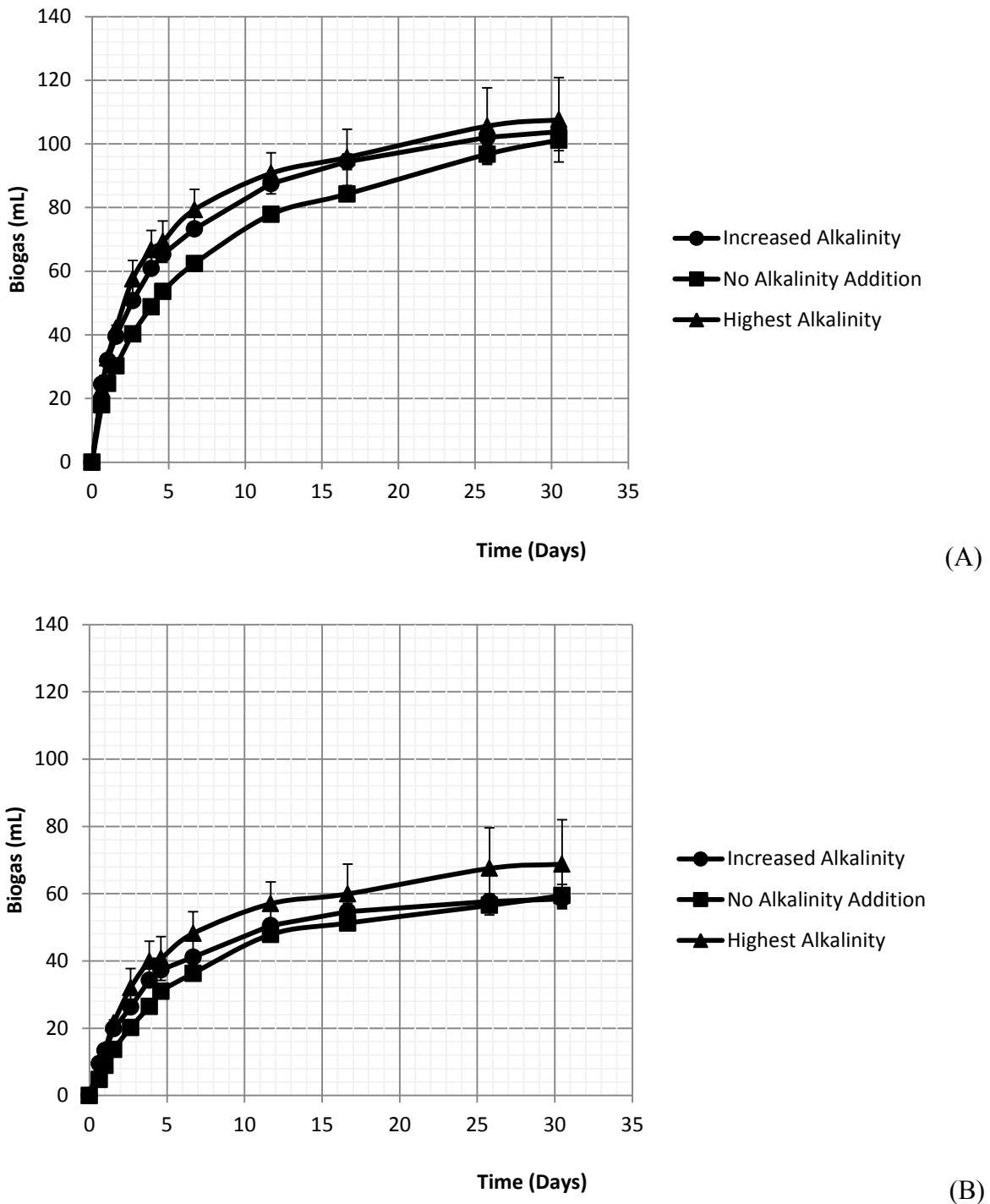


Figure 3.3. (a) Test 3 cumulative biogas production for varying alkalinites (b) Test 3 normalized cumulative biogas production for varying alkalinites.

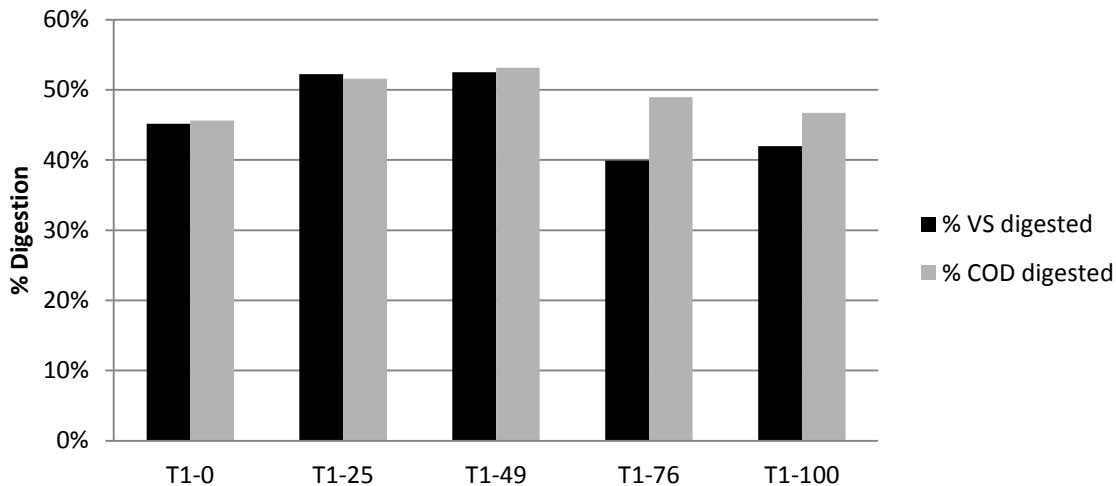


Figure 3.4. Percentage of initial VS and initial COD digested at end of test.

Table 3.3. Biogas Production Expressed per Mass of Initial and Digested VS and COD

Sample ID	Biogas produced (mL)	ml Biogas / mg VS digested	ml Biogas / mg COD digested	ml Biogas / mg initial TS	ml Biogas / mg initial VS	ml Biogas / mg initial COD
T1-0	81.13	0.62	0.44	0.21	0.28	0.20
T1-25	69.88	0.46	0.32	0.16	0.24	0.17
T1-49	91.00	0.59	0.39	0.19	0.31	0.21
T1-76	83.50	0.72	0.38	0.16	0.29	0.18
T1-100	80.00	0.65	0.36	0.15	0.27	0.17

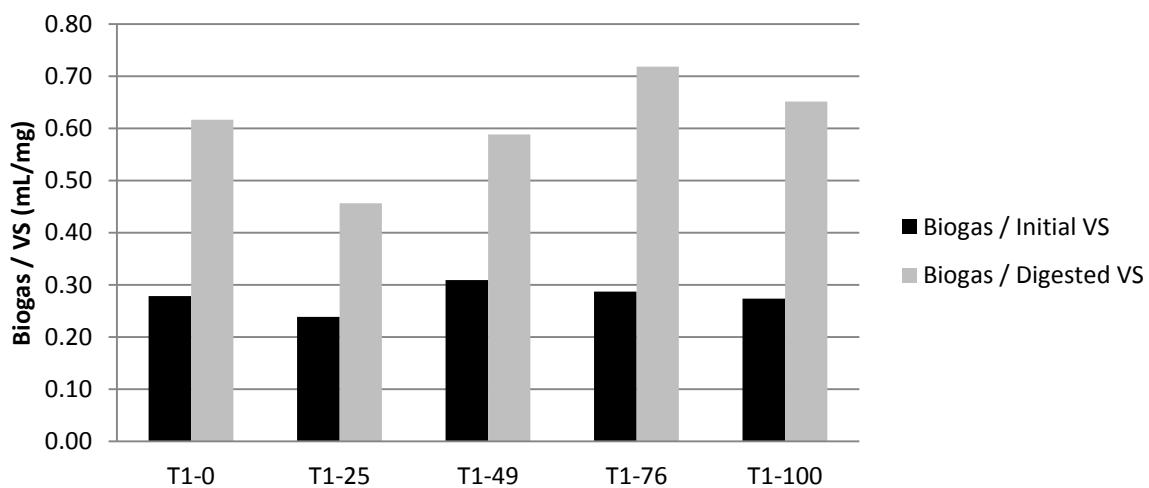


Figure 3.5. Volume of biogas produced per unit mass of initial and digested VS.

Table 3.4. Methane Yields

Sample ID	Methane produced (mL) ¹	Max CH ₄ Production based on Initial COD (mL) ²	Yield based on initial COD	Max CH ₄ Production based on Digested COD (mL) ²	Yield based on digested COD
T1-0	58.4	158.1	37%	72.1	81%
T1-25	50.3	165.9	30%	85.6	59%
T1-49	65.5	173.9	38%	92.4	71%
T1-76	60.1	179.3	34%	87.8	68%
T1-100	57.6	187.2	31%	87.4	66%

¹ Calculated based on 72% of biogas as methane

² Calculated based on 35°C = 0.395 L CH₄/g COD, at 20°C = 0.376 L CH₄/g COD, and at 10°C = 0.363 L CH₄/g COD

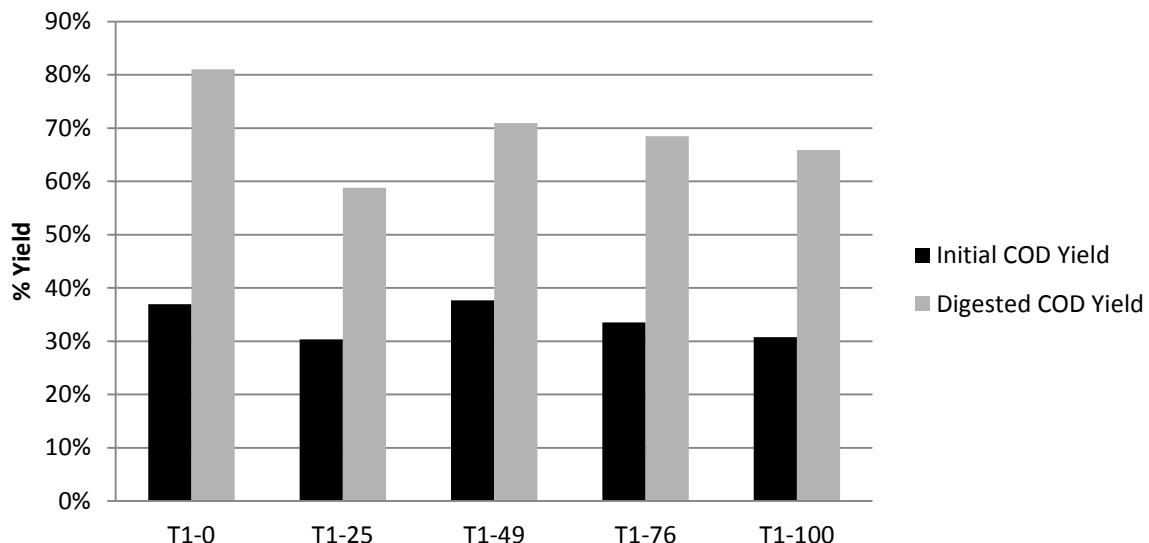


Figure 3.6. Percent yield of methane compared to theoretical maximum methane production based on initial COD and digested COD.

3.2 NUTRIENT AND COLIFORM LEVELS

Results for nutrient testing are shown in Table 3.5 and Figure 3.7. These tests were conducted on the post-digestion digestate and measured the amount of total nitrogen and total phosphorous remaining in the digestate.

The results of coliform testing are presented in Table 3.6 and Figures 3.8 thru 3.9. Table 3.6 displays coliform levels on a liquid digestate mass. Figure 3.8 compares the difference in initial and final total and fecal coliform levels, and Figure 3.9 shows the difference on a log scale.

Table 3.5. Digestate Nutrients: Nitrogen and Phosphorous

Sample ID	Total Nitrogen (% of digestate, g N-NO ₃ /g digestate)*	Total Phosphorous (% of digestate, g P-PO ₄ /g digestate)*	Total Phosphorous (% of digestate, g P ₂ O ₅ /g digestate)*
T1-0	18.98%	6.51%	14.94%
T1-25	12.60%	4.54%	10.42%
T1-49	11.55%	4.19%	9.60%
T1-76	11.34%	3.69%	8.45%
T1-100	11.26%	3.17%	7.26%
T2-10	10.57%	3.78%	8.66%
T2-20	12.70%	3.79%	8.70%
T3-N	11.45%	4.37%	10.02%
T3-I	6.70%	2.71%	6.20%
T3-H	4.82%	2.00%	4.60%

*Nutrients of digestate at the end of the digestion period

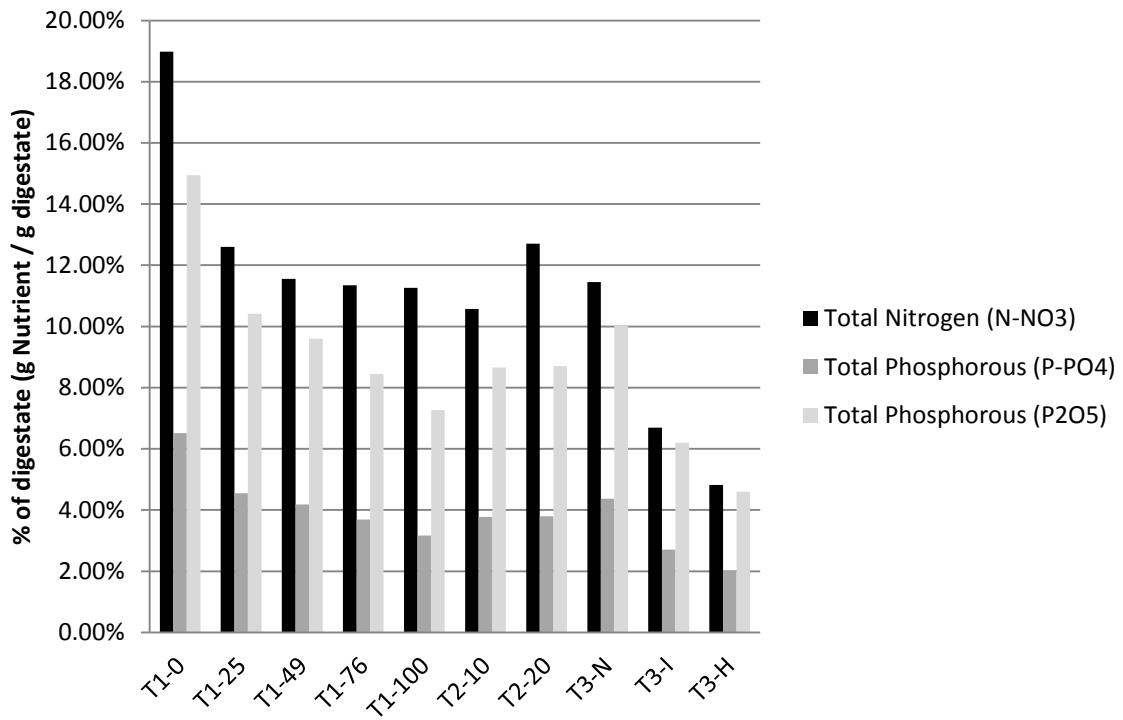
**Figure 3.7. Percentage of nutrients (N and P) in digestate.**

