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ANAERBIC DIGESTION OF WASTEWATER: EFFECTS OF INOCULANTS

AND NUTRIENT MANAGEMENT ON BIOMETHANE

PRODUCTION AND TREATMENT

by

Jason J Peterson

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biological Engineering

Approved:

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UTAH STATE UNIVERSITY
Logan, Utah

2017

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ABSTRACT

Anaerobic Digestion of Wastewater: Effects of Inoculants and Nutrient Management on Biomethane Production and Treatment

by

Jason J Peterson, Master of Science

Utah State University, 2017

Major Professor: Dr. Ronald C. Sims
Department: Biological Engineering

Due to population expansion and the increased awareness of the impact on the environment by wastewater treatment, improved wastewater treatment systems are needed to treat municipal and agricultural wastewater. Aerobic treatment of wastewater decreases organic compounds at the expense of energy to move organic chemicals and oxygen to be in contact with each other for treatment. Anaerobic digestion of wastewater can reduce the cost by utilizing methanogens to treat high amounts of organic chemicals in wastewater without the need for oxygenation. Anaerobic digestion also provides methane, a renewable energy source.

Wastewaters with high carbon content have been shown to produce higher amounts of methane when balanced with nitrogen. It has been suggested that microalgae be added to increase the nitrogen content to help balance the high carbon to nitrogen ratio of the wastewater. One challenge with the use of algae is the initial degradation of microalgae. Using a digester with algalytic microbes algae can be biodegraded and production of methane enhanced. The augmentation of wastewater by microalgae with algalytic microbes could provide the balance needed for the methanogens to treat wastewater and provide methane.

A biomethane potential test was used to compare the ability of two inocula, facultative lagoon sediment and anaerobic digester sludge, to digest algae under anaerobic conditions and produce biomethane. Each inoculant treated, dairy, swine, municipal, and petrochemical wastewaters augmented with algae and sodium acetate to increase and balance the carbon to nitrogen ratio. The ability to degrade augmented wastewaters and produce methane was determined by measuring the volume and composition of biogas produced over time.

Both treatments were successful in the production of methane. Facultative lagoon sediment showed a higher ratio of methane produced per carbon dioxide than anaerobic digester sludge. Facultative lagoon sediment showed a larger reduction in biological oxygen demand, where anaerobic digester sludge showed a larger decrease in volatile solids. Facultative lagoon sediment showed more methane produced per gram of volatile solids than anaerobic digester sludge.

(122 pages)

PUBLIC ABSTRACT

Anaerobic Digestion of Wastewater: Effects of Inoculants and Nutrient Management on Biomethane Production and Treatment

Jason J Peterson

Due to population expiation and the increased awareness of the impact on the environment by wastewater treatment, improved wastewater treatment systems are needed to treat municipal and agricultural wastewater. Treating wastewater with oxygen decreases carbon compounds at the expense of energy to move carbon and oxygen to be in contact with each other. Anaerobic digestion of wastewater can reduce the cost by utilizing microbes to treat high amounts of carbon in wastewater without the need for extensive oxygen requirement. With a proper balance of nutrients, microbes also produce methane, a renewable energy source.

It has been suggested that microalgae be utilized to help balance the nitrogen content of wastewater for treatment by microbes. One challenge with the use of algae is the initial breakdown of algae cells. Using a digester with microorganism that can produce methane and decompose algae could enhance production of methane from the digestion of algae. The combination of wastewater, which is high in carbon content relative to nitrogen, with algae, which is high in nitrogen, could provide the balance needed for the microbes to treat wastewater and provide methane.

A biomethane potential test was used to compare the ability of two microbe communities, facultative lagoon sediment and anaerobic digester sludge to digest algae. Each microbe community treated dairy, swine, municipal, and petrochemical wastewater augmented with algae and acetate. The ability to degrade augmented wastewater and produce methane was determined by measuring the volume and composition of biogas produced over time. Both treatments were successful in production of methane. Facultative lagoon sediment showed more methane produced per carbon dioxide than anaerobic digester sludge.

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Jason J Peterson

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INTRODUCTION AND OBJECTIVES

Introduction

Renewable energy resources are becoming a high priority as alternatives to fossil fuels. Anaerobic digestion can be used to treat wastewater with the added benefit of methane production as a bioenergy resource. Along with wastewater, microalgae can be utilized as a substrate for methane production. However one challenge with the digestion of microalgae to methane (also referred to as biomethane) is initial breakdown of microalgae cells causing slower digestion (Cho et al., 2013) (Schwede et al., 2013). Another challenge with digesting microalgae is the low carbon to nitrogen (C:N) ratio resulting in ammonia toxicity (Sievers and Brune, 1978) as well as limiting digestion (Yen and Brune, 2007).

Using a digester with methanogenic and algalytic microorganisms could enhance production of methane from the breakdown of microalgae. Combining carbon rich wastewater with microalgae could mitigate ammonia toxicity resulting from digestion of microalgae alone. Inefficient microbial consortium may leave digesters operating at a lower than ideal conditions. Locations containing facultative lagoons, which facilitate the growth of microalgae using large ratios of surface area to volume by utilizing sunlight as the energy source for cultivation of autotrophs, may have evolved to cohabitate methanogenic microbes in lagoon sediments, with the ability with the ability to metabolize and digest microalgae over time. Anaerobic digester sludge may have lost the capacity to degrade microalgae due to low pressure on microbes with algalytic activity, resulting in algalytic microbes being washed out over time.