Table 3.6. Reduction of Total and Fecal Coliforms per Gram of Total Solids

Sample ID	Initial TC (CFU / g TS)	Final TC (CFU / g TS)	TC Reduced (CFU / g TS)	Initial FC (CFU / g TS)	Final FC (CFU / g TSf)	FC Reduced (CFU / g TS)	% FC reduced			FC Log Reduction		
							% TC reduced	% FC reduced	TC Log Reduction	FC Log Reduction		
T1-0	6123044.6	1106555.6	5016489.1	1882033.2	232694.4	1649338.7	81.93	87.64	0.7	0.9		
T1-25	4698328.9	25682.5	4672646.4	1343180.0	5634.9	1337545.1	99.45	99.58	2.3	2.4		
T1-49	3521258.1	4904.9	3516353.2	898160.2	1963.7	896196.4	99.86	99.78	2.9	2.7		
T1-76	2463283.9	1106.2	2462177.7	487842.1	141.5	487700.6	99.96	99.97	3.3	3.5		
T1-100	1619398.9	398.0	1619000.8	169570.6	30.4	169540.2	99.98	99.98	3.6	3.7		
T2-10	4954916.3	2649835.7	2305080.6	1236439.1	152554.3	1083884.8	46.52	87.66	0.3	0.9		
T2-20	4954916.3	238804.7	4716111.6	1236439.1	28853.1	1207586.0	95.18	97.67	1.3	1.6		
T3-N	4299370.3	192022.3	4107348.1	1185549.4	44643.4	1140906.0	95.53	96.23	1.4	1.4		
T3-I	4299370.3	123588.2	4175782.2	1185549.4	4025.7	1181523.6	97.13	99.66	1.5	2.5		
T3-H	4299370.3	855.7	4298514.7	1185549.4	1.9	1185547.4	99.98	100.00	3.7	5.8		

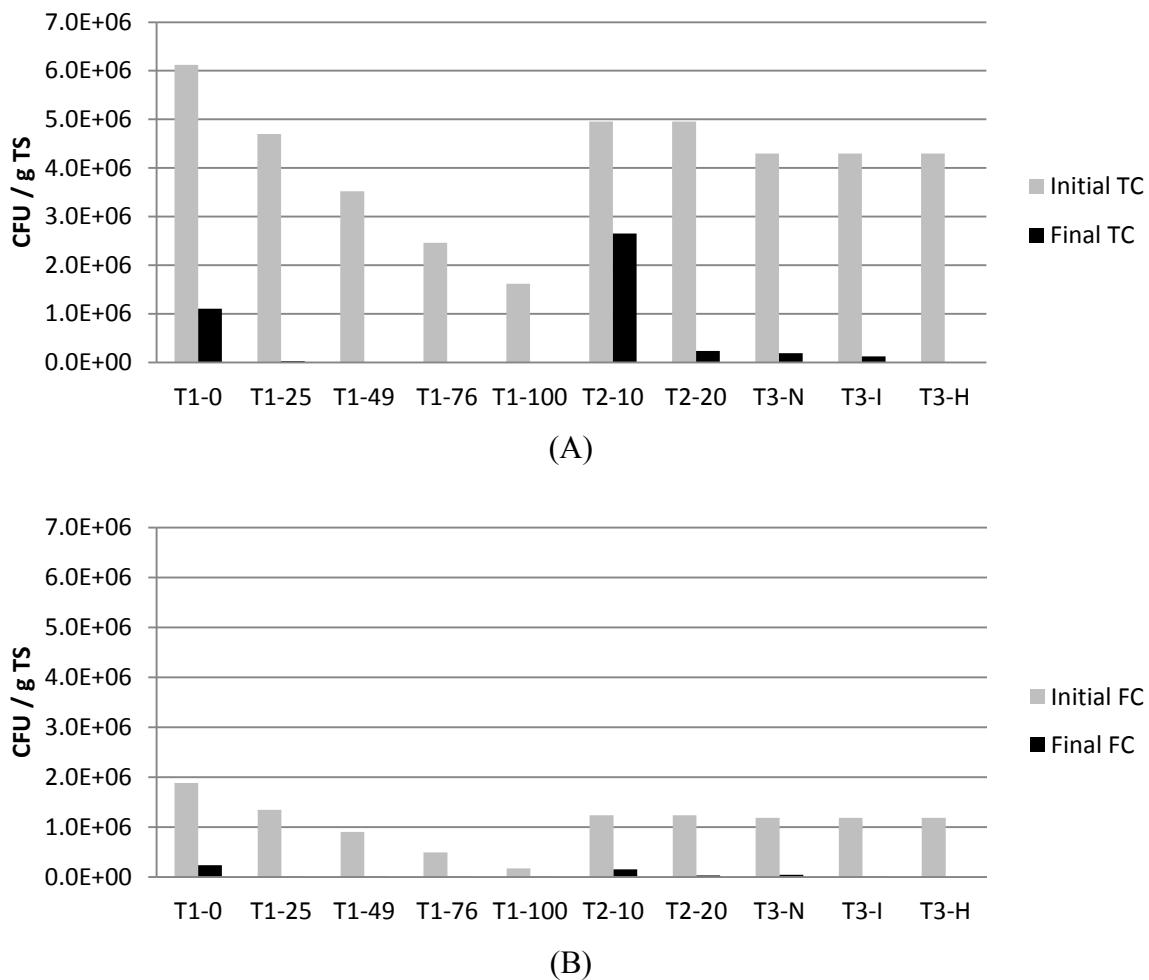


Figure 3.8. (a) Initial and final total coliform levels per unit mass of total solids (b) Initial and final fecal coliform levels per unit mass of total solids.

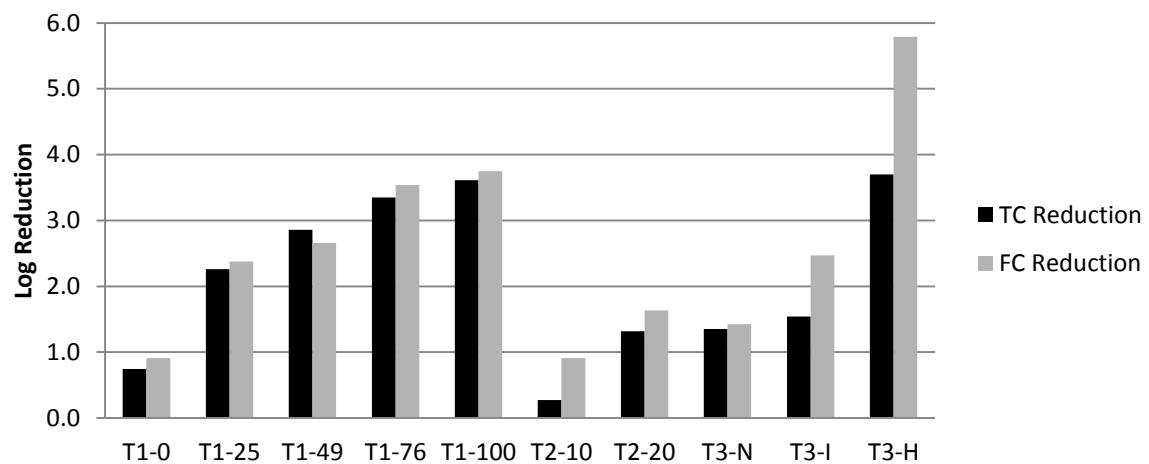


Figure 3.9. Log reduction in total and fecal coliforms per unit mass of total solids.

CHAPTER 4

DISCUSSION

A detailed analysis and interpretation of the results is presented in this chapter.

4.1 BIOGAS PRODUCTION OF VARYING PROPORTIONS OF SLUDGE AND ALGAE

The first objective to be completed for this research was to investigate the change in production of methane gas during anaerobic co-digestion varying proportions of wastewater sludge and *Scenedesmus sp*. Figure 3.1 shows the difference in the volumes of biogas produced by the varying proportions of sludge and *Scenedesmus*, with T1-49 producing the greatest volume. The least amount of biogas was produced by T1-25, which produced 23% less biogas than T1-49. The other proportions produced biogas volumes in between those two and had no significant difference between each other. Moreover, there was no significant difference between T1-49 and T1-100, T1-76, and T1-0. Thus, there is no clear synergistic effect of biogas production increasing as the proportion of algae is increased, but the significant difference between T1-49 and T1-25 demonstrates that the addition of algae to anaerobic digestion can enhance the production of biogas.

Another result to note is the rate of biogas production and the amount of time it takes to reach maximum biogas production. During the first 5 days of digestion, T1-25 and T1-49 have similar rates of biogas production. After that, the T1-25 production rate slows down and starts to plateau at about the 30th day. It is the first proportion to reach its maximum biogas volume at about 36 days. On the other hand, T1-49 does not plateau until about 47 days, where it reaches its maximum volume of biogas produced. Thus, it appears that increasing the proportion of algae in the digestant from 25% to 49% not only generates a greater volume of biogas, but it also increases the rate of biogas production.

In addition to looking at the total amount of biogas generated in each of the different test cases, normalizing it to the amount of VS and COD is important because each test case varies in the amount of VS and COD added and an accurate comparison can only be made by taking this into account. Moreover, anaerobic digestion is incapable of digesting all of the VS/COD present, and the various amounts of digestion can be seen in Figure 3.4. The

percent of VS digested ranged from 53 to 40% and for COD, 53 to 46%. T1-49 had the highest percent digestion, with the extreme proportions showing a lower percent digestion. This is may be due to the C:N ratios of each test, as T1-0 has too much C from the sludge and T1-100 has too much N from the algae. The literature has shown that a 20-25 C:N is optimal for anaerobic digestion (Yen and Brune 2007). Also, algae can be resistant to bacterial degradation (Foree and McCarty 1970), which may be reflected in the lower percent VS digested in the 75% and 100% proportions. The fact that algae may be more difficult to digest is tempered by using it as a material to optimize a digester's C:N ratio.

Normalizing the biogas produced by the VS is shown in Figure 3.5 and Table 3.3. The results show a range of 0.46 to 0.72 mL biogas per mg of VS digested (0.33 to 0.52 mL methane, assuming 72% methane composition of biogas determined by GC analysis), which is similar to the 0.45 mL methane per mg of algal (combination of *Scenedesmus* and *Chlorella*) VS digested and 0.47 mL methane per mg of TWAS VS digested determined by Golueke, Oswald, and Gotaas (1957).

Normalizing by the amount of VS added, a range of 0.24 to 0.31 mL biogas per mg of initial VS is obtained (0.17 to 0.22 mL methane). This also falls in line with the literature, where Golueke, Oswald, and Gotaas (1957) saw 0.17 mL methane per initial VS for the algal mixture and 0.28 mL methane per initial VS for the TWAS. Others like Yen and Brune (2007) have seen higher results such as 0.32 mL methane per initial VS with a *Chlorella/Scenedesmus* and paper mixture and lower results such as Salerno, Nurdogan, and Lundquist's (2009) 0.12 mL methane per initial VS.

Stoichiometrically, the COD of methane is 64 g. That is, the conversion of 1 mg of COD yields 0.395 mL methane at 1 atm and 35°C. As seen in Table 3.3, the results normalized by COD produced a range of 0.32 to 0.44 mL biogas per COD digested, which using the 72% biogas methane composition mentioned earlier, converts to 0.23 to 0.32 mL methane per mg COD digested. Thus, actual the methane conversion efficiency ranges from close to the ideal calculated to almost half of it. In addition, the COD conversion can be used to determine the methane yield for the amount of COD that was digested during the tests. For this research, Table 3.4 and Figure 3.6 show that it ranged from 59 to 81%. Ras et al. (2011) saw a 51% reduction of COD and a 49% methane yield. Others have seen a microalgae yield

topping out at 60% and that for sludge up to 80% (P. H. Chen 1987), which correlates well with this research.

4.2 INFLUENCE OF OPERATIONAL PARAMETERS

The second objective of this research was to investigate the effects of other various operational parameters on biogas production. The first parameter tested was temperature, in Test 2. Temperature plays a major role in anaerobic digestion, as it has a direct effect on the activity of the bacteria performing the anaerobic digestion. Two temperatures, 10°C and 20°C, were tested, and the results can be seen in Figure 3.2. Lowering the temperature has a clear detrimental effect on the volume of biogas produced – anaerobic digestion at 10°C produced less than a third of the biogas produced by anaerobic digestion at 20°C. At the end of the digestion period at about day 30, the 20°C digesters had produced about 36 mL of biogas and the 10°C ones about 9 mL. Going back to Figure 3.1(b), the T1-49 proportion took only about 10 days to produce 36 mL of biogas and produced about 64 mL at day 30.

So the 43% drop in temperature from 35°C to 20°C resulted in the same percent reduction in biogas generated after 30 days. The 50% reduction in temperature from 20°C to 10°C resulted in a 75% reduction in biogas generation. Thus, it appears that anaerobic digestion is much more sensitive to temperatures that are below room temperature range, and operation of it at those temperatures makes it an inefficient means of energy generation. This supported by the fact that degradation of long chain fatty acids is often rate limiting in the 10°C to 20°C range, and their accumulation can lead to reactor foaming (Tchobanoglous, Burton, and Stensel 2003). However, a reasonable amount of biogas is still generated at 20°C and above, so it may still be economically viable to perform anaerobic digestion at that temperature range.

The other part of the second research objective was to test the effect of alkalinity on biogas production. Alkalinity is another parameter that also plays an important role in anaerobic digestion. As anaerobic digestion proceeds, alkalinity is primarily consumed by the generated CO₂ (Tchobanoglous, Burton, and Stensel 2003) and low alkalinity levels will result in the pH dropping and souring the digester. In Test 3, the alkalinity was increased to two higher levels of alkalinity.

During the first 12 days the increased alkalinity digesters did show a significant increase in biogas production, as seen in Figure 3.3, but then by the end of the test at 30 days, the difference was insignificant. This was surprising because the alkalinity of the digesters without any alkalinity addition was measured at 70 mg/L CaCO₃, whereas the typical anaerobic digester requires alkalinity in the order of 2,000 to 4,000 mg/L as CaCO₃ to maintain proper pH (Tchobanoglous, Burton, and Stensel 2003). However, the alkalinity of all the digesters did increase at the end of the test, indicating that the process was adding alkalinity to the system, likely the digestion of the algae. This can occur through the ammonification of gaseous ammonia produced from the breakdown of proteins in the algae to ammonia ions and bicarbonate (Tchobanoglous, Burton, and Stensel 2003), with *Scenedesmus* already known to have a protein large percentage composition of 50-56% (Becker 2004). S. Park and Li (2012) found that the additional alkalinity provided by algal biomass residue helped offset the lack of lipid alkalinity and retain its degradation efficiency. If this was the case, this would explain how the no-alkalinity-addition digesters were not limited in biogas production despite having such low initial alkalinity. Not having to add an additional source of alkalinity would be a significant efficiency advantage for anaerobic digester operators.

4.3 RESIDUAL QUALITY

High levels of total nitrogen and total phosphorous were detected in the digestate. Scotts Miracle-Gro Company, the world's largest marketer of branded consumer lawn and garden products, sells lawn fertilizer products that range from 11% to 32% total nitrogen and 4% to 25% available phosphate (P₂O₅). From Table 3.5 and Figure 3.7, the range in the digestate varied from 4.82% to 18.98% total nitrogen and 4.60% to 14.94% total phosphorous, which compares well with the commercial fertilizer product. The nutrient levels trended in a fashion such that an increasing TWAS proportion increased the amount of nutrients present and the opposite for an increasing proportion of algae. This indicates that although algae addition decreases the nutrient content of an anaerobically digested sludge, it still provides level of nutrients would make it a good fertilizer.

The ability of anaerobic co-digestion of algae and TWAS to reduce bacterial levels was also evident. Besides for T1-0 and T2-10, every test saw at least a one log reduction in

TCs and FCs, with the maximum being a 5.6 log reduction in FC shown in Figure 3.9. This means that every test would meet USEPA 40 CFR Part 503 regulations to be classified as a Class B biosolid, and in some cases, even Class A. These classifications allow the sludge to be used in certain land applications or to be disposed of in a landfill, which helps to improve the value of algal and TWAS co-digestion. Moreover, increasing the proportion of algae increased the log reduction of both types of coliforms.

CHAPTER 5

SUMMARY, SIGNIFICANCE AND RECOMMENDATIONS

This chapter will summarize the major findings of the studies and provide recommendations for future research.

5.1 SUMMARY

This study shows that the microalgae, *S. quadricuada*, can produce at least the same amount of methane as wastewater sludge. The combination of 49% algae with 51% TWAS produced the highest volume of biogas and seemed to be the most optimal combination of algae and sludge. The percentage of VS and COD digested ranged from 40 to 53% and 46 to 53%, respectively, and 0.33 to 0.52 mL methane was produced per VS digested (0.17 to 0.22 mL methane per initial VS), which was about average compared to other studies.

Temperature had a significant effect on biogas production, as the lower temperatures greatly reduced biogas yields, but alkalinity showed no significant influence on the amount of biogas produced. Total nitrogen and phosphorous levels in the digestate ranged from 4.82% to 18.98% and 4.6% to 14.94% respectively, comparable to commercial fertilizer. Moreover, the TC and FC levels were reduced significantly enough after digestion for the digestate to qualify as a Class B biosolid per USEPA standards, rendering it safe for land application.

5.2 SIGNIFICANCE

The results and analysis of this research show that the co-digestion of microalgae and sludge is a practical technology with multiple areas of benefit. Although the volume of methane produced by the co-digestion of microalgae and sludge was only similar to that of standard WWTP sludge AD, it was an important finding to show that WWTPs are not losing energy productivity when sludge is displaced by algae in AD. Since microalgae have simple requirements, they can be grown on site at WWTPs using existing inputs and outputs of the current treatment process such as wastewater and CO₂.

This has multiple ramifications for WWTPs. By growing algae on site and using it with sludge as a feedstock for AD, they can (a) increase energy production with the increased biomass, (b) decrease greenhouse gas emissions and increase renewable energy generation, (c) improve wastewater treatment without adding any chemical or physical processes, and (d) do this without increasing production of a hazardous or unusable waste that must be properly disposed of.

These benefits align well with the current trend of increasing environmental regulations and public awareness of reducing human's environmental footprint, especially concerning greenhouse gases. A WWTP setup like in Figure 5.1 is a simple example of how this technology could be utilized to maximize these benefits and comply with future regulations, while improving public perception of a "dirty" industry.

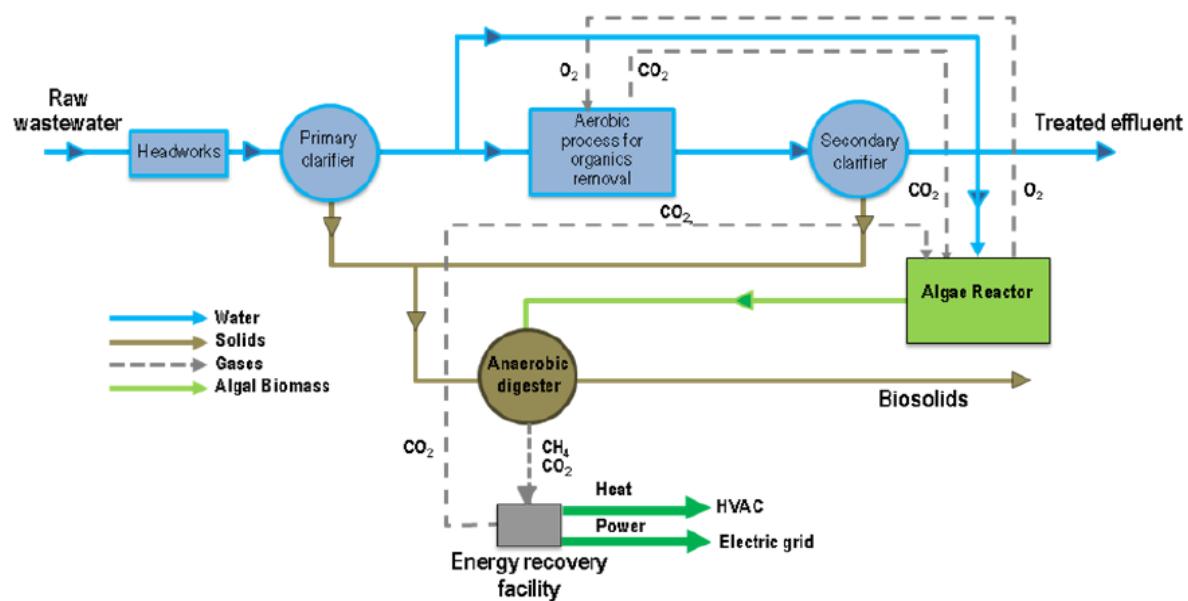


Figure 5.1. Wastewater treatment process with integrated algae reactor and AD.

This setup is self-contained - the algae reactor uses the plant's own wastewater and CO_2 streams to grow the algae, which is then fed to the AD to generate CH_4 . Compared to conventional wastewater treatment, this leads to (a) additional biomass for the AD to process and hence generate more energy, (b) elimination of CO_2 being emitted to the atmosphere, (c) additional biological treatment of wastewater, and (d) biosolids that are low in pathogens and high in nutrients that can be used as fertilizer.

In other words, this technology means that WWTPs can become carbon-neutral, renewable energy sources without many of the pitfalls of popular renewable energy sources such as solar and wind energy. These more traditional energy sources require massive quantities of land in often remote locations, miles of power transmission lines, and can harm local wildlife, unlike WWTPs which already exist in close proximity to population centers.

In summary, the anaerobic co-digestion of *S. quadricauda* and sludge has the potential increase renewable energy generation that is not only carbon neutral, but also works within existing wastewater treatment infrastructure, reducing costs, financially, physically, and environmentally.

5.3 RECOMMENDATIONS

The results of this research demonstrated that an approximately 50/50 blend of algae and sludge produces the optimal amount of methane. In addition, temperatures of at least 35°C should be maintained during AD and no alkalinity addition is necessary. Furthermore, the digestion of algae needs to be optimized, as shown by the fact that less than 55% of VS and COD are digested at the end of the digestion periods. With nearly half of the algae/sludge mixture being undigested and algae already having been shown to be resistant to degradation and intact cells still present in the digestate (Golueke, Oswald, and Gotaas 1957; Tartakovsky et al. 2013), methane production could be significantly increased if energy-efficient techniques could be developed to boost algal digestion.

Finally, although an overview of this technology would suggest that it would be advantageous for WWTPs to integrate it into their operations, a complete life cycle analysis including the capitals costs of integrating it into existing WWTPs would be needed to confirm that it is an economically feasible technology. Although various life cycle analyses have been completed on different algae cultivation methods (Resurreccion et al. 2012) and anaerobic digestion of algae (Collet et al. 2011; Zamalloa et al. 2011), there has not been one completed to determine the cost of the entire system from cultivation to digestion. This would provide the clearest picture of the advantages of using this technology.

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APPENDIX A**STANDARD OPERATING PROCEDURE**

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0001:
Overall Experimental Setup of Sludge/Algae Co-digestion BMP test**

1. PURPOSE

This protocol describes the process of testing the bio-chemical methane potential (BMP) of microalgae co-digested with wastewater sludge.

2. MATERIALS

- 2.1. 250mL reactor bottles
- 2.2. Caps, (BC-322N Western Analytical) BOTTLE CAP GL45 3-ported 1/4-28 PTFE insert (replace with EPDM gasket), blue Polyethylene collar
- 2.3. Hard tubing, (EW-06605-27 Cole Parmer) PTFE tubing, 1/16"ID x 1/8"OD
- 2.4. Soft tubing, ¼" OD
- 2.5. Connectors/Adaptors:
 1. (AQ-115X) TUBING CONNECTION 1/8"" OD tubing x 1/4-28 Blue Polypropylene fitting natural Tefzel ferrule
 2. (PL-104X 10/P) PLUGS 1/4-28 natural PFA Tef
 3. Zip ties
- 2.6. Nitrogen gas
- 2.7. Plastic 30 ml syringes
- 2.8. Glass syringes, (Micro-mate) 20mL
- 2.9. Graduated cylinders, from 10-50mL
- 2.10. Can koozies

3. MACHINERY

- 3.1. Incubator(s) with shakers

4. PROCEDURES FOR OVERALL EXPERIMENT SET-UP

4.1. VS Inoculum to VS Sludge Ratio Testing

4.1.1. Determine the VS, TS, and COD of Inoculum and TWAS (See SOP #0005 and SOP #0006)

4.1.2. Determine volume of inoculum and TWAS to be added to each digester based on various ratios of VS of inoculum to VS of TWAS - 1:1, 1:1.5, and 1:0.5

Note: We used 43mL, 65ml, and 22mL of TWAS with 135mL of inoculum and the remainder DI water to 200mL

4.1.3. Follow Digester Setup Procedure (Section 5) and Monitor and Sample as described below (Section 6)

Note: The purpose of this ratio test is to ensure CH₄ production is not limited by substrate or inoculum availability. Therefore, multiple substrate to inoculum ratios should be tested. After correction for endogenous methane production, results are normalized to volume of gas per mass of assayed COD. Normalized results between ratios are compared to insure the assay was not substrate limited. The substrate to inoculum ratio should not affect the final volume of methane produced, but should control the rate at which methane is produced.

4.2. Co-Digestion of Algae and Sludge

4.2.1. Pour appropriate amount of algae into glass bottle and cap

4.2.2. Place on bench overnight to allow settling

4.2.3. Syphon off supernatant

4.2.4. Use the remaining dense algae for testing

4.2.5. Determine VS, TS, and COD (See SOP #0005 and SOP #0006) of each algae

(chorella and scenedesmus) and correlate to Optical Density (optional, See SOP #0008)

4.2.6. Determine mL of Algae, TWAS, and inoculum to add to each digester

4.2.6.1. Use the VS inoculum to VS substrate ratio determined in Section 4.1

- 4.2.6.2. Based on this VS/VS ratio, calculate the volume needed for each algae/TWAS ratio

Exp. Run	Target Algal Species	Fraction of by VS (%) of Substrate	
		Wastewater Sludge	Algal Biomass
1	<i>Chlorella</i>	0	100
2		25	75
3		50	50
4		75	25
5		100	0
12	<i>Control</i>	0	0

- 4.2.6.3. Follow Digester Setup Procedure (Section 5) and Monitor and Sample as described below (Section 6)

4.3. Alkalinity Test

- 4.3.1. Using optimum algae:sludge ratio found in section 4.2, follow Digester Setup Procedure (Section 5) for 3 sets of digesters (4 digesters each set): control (no alkalinity adjustment), and two sets with above average operating alkalinity (3000 mg/L and 5000 mg/L). Also set up a triplicate control for each set without substrate (just inoculum and DI).
- 4.3.2. Determine the alkalinity (See SOP #0004) of all the digester components (inoculum, TWAS, chlorella, scenedesmus)
- 4.3.3. Calculate the alkalinity of each digester based on the alkalinity and amounts of the components.
- 4.3.4. Make additional digesters (duplicate) from at least 2 of the tests to check the alkalinity against the calculated values.
- 4.3.5. If values are similar, calculate the amount of NaHCO₃ needed for each set.
- Note:** amount of NaHCO₃ to add (in grams) =

[Alkalinity Increased Desired (mg/L) / 50,000 (mg CaCO₃/eq)] * 61 (g HCO₃/eq) *

[1 + 23/84] * volume (in liters) you are adding to

4.3.6. Add enough NaHCO₃ to the first set of digesters to increase the alkalinity by 2,000 mg/L.

4.3.7. Add enough NaHCO₃ to the second set of digesters to increase the alkalinity by 4,000 mg/L.

4.3.8. Test the pH of the additional digesters; this is the starting pH.

4.3.9. Retest the additional digesters to ensure they are at the desired alkalinity.

4.3.10. Put into incubator as above and do not touch 3 digesters from each set until final day

4.3.11. Monitor and Sample as described below (Section 6)

4.3.12. Halfway through the experiment (15th day in this case) sacrifice 1 digester from each set and test the alkalinity and pH

4.3.13. At the end of the test (day 30), analyze pH and alkalinity of remaining 3 digesters from each set.

4.4. Temperature Test

4.4.1. Using optimum algae:sludge ratio found in section 4.2, follow Digester Setup Procedure (Section 5) for 3 sets of digesters (3 digesters each set) (plus a set of controls for each) (total 18 digesters)

4.4.2. Place one set in an incubator at 10°C and one at 20°C

4.4.3. Monitor temperature with an external thermometer

4.4.4. Monitor and Sample as described below (Section 6)

5. PROCEDURES FOR SETTING UP DIGESTERS

5.1. Clean and dry all bottles, caps, syringes, tubing, and graduated cylinders

5.2. Prepare caps

5.2.1. One port capped, two ports with 1/8" OD hard tubing attached with ferrule and tubing connection – one with longer soft tubing (for syringe) and one with shorter soft tubing.

5.2.2. Zip tie soft tubing around hard tubing by double wrapping and plug soft tubes with 2-way valves.

5.2.3. Keep hard tubing ends in headspace.

5.2.4. *Put a small piece of red tape around the longer tube to mark it for the syringe.*

Note: Optional, may cause stickiness

5.2.5. Tighten all fittings.

5.3. Prepare each test in triplicate

5.4. Label caps 1 – X and record in lab book and excel what will go in which digesters.

5.5. Cover bottles with a koozie and electrical tape or heavy duty aluminum foil to inhibit light transfer.

5.6. Add determined amount of substrate to the 250mL bottles

5.7. Add DI water (calculated amount to fill to 200mL once inoculum is also added)

5.8. Cap digesters

5.9. Purge with N₂ gas at a flow rate of at least 1 L/min for at least 2 minutes for all digesters

5.9.1. Flush bottles in the hood with hood exhaust turned on.

5.9.2. Connect tubing from N₂ canister to the valve on the longer tubing and open the valve on the short tubing to allow gas to flow out

5.9.3. Close short tube valve, close long tube valve, release Nitrogen gas tubing

5.9.4. Open short tube valve to release extra pressure and then close.

5.10. Add inoculum

5.10.1. Cap inoculum bottle with a customized 3-port cap to allow inoculum extraction, nitrogen entering, and nitrogen exiting (if tubes are in the head space)

5.10.2. Use soft tubing connected to a 30 ml plastic syringe to extract inoculums

5.10.3. *Keep nitrogen gas blowing into tubing of inoculum bottle while extracting*

Note: Optional, will only work if tubes are in head space and using a low gas flow rate

- 5.10.4. Start with digester 1 and continue in sets of 9
- 5.10.5. Shake inoculum bottle before each extraction
- 5.10.6. Extract required amount of inoculum and insert into short tubing valve of digester
- 5.10.7. Attach syringe, open long tube valve to release pressure, open short tube valve and insert inoculum
- 5.10.8. Close valves and detach syringe
- 5.10.9. Repeat if more inoculum is needed
- 5.10.10. Go back to hood with nitrogen gas
 - 5.10.10.1. Open long tube valve
 - 5.10.10.2. Open short tube valve
 - 5.10.10.3. Insert nitrogen gas into short tube and flush for 10-20 seconds.
 - 5.10.10.4. Close short tube valve, close long tube valve, release Nitrogen gas tubing
 - 5.10.10.5. After all valves are closed, quickly open and close the longer tube valve to release any excess pressure. Do this multiple time in a half circle motion until all gas pressure is released.
- 5.11. After completing 9 digesters, cover bottles, put into incubator and record start time
- 5.12. Place on a shaker at 150 rpm at desired temperature

Note: For the purpose of accuracy, never add less than 10mL of substrate or inoculum to a digester. If less is called for, use a dilution and always add at least 10mL to the digester and dilution.

6. MONITORING AND SAMPLING

6.1. Volume Measurement

- 6.1.1. Attach a clean, dry glass syringe to the digester to be sampled

6.1.2. While bracing the plunger, turn valve to release gas

6.1.3. If more than 20mL of gas appears to have been produced, push plunger to about the 15mL mark and close valve. Release plunger. Read gas level and repeat until all gas has been released.

6.1.4. Record reading on Volume Data Sheet

6.1.5. Save at least 20mL if testing the gas constituents (6.2 below), or perform GC measurement first before wasting gases.

6.2. Gas Chromotography Measurement

See SOP #0012

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0002:
Gas Chromatography Calibration with TCD**

1. PURPOSE

This protocol describes the process of calibrating the Gas Chromatography with TCD for CO₂ and CH₄.

2. MATERIALS

- 2.1. Standard CO₂, 99.8 % grade (Scotty)
- 2.2. Standard CH₄, 99.0 % grade (Scotty)
- 2.3. 30/balance CO₂/CH₄ calibration grade (Praxair)
- 2.4. 20mL Glass Syringe, (Micro-mate)
- 2.5. 5x Summa Canisters
- 2.6. Fischer Scientific Digital Flow Sensor

3. MACHINERY

- 3.1. GC (HP 6860) with TCD and sample injection valve
- 3.2. GC Control Computer
- 3.3. Vacuum Pump

4. PROCEDURE FOR GC CALIBRATION

4.1. FLUSH CANISTERS

- 4.1.1. Place canisters in oven at 60°C and keep them inside during the whole procedure
- 4.1.2. Attach flow sensor connected to vacuum pump to first canister and vacuum until -750 mm Hg pressure is reached

4.1.3. Switch valve to nitrogen side and fill canister with Nitrogen gas until about 20 mm Hg pressure

4.1.4. Repeat filling and vacuuming seven times

4.1.5. During last nitrogen fill cycle, fill to 100 mm Hg pressure

4.1.6. Take sample, making sure to purge once, and inject into GC to test for any residual gases

4.2. CREATE CALIBRATION STANDARD DILUTIONS

4.2.1. At room temperature, vacuum nitrogen out of clean canisters until -750 mm Hg and fill with CO₂ and CH₄ to a total pressure of 150 mm Hg in the following ratios: 90 mm Hg:810 mm Hg (CO₂:CH₄/10:90), 180 mm Hg:720 mm Hg (CO₂:CH₄/20:80), 270 mm Hg:630 mm Hg (CO₂:CH₄/30:70), 360 mm Hg:540 mm Hg (CO₂:CH₄/40:60), 450 mm Hg:450 mm Hg (CO₂:CH₄/50:50)

4.2.2. Extract three 18-20mL samples for each canister and run in GC according to SOP 12.

Run each set in triplicate and flush with gas to be filled before filling each time. Use this data to make a GC calibration curve plotting moles of CO₂ and CH₄ against peak area.

Note: Moles of sample_gas = ((1*0.002)/(0.0821*293))*Percent-of-Gas(in decimal)

4.2.3. Run seven 18-20mL samples of the 30:70 pre-made standard and compare results to self-made 30:70 standard to ensure they are very similar. Make a control chart from these seven samples to use as a calibration check each sample day.

5. QA/QC

5.1. Always make sure reference gas is flowing through before turning on the detector, otherwise you will burn the detector.

5.2. Always inject the gas sample at a slow, consistent rate.

5.3. An injection sample of 20mL is preferred with a minimum sample size of 10mL

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0004:
Test the Alkalinity of a Liquid**

1. PURPOSE

This protocol describes the process of testing the alkalinity of digester sludge solution.

2. MATERIALS

- 2.1. HCl (0.02 N)
- 2.2. 100-200 ml Beaker
- 2.3. 50-100 mL Beaker (x2)
- 2.4. 50 ml Pipette with turn valve
- 2.5. Pipette clamp and stand

3. MACHINERY

- 3.1. pH meter

4. PROCEDURE FOR ALKALINITY TESTING

- 4.1. Attach pipette to clamp on stand.
- 4.2. Pour approximately 10-20 mL of HCL into a small beaker and pour this into the pipette to clean it.
- 4.3. Open the valve and let the HCL go into a waste beaker.
- 4.4. Fill the large beaker with 20 ml of digester sludge solution, insert pH probe, and place under pipette.
- 4.5. Add 10 ml of HCl to pipette.
- 4.6. Slowly add HCl to digester solution until pH reaches 4.5.

- 4.7. Once pH 4.5 is reached, determine amount of HCl added.
- 4.8. Multiple volume of HCl added by its normality and 50,000 and divide by the mL of solution tested (20 mL in this case) to get the alkalinity in mg CaCO₃/L.

NOTE: The pH will naturally vary during digestion, which we will measure with a pH meter at the beginning and end of the alkalinity digestion test. No manual adjustment of the pH will be made.

5. COMMENTS

- 5.1. Be sure to wear safety goggles, gloves, and lab coat when handling HCl.
- 5.2. Procedure based on Standard Methods 2320 B. Titration Method (20th Edition, page 2-28)

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0005:
Test the COD**

1. PURPOSE

This protocol describes the process of testing the COD of a sample.

2. MATERIALS

- 2.1. Hach 0-1500 mg/L COD Reagent Vials
- 2.2. 40 ml Vials
- 2.3. 1000 mg/L COD Standard Solution
- 2.4. Potassium acid phthalate (KHP)
- 2.5. DI Water
- 2.6. Kim Wipes

3. MACHINERY

- 3.1. COD Reactor
- 3.2. Spectrophotometer

4. PROCEDURE FOR COD CALIBRATION

- 4.1. Make a 3000 mg/L COD standard from the KHP by weighing out the appropriate amount and adding it to DI water.
- 4.2. Dilute the 3000 mg/L stock to make concentrations of 250, 500, 750, 1000, 1250, and 1500 mg/L.

Note: Do not dilute one standard to make another. Use the 3000 mg/L base for all of the standards. Check the dilution by also running the 1000 mg/L standard to ensure accuracy.

- 4.3. Follow the Procedure in Section 5 below to determine the COD of these standards.
- 4.4. Create a COD calibration curve (COD (mg/L) vs Absorbance at 620nm

4.5. Create a COD-OD calibration curve by plotting the OD readings of the 0, 250, 500, 750, 1000, 1250, and 1500 mg/L standards versus their COD concentration. This curve can be used to determine the COD of unknown samples by reading their OD.

5. PROCEDURE FOR COD DETERMINATION

5.1. Standard Procedure

5.1.1. Hold the vial at a 45 degree angle and add 2 ml of the diluted(2x or 3x) sample or standard to be tested to the Hach COD reagent vial.

5.1.2. If you are blanking with reacted reagent, add 2 ml of DI water to another COD reagent vial to act as a blank.

Note: we created our calibration curve by blanking with DI water (unreacted); therefore, we zero our spectrometer with unreacted DI water before each reading.

5.1.3. Cap the vials and invert to mix.

5.1.4. Place sample, standards, and blank vials into the COD reactor at 150°C for 2 hours.

5.1.5. After reactor has finished its 2 hour run, wait for it to cool to 120°C, invert all vials to mix, and let cool to room temperature.

5.1.6. Once cool, clean the vials with a kim wipe and use the spectrophotometer to get absorbance readings of the vials at 620nm.

5.1.7. Use the COD calibration curve to determine the COD

5.2. Using Optical Density

5.2.1. Use the COD-OD calibration curve created in step 4.3 above to determine the COD of unknown samples based solely on the optical density.

6. QA/QC

6.1. Run a 1,000 mg/L COD standard each test day to ensure consistency

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0006:
Measure VS and TS**

1. PURPOSE

This protocol describes the process of determining the volatile solids (VS) and total solids (TS) content of a sample.

2. MATERIALS

- 2.1. Evaporating Dishes
- 2.2. Graduated Cylinder or 20 ml Syringe
- 2.3. Pipette and tips for 10 mL

3. MACHINERY

- 3.1. 105°C Oven
- 3.2. 550 °C Oven
- 3.3. Dessicator
- 3.4. Scale

4. PROCEDURE FOR VS/TS TESTING (Based on Standard Methods 2540-B, 2540-E and 2540-G)

- 4.1. Clear the evaporating dish of any contaminants by heating it at 550°C for 1 hour, then let dish cool to room temperature and store in desiccator until needed. Weigh dish immediately before use.
- 4.2. Transfer 10 ml of sample onto dish, weigh and then place dish in drying oven at 105 °C for at least 2 hours. This weight minus dish weight is total sample weight.

Note: According to standard methods, the sample should be evaporated on a water bath or in a drying oven first, and then dried at 103-105C for at least 60 minutes.

Note: The sample size should yield a total solids residue between 2.5 and 200 mg.

- 4.3. Remove from oven, allow dish to cool in dessicator to room temperature and weigh.
- 4.4. Repeat drying and cooling until weight change is less than 4% of previous weight or 0.5 mg, whichever is less. This weight minus the dish weight is the TS weight.
- 4.5. Place dish into furnace at 550°C for 45 minutes.

Note: If semi-solid samples are to be measured, such as wastewater sludge, follow standard method 2540-G: use 25-50 grams of wet sample and, after TS determination, heat at 550°C for 1 hr. If the residue contains large amounts of organic matter, first ignite it over a gas burner and under an exhaust hood in the presence of adequate air to decrease losses due to reducing conditions and to avoid odors in the laboratory.

- 4.6. Remove from furnace and partially cool the dish in air. Then transfer to dessicator for final cooling to room temperature.
- 4.7. Weigh dish as soon as it is cooled, and repeat heating and cooling cycle until weight change is less than 4% of previous weight or 0.5 mg, whichever is less. The TS plus dish weight minus this weight is the VS weight.
- 4.8. % TS = TS weight / Total sample weight found in step 4.2
- 4.9. % VS = VS weight / Total substrate weight found in step 4.2

5. COMMENTS

- 5.1. TS cycle: 120 minutes, 30 minutes, if <4% change finished, else continue 30 min cycles
- 5.2. VS cycle: 45 minutes, 15 minutes, if <4% change finished, else continue 15 min cycles

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0007:
Growing *Scenedesmus sp.***

1. PURPOSE

This protocol describes the process of growing algae for use in laboratory experiments.

2. MATERIALS

- 2.1. 4 L Flask or larger container
- 2.2. Primary Wastewater Effluent
- 2.3. Magnetic Stirrer Bar
- 2.4. 99.9% CO₂ tank with regulator and associated connectors, valves, and tubing
- 2.5. Air source
- 2.6. Diffuser
- 2.7. Aluminum Foil
- 2.8. Culture of *Scenedesmus sp.*

3. MACHINERY

- 3.1. Stir Plates
- 3.2. T-12 Fluorescent Light Fixture and 2x 40W Bulbs
- 3.3. Air and CO₂ Flow Regulators

4. GROW ALGAE

- 4.1. Setup up the light fixture so that it will be no more than two inches above the 4 L flask.
- 4.2. Setup a 5% CO₂ stream by connecting the CO₂ tank and air source to their flow regulators set at 1 ml/min and 20 ml/min, respectively. Then combine the two flows into one tube that is capped with a diffuser.

Note: We are using a N₂ flow controller instead of a CO₂ controller. Therefore, we adjust the flow based on gas K factor (see flow meter manual) so that 1 mL/min on the N₂ flow controller equals 0.739 mL/min CO₂ in reality.

- 4.3. Place a stirrer bar into the 4 L flask and place the flask on top of the stir plate. This unit should be centered under the light fixture.
- 4.4. Fill the flask with 0.5 L of algae solution and 2 L of primary effluent and place the tube with diffuser as close to the bottom as possible. Cover the mouth of the flask with foil.

Note: We started with about a 20 mL culture in 1 L of effluent

Note: We use a glass rod to secure the tubing to in order to get the diffuser to the bottom
- 4.5. Turn on the stirrer so that a gentle swirl is created in the flask(lowest setting or slightly higher depending on density of algae within flask). This should prevent any algae from settling.
- 4.6. Add fresh effluent to the flask at least once every two weeks. If the flask is full, pour out half of the solution and replace with effluent. Remember to let the algae settle first then you can siphon off half of the solution and replace the other half with primary effluent.
- 4.7. Continue growing this poured half in another bottle if needed.

Note: pH of the algae can be buffered with Na₂CO₃ if needed to prevent acidification. pH of *Scenedesmus sp.* should be between 7 and 8. For maximum growth, light is on 24/7.

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0010:
Collecting and Storing TWAS and Inoculum**

1. PURPOSE

This protocol describes the process of collecting and storing thickened waste activated sludge (TWAS) and inoculum.

2. MATERIALS

- 2.1. 2 L Glass Bottle
- 2.2. Ported cap with attached 30mL plastic syringe
- 2.3. Wide-Mouth Container
- 2.4. Cooler

3. MACHINERY

- 3.1. None

4. PROCEDURE FOR TWAS AND INOCULUM COLLECTION AND STORAGE

- 4.1. For collecting TWAS, use a wide mouth bottle to make it easier to collect since it must be manually scooped up.
- 4.2. Use a standard 2 L glass bottle for inoculum, equipped with cap with pressure releasing syringe attached. When collecting it, leave as little headspace as possible in the bottle and make sure the cap is airtight to minimize oxygen in the bottle.
- 4.3. Keep the bottles in a cooler during transport to minimize temperature fluctuations. This is more important for the inoculum since temperature affects its performance.
- 4.4. TWAS can be stored in a 4°C room or refrigerator for up to 3 days.
- 4.5. Inoculum should be used as soon as possible, but can be stored for up to 24 hours in an incubator at 35 °C or whatever temperature you are testing.

NOTE: Be sure to vent the inoculums bottle frequently or gas pressure will build up inside the bottle. A cap vented with a syringe may also be used to prevent gas buildup.

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0012:
Gas Chromatography Analysis with TCD**

1. PURPOSE

This protocol describes the process of measuring the proportions of CO₂ and CH₄ with a Gas Chromatography with TCD.

2. MATERIALS

- 2.1. Sample in glass syringe with valve
- 2.2. 30/70 CO₂/CH₄ calibration check sample

3. MACHINERY

- 3.1. GC (Agilent 6890) with TCD and sample injection valve
 - 3.1.1. Column Specifications:
ALL TECH Chromosorb 106 80/100
6"x1/8" x .085" SS
Part # C-5000
Bath Serial # 156-705100057
MAOT 275°C
- 3.2. Computer with Agilent ChemStation software

4. PROCEDURE FOR GC MEASUREMENT

- 4.1. Make sure GC and computer are on and open method “Digester” in the ChemStation software
- 4.2. Ensure all gases are flowing and all temperatures are as needed
 - 4.2.1. Sample valve temperature should be 70°C ± 2°
 - 4.2.2. Keep FID oven on 250°C even though not using
 - 4.2.3. Sample valves should look like this: oxidizer \ / methanizer
 - 4.2.4. Aux 3 is the Nitrogen carrier gas

4.2.5. Use the method “DIGESTER.M” -> for details on this method see the Comments below.

4.3. Create new Sequence

4.3.1. Edit Sequence Table and Parameters

4.3.2. Click “Run” on the Sequence Table

4.4. Load 20mL sample in glass syringe and inject into GC sample valve at steady speed (about 10 seconds for 20mL)

4.5. Press “Start” on the GC

4.6. Look at the peak graphs to make sure everything is looking right

4.7. Flush syringe with N₂ or ambient air before next sample

4.8. When run is finished, wait until temperature is cooled and run next sample

4.9. After finished with all runs, turn off the filament and close the method on the software

5. QA/QC

5.1. Always make sure reference gas is flowing through before turning on the detector, otherwise you will burn the detector.

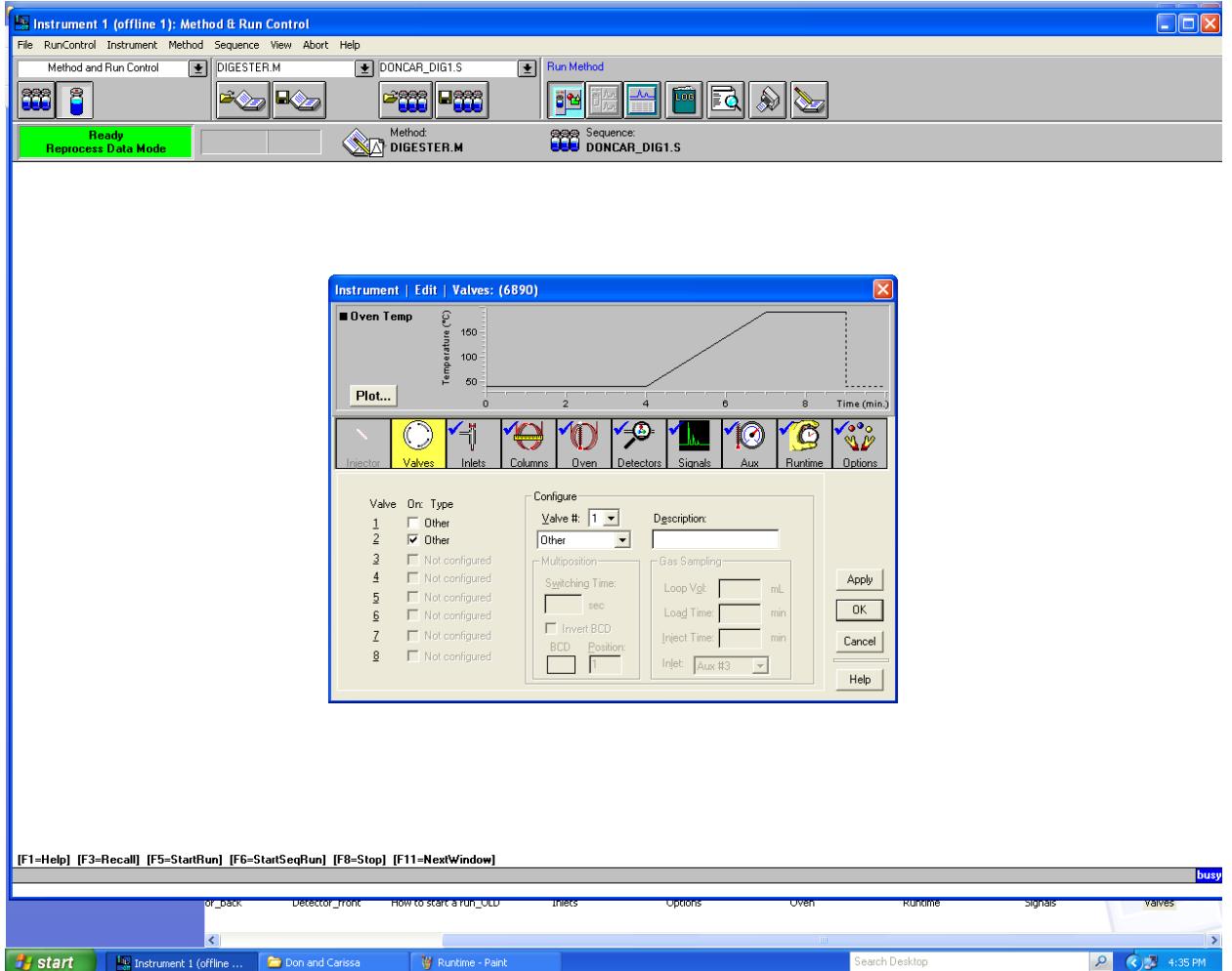
5.2. Always inject the gas sample at the same steady speed.

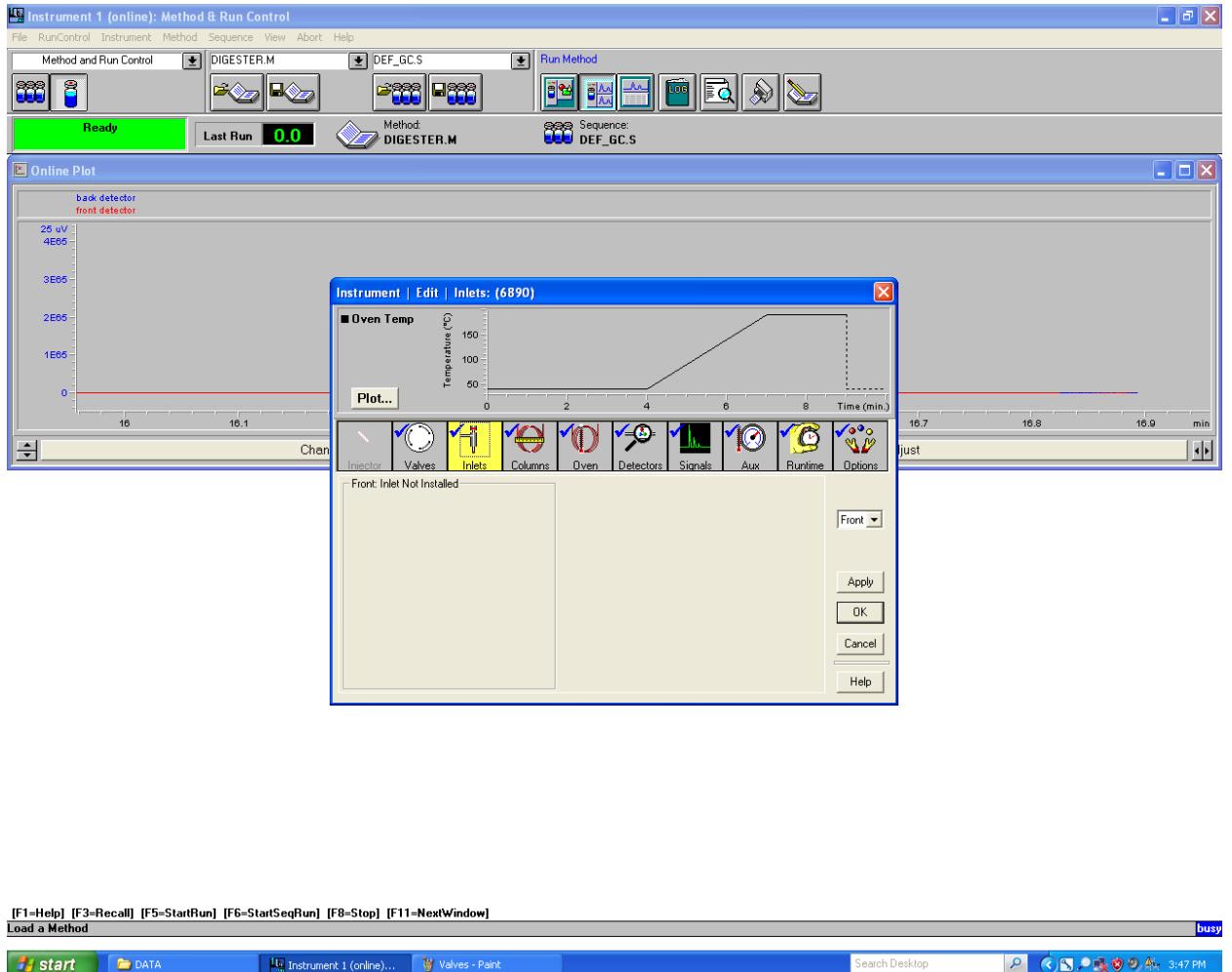
5.3. An injection sample of 20mL is preferred with a minimum sample size of 10mL

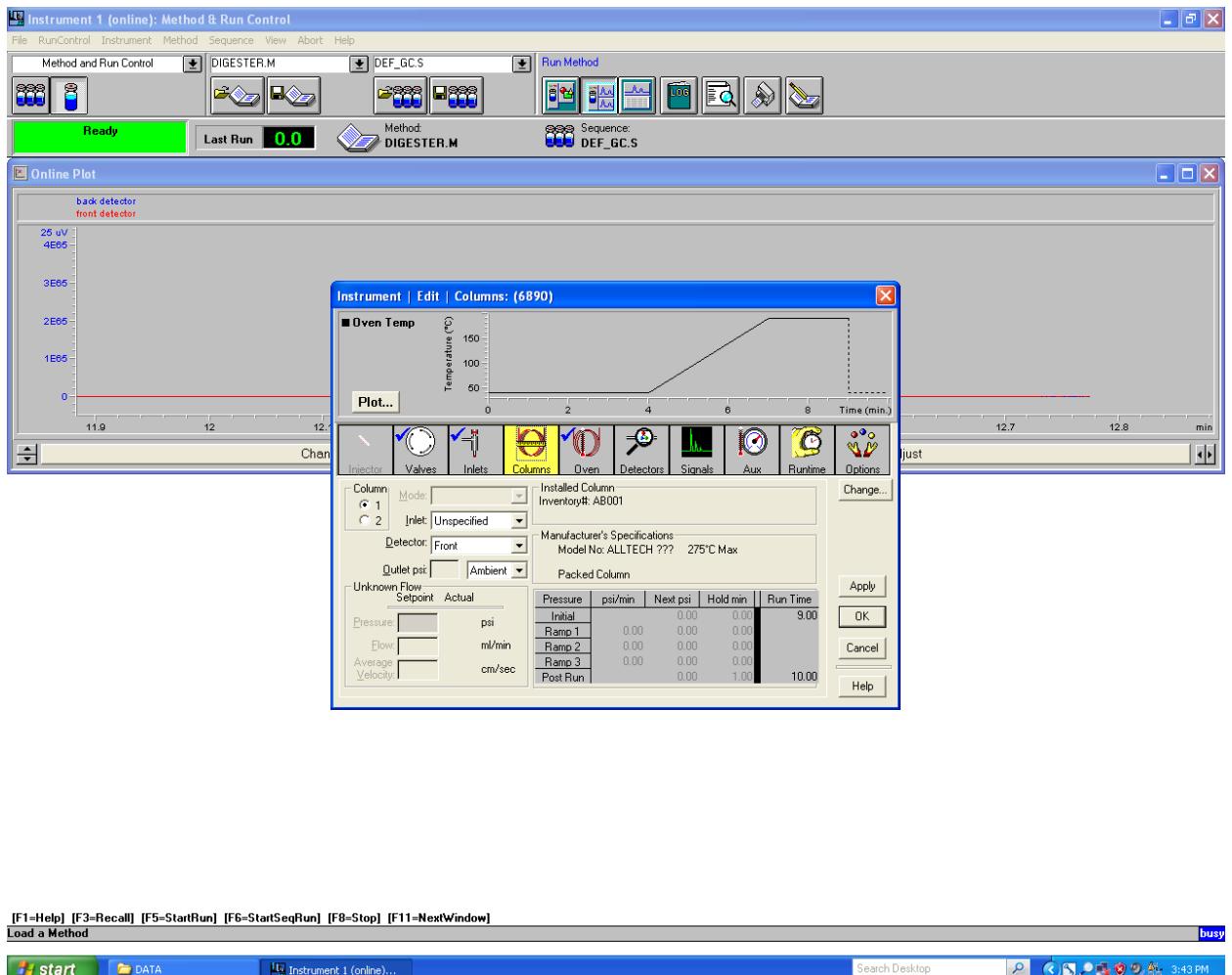
5.4. Run a calibration check sample as the first run each sampling day and check against control chart. Use the 30:70 standard as the calibration check.

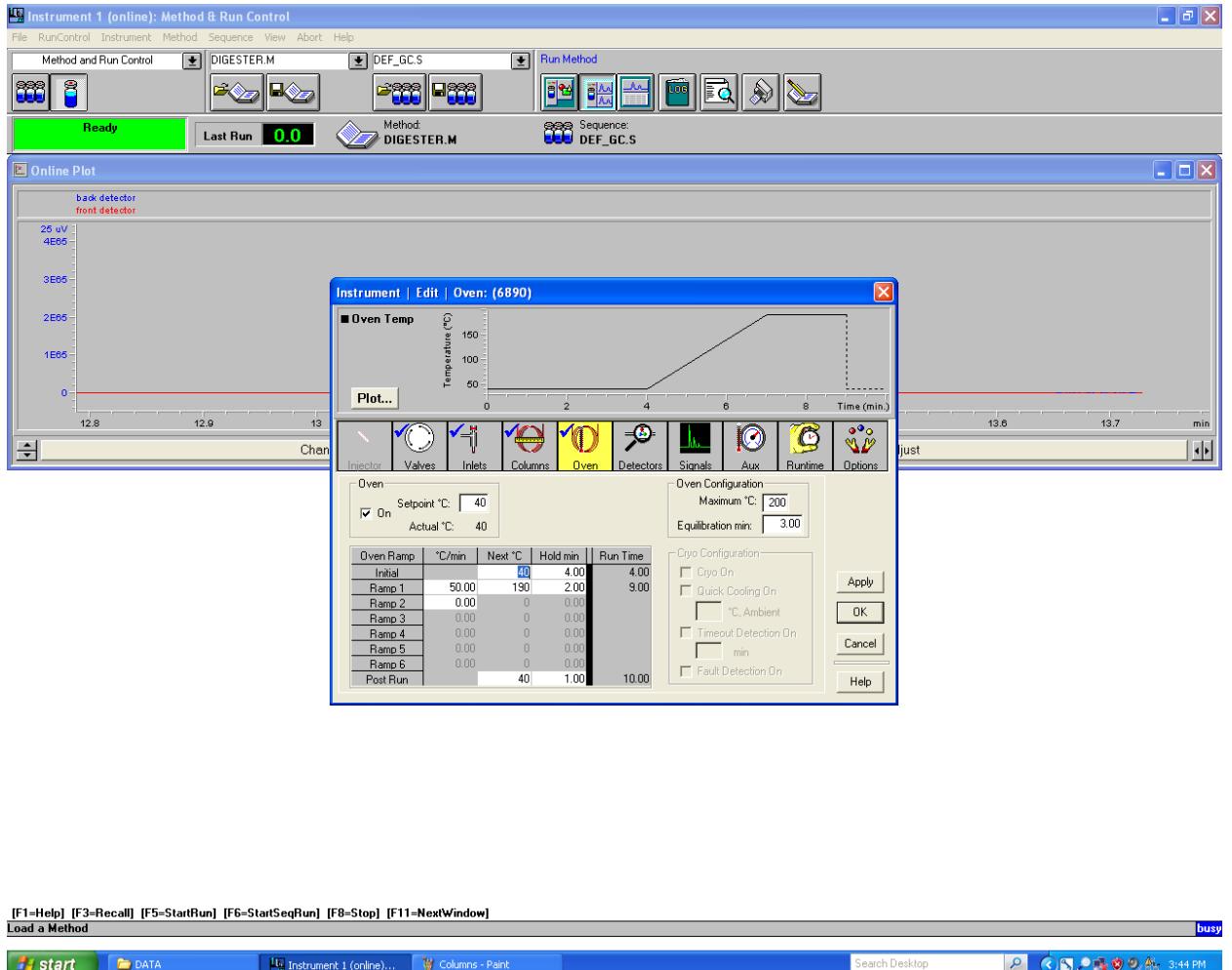
6. COMMENTS

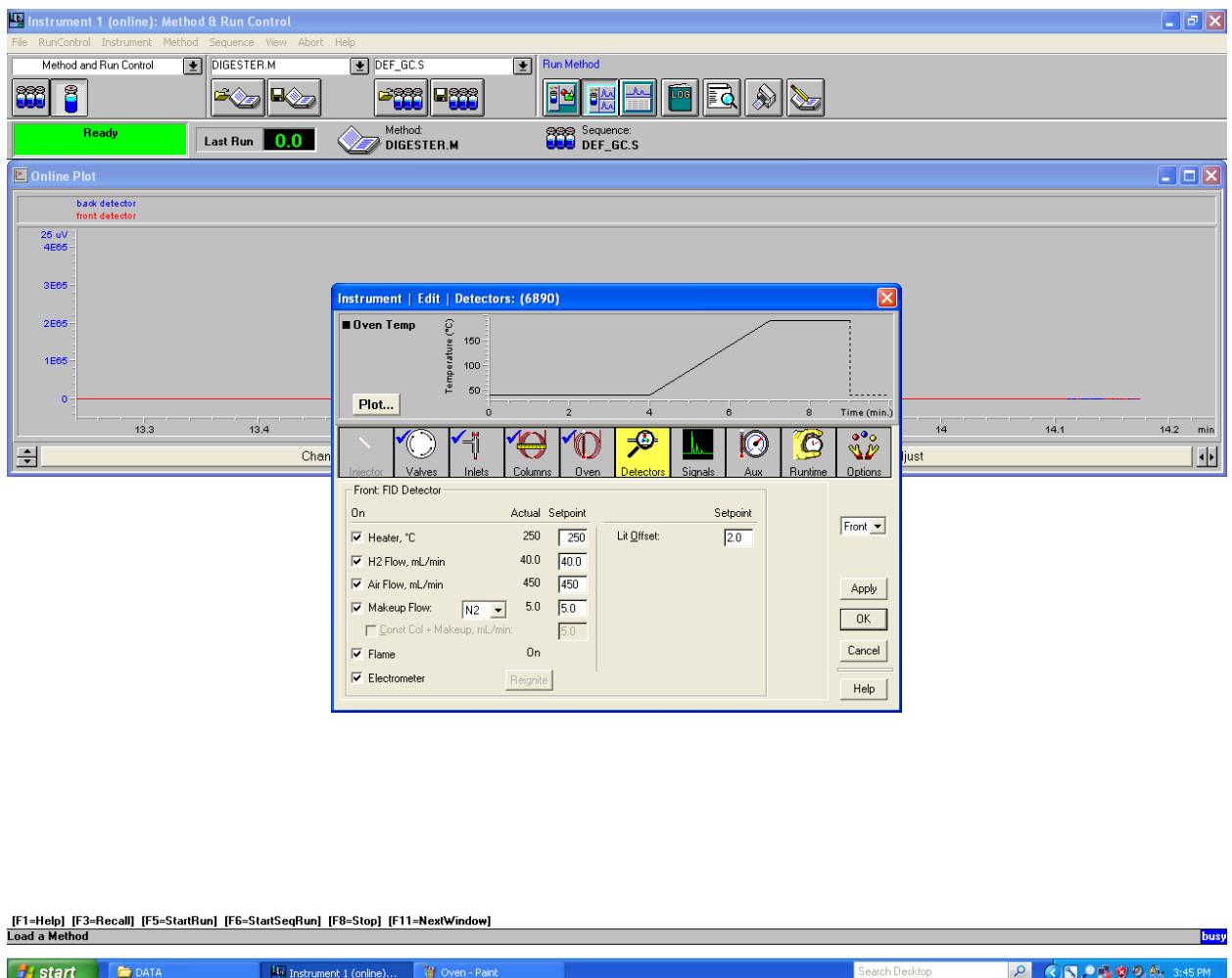
Method Details



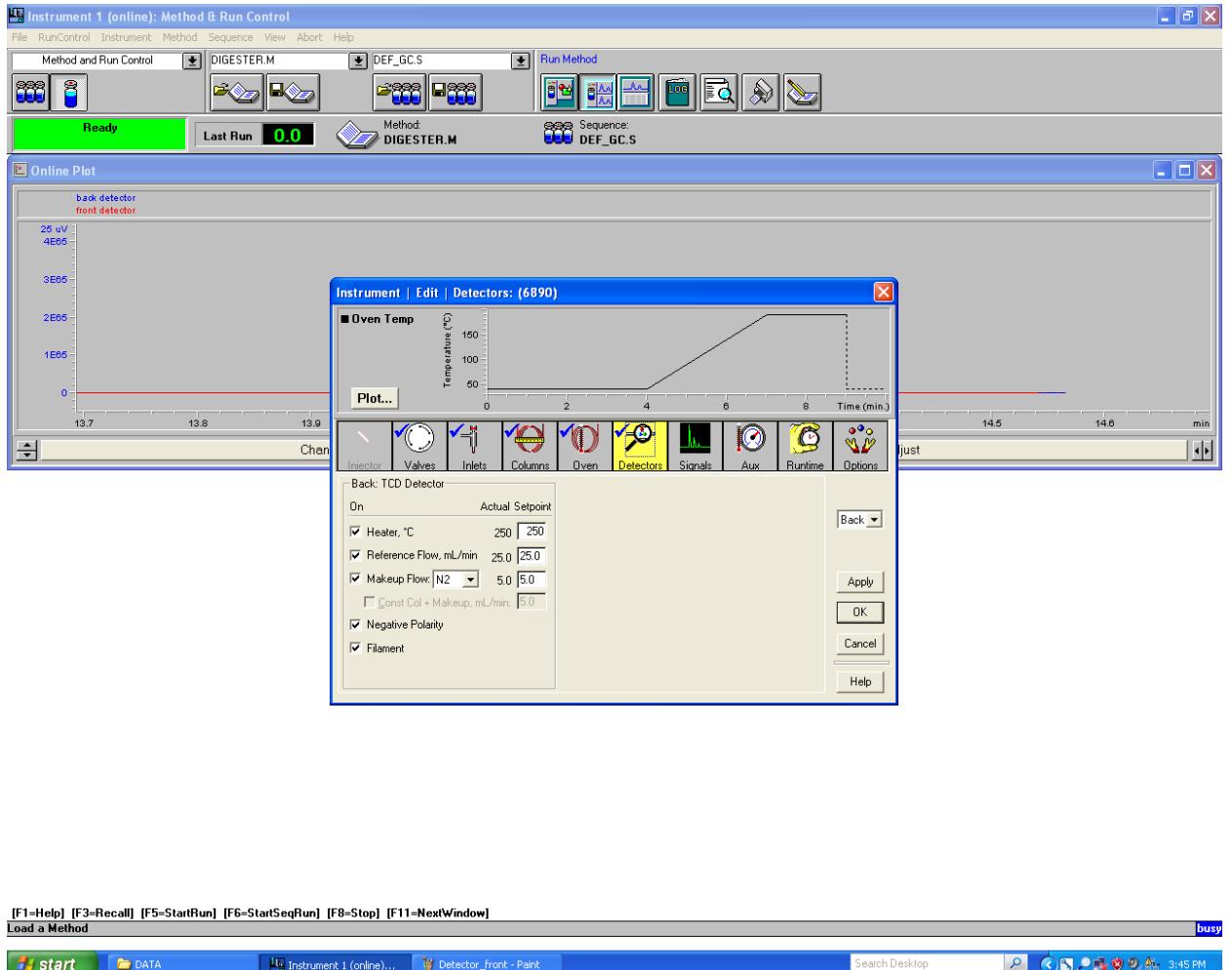


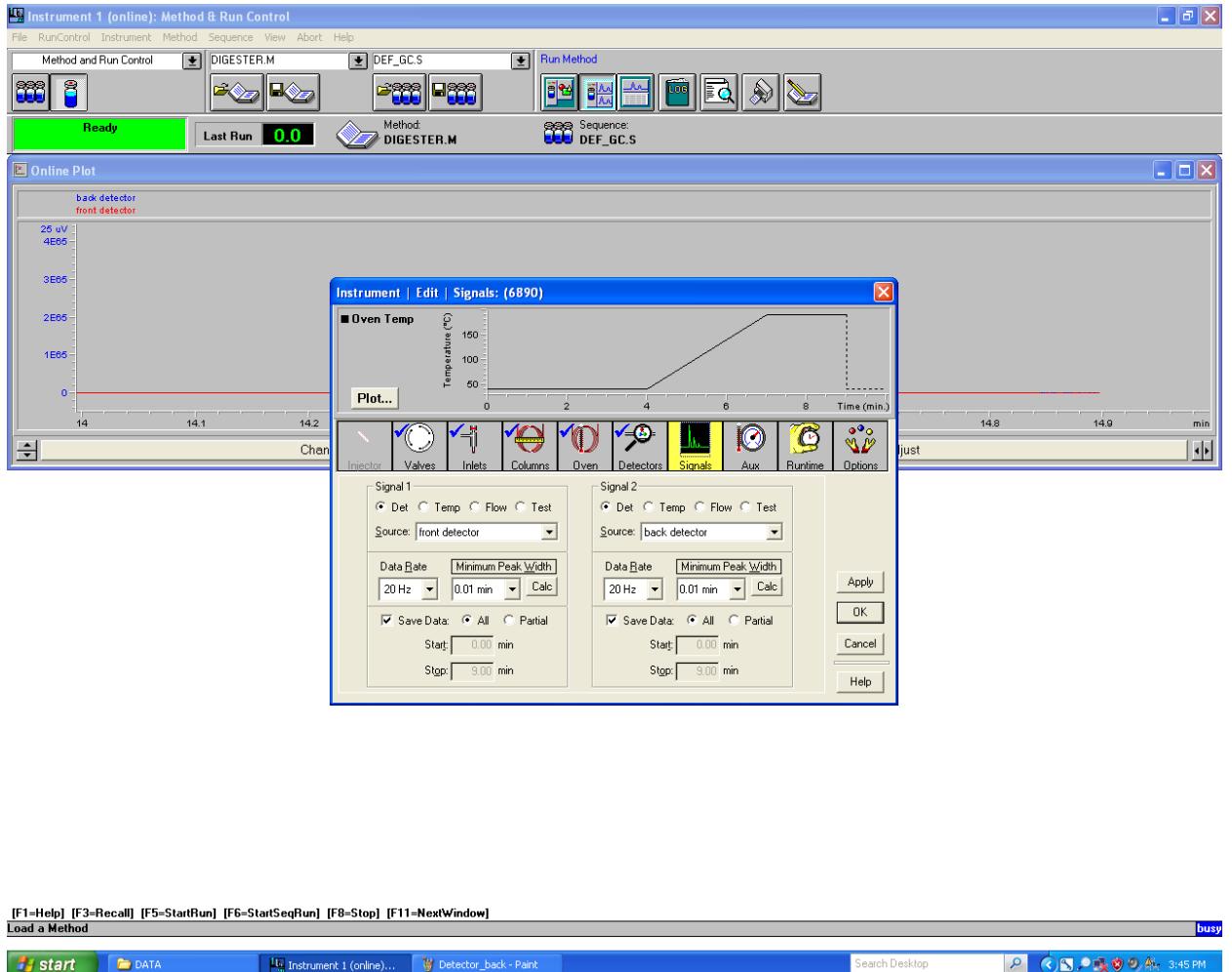


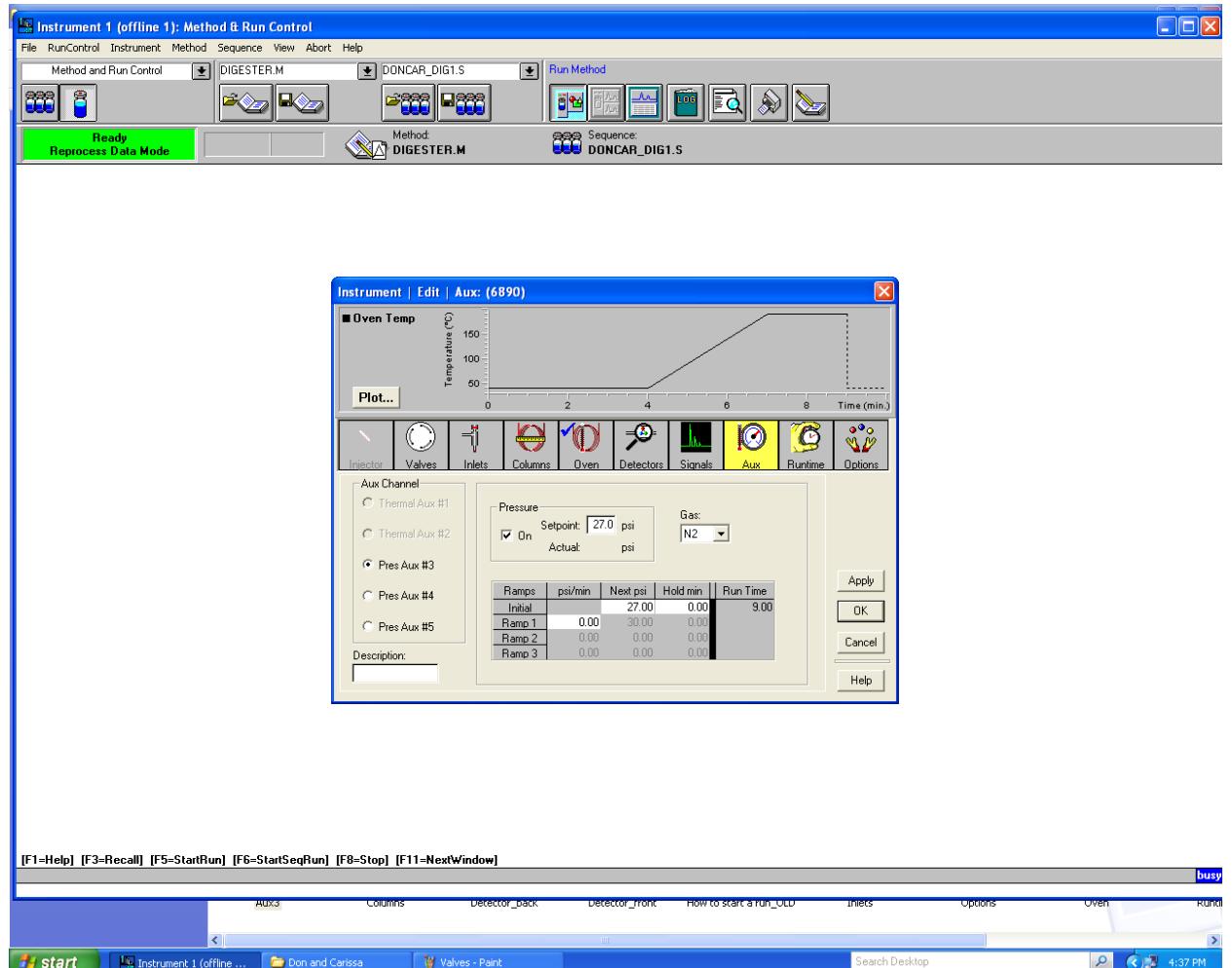


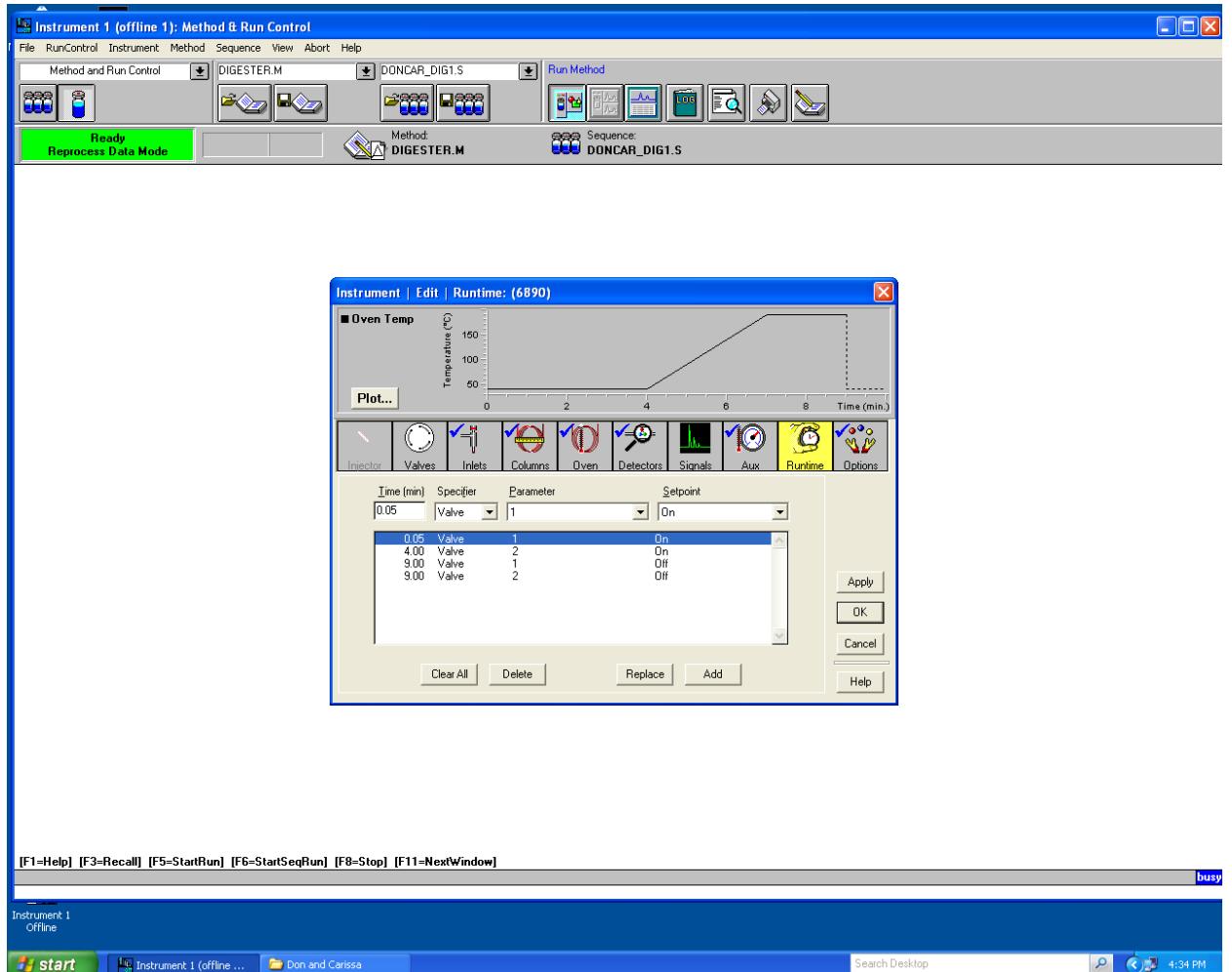


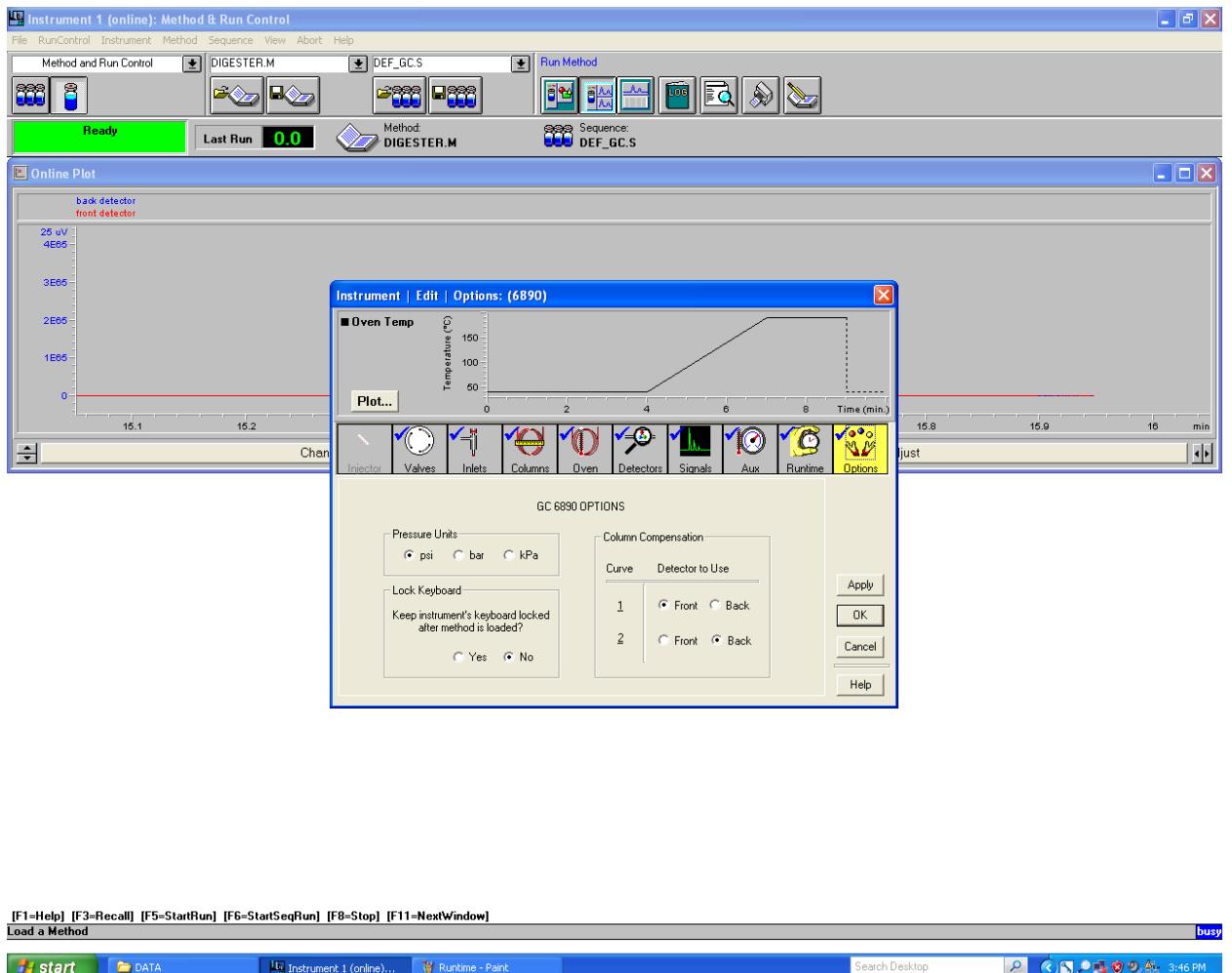
We were not using the FID detector so only the Heater and Makeup flow were checked (on)











**Environmental Engineering / San Diego State University
Standard Operating Procedure #0017:
Determination of Total and Fecal Coliforms with Colisure**

1. PURPOSE

This protocol describes the process of determining the total (TC) and fecal coliform (FC) counts (CFU) in digester liquid using the IDEXX Colisure method.

2. MATERIALS

- 2.1. Quanti-Tray/2000 and Colisure packs (IDEXX)
- 2.2. 100 mL bottles (number depends on number of dilutions) – autoclaved
- 2.3. 100 mL volumetric flask (x2) – autoclaved
- 2.4. ~500 mL beaker (x2) – one for waste, the other autoclaved
- 2.5. Autoclaved DI water (~550 mL per test)
- 2.6. 1 ml and 10 ml pipettes and autoclaved tips
- 2.7. Marker and labeling tape
- 2.8. 70% ethanol for sterilizing

3. MACHINERY

- 3.1. Incubator at 35°C
- 3.2. Autoclave
- 3.3. Laminar flow hood
- 3.4. Quanti-Tray sealer
- 3.5. 6 W, 365 nm UV light (IDEXX)

4. PROCEDURE FOR SETTING UP COLISURE TEST

- 4.1. After autoclaving the required supplies, sterilize the entire laminar flow hood with ethanol solution and then the UV light for 15 minutes.

Note: The entire procedure must be performed in the hood.

- 4.2. Spray pipette bodies down with ethanol as well as gloved hands. Alternatively, use antibacterial gel on skin if no gloves are worn.

- 4.3. For this example, we will be doing 100x, 1000x, or 2000x dilutions.

For **100x**, 1 ml of sample plus 99 ml of DI water. To do this, measure out 100 ml DI water with the volumetric flask, then remove 1 ml with the 1 ml pipette. Afterwards, pour the 99 ml DI water into a 100 ml bottle. Add 1 ml of sample with the pipette. Prepare two more bottles to complete the triplicate.

For **1000x**, to do this, in a small beaker make a 10x dilution, (90ml H₂O+10ml sample). Remove 1ml from the bottles with 100x dilution. Then add 1ml of the 10x dilution in the small beaker to the 100x dilution bottle which should have 99ml of solution in it. (99ml of 100x + 1ml of 10x). Prepare two more bottles to complete the triplicate.

For **2000x**, to do this, next, in a small beaker make a 20x dilution, (190ml H₂O+10ml sample). Remove 1ml from the bottles with 100ml water. Then add 1ml of the 20x dilution in the small beaker to the 100x dilution bottle which should have 99ml of solution in it. (99ml of 100x + 1ml of 20x). Prepare two more bottles to complete the triplicate.

Note: Take care not to let the pipette body touch the volumetric flask when removing the 1 mL of DI water. If this is not possible, the 100 mL of DI water may be poured into the bottle and then the 1 mL removed. **IMPORTANT:** If this is done with a used bottle, make sure to dispose of the pipette tip afterwards, do not continue to use it for DI water.

- 4.4. Add one pack of Colisure to each bottle and swirl until no large media particles remain. Try to minimize foam production.

- 4.5. Open a Quanti-Tray/2000 by curling the tray back and pour in the contents of one 100 ml bottle. Take caution to not touch the inside of the Quanti-Tray. At this point, you may reuse the bottle for the same digester if you do not have enough bottles by rinsing it at least twice with DI to remove as much foam as possible.

- 4.6. Place the tray in the Quanti-Tray sealer insert and seal the tray in the sealer. Remember that the sealer needs about 10 minutes to warm up after turning it on and is ready when the green light is on.
- 4.7. Place the tray in a 35°C incubator in a dark location for 24 hours.

5. PROCEDURE FOR READING QUANTITRAYS

Total Coliforms

- 5.1. After 24 hours, read trays by counting the number of red or magenta wells (positive). If a well is still yellow after 24 hours, it is negative.
- 5.2. If there are orange or pink wells, continue incubating for another 24 hours (up to 48 hours total).
- 5.3. At 48 hours, count the number of magenta or red wells as positive. At this time, wells that are still orange or pink are negative.

Fecal Coliforms

- 5.4. After counting TC, count the number of large and small wells that fluoresce by using a portable UV lamp in a dark room.

Total and Fecal Coliforms

- 5.5. Record the number of positive large wells and positive small wells for both Total and Fecal.
- 5.6. Use the given chart to find the MPN for Total and Fecal Coliforms based on the number of positive large and small wells.
- 5.7. Quantitray-2000 can read up to 2,419 MPN, so dilutions may be necessary. If this is the case, multiply the MPN found on the chart by the dilution factor to obtain the MPN.

6. QA/QC

- 6.1. Tests should be done in at least triplicate

- 6.2. Take care to keep hands, surfaces and tools sterile

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0019:
Post-digestion Testing**

1. PURPOSE

This protocol describes the process of tests to be run at the end of a digestion cycle.

2. MATERIALS

- 2.1. See SOPs #0005, 0006, 0017, 0021, and 0022

3. MACHINERY

- 3.1. See SOPs #0005, 0006, 0017, 0021, and 0022

4. PROCEDURE FOR POST ANALYSIS

- 4.1. Autoclave necessary glassware, D.I. Water, and pipet tips day before starting coliforms.

- 4.2. Test each digester (excluding controls) for Total Coliforms and Fecal Coliforms using the Quantitray 2000 method in triplicate (see SOP #0017)

Note: This should be done first so that the digesters are not exposed to open air before being sampled for coliforms.

- 4.3. Test each digester for COD in triplicate (see SOP #0005)

- 4.3.1. Run triplicate of 1000 standard calibration check each testing day

- 4.4. Test each digester for TS/VS in duplicate(triplicate if possible) (see SOP #0006)

- 4.4.1. Keep samples in the cold room for up to one day while waiting for oven space

- 4.5. Test each digester for Total Nitrogen (see SOP #0021) and Total Phosphorous (see SOP #0022) in duplicate

- 4.5.1. Run single calibration standard for both N and P (~50 mg/L) each testing day

5. QA/QC

5.1. Do not open digesters until in the laminar flow hood. Make the dilutions needed for the coliform testing before exposing the digestate to the air to do the other tests.

6. COMMENTS

Place all used digesters and dilutions in cold room for one week post testing in case tests need to be re-run.

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0021:
Total Nitrogen Determination**

1. PURPOSE

This protocol describes the process of determining the total nitrogen in a sample (specifically anaerobically digested substrate) by the Hach persulfate digestion method (Method 10072). This is similar to method 4500-N C of the *Standard Methods for Water and Wastewater*.

2. MATERIALS

- 2.1. Assorted glass beakers for dilutions and holding chemicals
- 2.2. HCL for acid washing
- 2.3. Nitrate-nitrogen standard
- 2.4. HACH Total Nitrogen Test N Tube High Range, 10-150 mg/L (cat # 27141-00)
- 2.5. Kim wipes

3. MACHINERY

- 3.1. Digester – DRB 200
- 3.2. Spectrometer

4. PROCEDURE FOR TOTAL NITROGEN TNT METHOD (Method 10127)

- 4.1. Turn on the DRB 200 and heat to 105°C
- 4.2. Use a funnel to add one Total Nitrogen Persulfate Powder Pillow to each HR Total Nitrogen Hydroxide Digestion Reagent vial
- 4.3. Add 0.5 mL of sample to each vial

Note: Prepare diluted sample as appropriate, normally (2x)

- 4.4. Cap tightly and shake vigorously for at least 30 seconds to mix

Note: The persulfate reagent may not dissolve completely after shaking. This will not affect accuracy.

- 4.5. Insert the vials in the DRB 200 reactor and close the cover
- 4.6. Heat for EXACTLY 30 minutes
- 4.7. After the timer expires, immediately remove the hot vials from the reactor and allow them to cool in a rack to room temp (18-25°C)

Note: At this point you can stop the test and resume 4.8 the following day.

- 4.8. Add the contents of one Total Nitrogen Reagent A Powder Pillow to each vial

- 4.9. Cap and shake for 15 seconds

- 4.10. Start the timer (a three-minute reaction period will begin)

- 4.11. Add the contents of one Total Nitrogen Reagent B Powder Pillow to each vial

Note: The reagent will not completely dissolve. This will not affect accuracy. The solution will begin to turn yellow.

- 4.12. Cap and shake for 15 seconds

- 4.13. Start the timer (a two-minute reaction period will begin)

- 4.14. After the timer expires, pipet 2 mL of digested, treated sample into one TN Reagent C vial(separate vial)

- 4.15. Cap the vials and invert ten times to mix. Use slow, deliberate inversions for complete recovery.

Note: The tubes will become hot to the touch

- 4.16. Start the timer (a five-minute reaction period will begin)

- 4.17. Zero the spectrometer with DI, Wipe the vial with a Kim wipe, and Read in a spectrometer at 410 nm

Note: Make calibration curve (10-150mg/L NO₃⁻-N)

5. QA/QC

- 5.1. Run a standard each new day of testing

**Environmental Engineering / San Diego State University
Standard Operating Procedure #0022:
Total Phosphorous Determination**

1. PURPOSE

This protocol describes the process of determining the total phosphorous in a sample (specifically anaerobically digested substrate) by the HACH Molybdovanadate Method with Acid Persulfate Digestion (Method 10127Adapted from *Standard Methods for the Examination of Water and Wastewater* (4500 B-C)).

2. MATERIALS

- 2.1. Assorted glass beakers for dilutions and holding chemicals
- 2.2. HCL for acid washing
- 2.3. Phosphate Standard, 50 mg/L (might need to get 100 mg/L)
- 2.4. HACH Total Phosphate Test N Tube High Range, 1-100 mg/L (cat # 27672-45)
- 2.5. Kim wipes

3. MACHINERY

- 3.1. Digester – DRB 200
- 3.2. Spectrometer

4. PROCEDURE FOR PHOSPHATE TNT METHOD (Method 10127)

- 4.1. Turn on the DRB200 and heat to **150°C**

- 4.2. Add 5 mL of sample to a TNT vial

Note: Prepare diluted sample accordingly, normally (5x). ex. 5ml sample+20ml

H₂O

- 4.3. Use a funnel to add one Potassium Persulfate Powder Pillow to each vial

- 4.4. Cap tightly and shake to dissolve

- 4.5. Insert the vials in the DRB 200 reactor and close the cover

- 4.6. Set the instrument timer to 30 minutes and start (a 30 min heating period will begin)
- 4.7. After the timer expires, carefully remove the hot vials from the reactor and allow them to cool in a rack to room temp (18-25°C)

Note: At this point you can stop the test and begin with 4.8 the next day

- 4.8. Add 2 mL of 1.54N NaOH to each vial, Cap and invert to mix
- 4.9. Add 0.5 mL of Molybdovanadate reagent to each vial, , Cap and invert to mix
- 4.10. Start the timer for 7 minutes (A seven min reaction period will begin)

Note: Read the sample between 7 and 9 minutes after adding the Molybdovanadate reagent.

Note: Make calibration curve (0-100mg/L)

- 4.11. Zero the spectrometer with DI, Wipe the vial with a Kim wipe, and Read in a spectrometer at 420nm

5. QA/QC

- 5.1. Run a standard each new day of testing

6. COMMENTS

- 6.1. The final samples will contain molybdenum. In addition, the final samples will have a pH of less than 2 and are considered corrosive.

APPENDIX B

RESULTS FROM PRELIMINARY TESTS

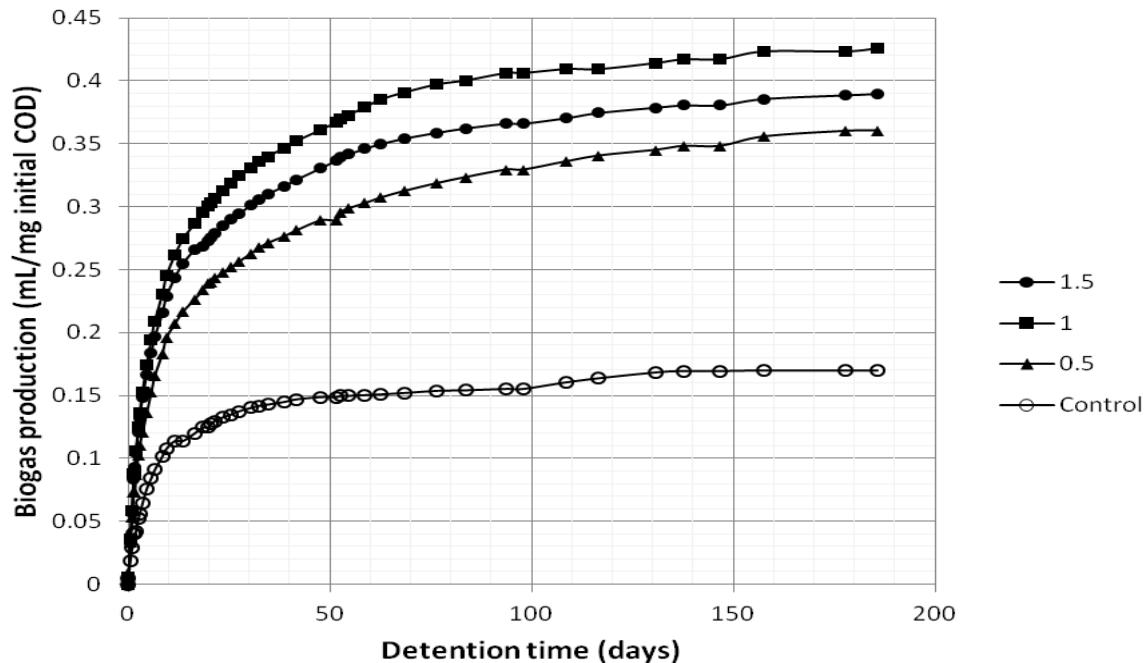


Figure B.1. Cumulative biogas production at various sludge to inoculum ratios.

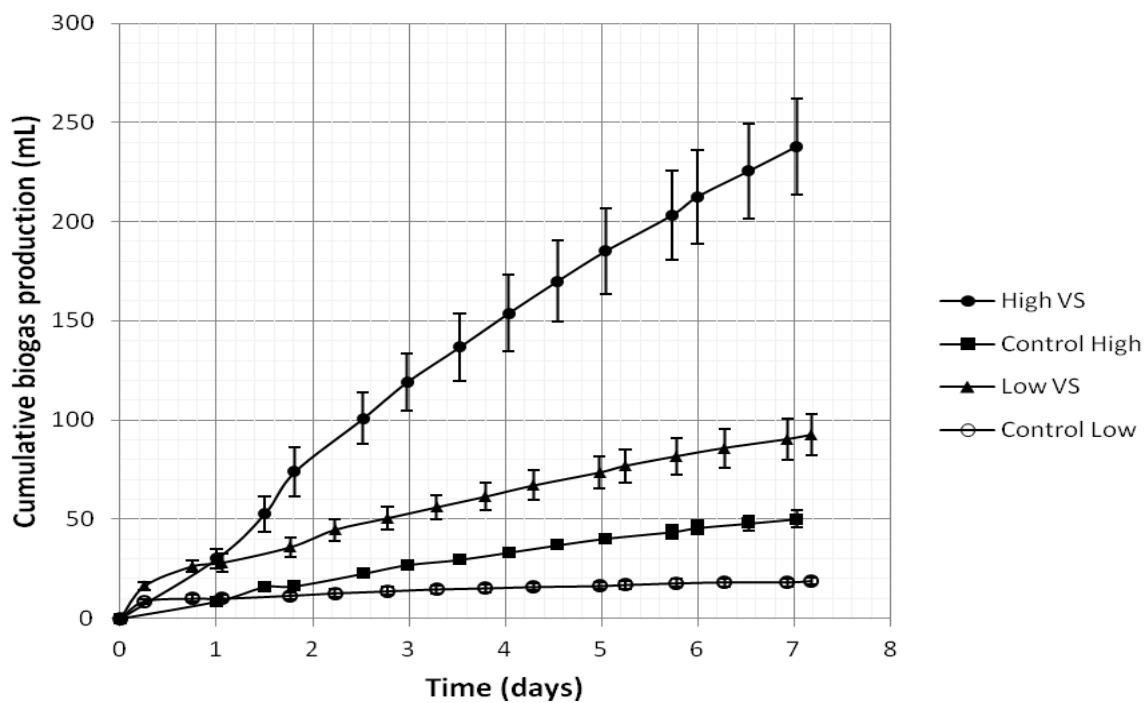


Figure B.2. Cumulative biogas production for high and low VS loadings.

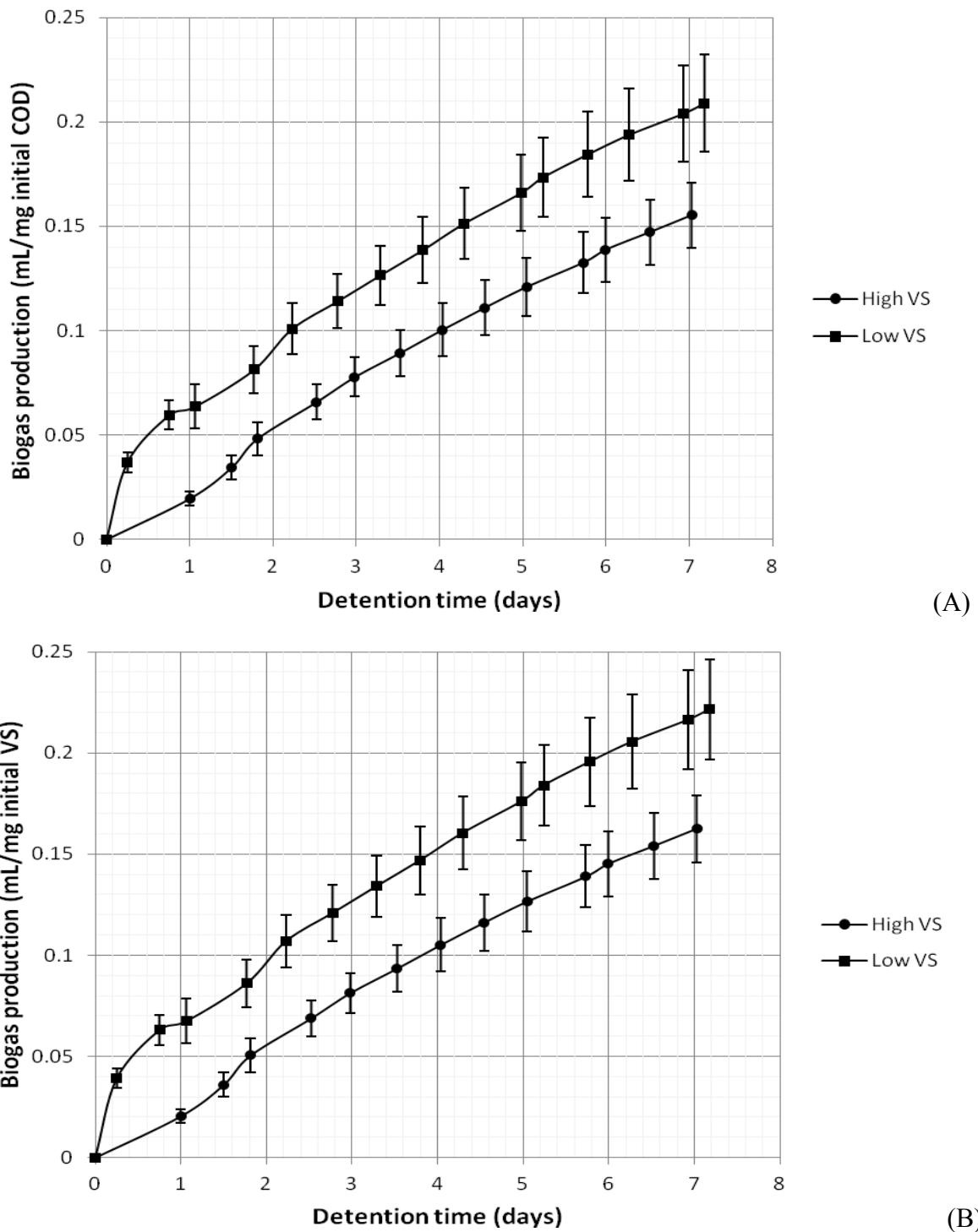


Figure B.3. (a) Biogas production per initial COD for high and low VS loadings (b) Biogas production per initial VS for high and low VS loadings.