It was hypothesized that facultative lagoon sediment (City of Logan, Utah, facultative lagoons) contains a greater ability to digest wastewater augmented with algae compared to anaerobic digester sludge (Central Weber Sewage District). A comparison of lagoon sediment to anaerobic digester sludge would reveal if one microbial community can outperform the other when digesting wastewaters augmented with microalgae. The four wastewaters selected were dairy (City of Wellsville, Utah, Caine Dairy), swine (City of Milford, Utah, Circle Four Farms), municipal (South Davis Sewer District), and industrial (City of Woods Cross, Utah, Refinery). Performance will be measured based on rate of biogas production, total volume of biogas produced, and the resulting composition of biogas.

Objectives

The objective of this study was to determine if bioaugmentation with two inoculants, facultative lagoon sediment (FLS) or anaerobic digested sludge (ADS), demonstrated an advantage in anaerobic digestion of four wastewaters for the production of biomethane. The wastewaters evaluated included swine, dairy, municipal, and petrochemical. The advantage was assessed based on two comparisons. The first comparison was to assess the capacity of each inoculant to digest each of the four selected wastewaters. The results show if either inoculant is applicable for the different wastewaters or if each inoculant is restricted in the type of wastewater it can digest. The second comparison was between inoculants for each wastewater with regard to biogas production. Treatability was determined by observing the production of biogas as well as the change in organic chemical concentration of each wastewater.

LITERATURE REVIEW

Anaerobic digestion of waste material is not a new science, though many aspects of it remain challenging. One challenge with treatment is to treat as much of the waste as possible while maximizing methane production. This is a daunting task do to the many parameters that contribute to operation of anaerobic digestion. Such complexity can leave digesters functioning at lower than ideal operating conditions (Lagerkvist and Morgan-Sagastume, 2012). Examples of challenges of treatment include fluctuations in the organic loading rate (Ward et al., 2008) and fluctuations in the carbon to nitrogen ratio (Yenigün and Demirel, 2013). Some researchers have suggested that mathematical models would be the best way to optimize performance of anaerobic digesters (Appels et al., 2011). Models can be used to help design and maximize the performance of digesters provided that critical information is available when developing the models.

A focus for anaerobic digestion has been to balance the C:N ratio of feed source to optimize performance (Wang, X. et al., 2014; Sievers and Brune, 1978). The optimal C:N ratio for anaerobic digestion is reported to be between 20:1 and 30:1 mass ratio (Ward et al., 2008; Esposito et al., 2012). Various C:N ratios have been tested using mixed paper as a carbon source and microalgae as the primary nitrogen source. Rates of Biogas production have been measured with the different C:N ratios. The highest yield of biogas observed when digesting microalgae and mixed paper was shown to be operating at a C:N mass ratio of 22.6:1 (Yen and Brune, 2007) and 21:1 mass ratio using algae and acetate (Soboh et al., 2016).

Biomethane Potential Test

The biomethane potential test (BMP) is becoming a popular choice for anaerobic digestion studies. This is attributed to the ease of setup and the ability to control treatment parameters resulting in valuable data that can be obtained from a small scale bench top digestion (Esposito et al., 2012). The BMP test is used to test substrate degradation under favorable conditions and show the maximum biomethane potential of the degraded substrate (Browne et al., 2013). Operating as a batch system, the BMP assay allows for a quick determination of substrate degradation. The data collected from the BMP could then be used to determine if the wastewater should be treated on a continuous flow system. Another benefit to the BMP test is the advantage of treating potential toxic wastewaters in a small working volume, reducing risks associated with toxic wastewaters in larger working volumes.

The BMP test may also under estimate the yield of methane generated from wastewater. Solids that are slower to degrade could still have the potential to be converted to methane in a continual flow digester (Thamsiriroj and Murph, 2011). The methane produced from the slow degrading solids in not accounted for due to early termination of BMP caused by low biogas production. The actual yield of methane produced from substrate in a continuous flow digester may be higher than what is produced from a BMP test. Soboh et al., 2016 reported a peak methane percentage of 82% in the BMP assay and showed greater than 90% methane with the same carbon to nitrogen ratio operating at steady state.

The parameters associated with collecting and measuring the formation of biogas from a BMP test can be accomplished in several ways. These ways range from commercial kits that mix contents and monitor the production of biogas, to simple syringe methods (Mshandete et al., 2006) used to collect biogas content. Other ways to measure biogas include: the volumetric displacement of water from a flask (Esposito et al., 2012), the use of a manometer system (Saha et al., 2011), and the capture of biogas in a gas tight bag (Murto et al., 2004). Each of these systems have strengths and weaknesses. Commercial equipment can be simple to use, but also expensive, costing as much as 27,000 dollars. Capturing biogas in a syringe is a cost effective way to monitor biogas formation, with the added challenge of accounting for the difference of friction between syringe and the challenge of determining the pressure of biogas in each syringe. All systems can be effective for measuring biogas production, but many are time and labor intensive (Wang, B. et al., 2014).

Both temperature and mixing can change the rate of biogas formation during the BMP test. A temperature range of 22 to 60 degrees Celsius has been applied to anaerobic digestion, (Gunaseelan, 1997) depending on the substrate being digested. Typical mesophilic anaerobic digestion operates at a temperature of 35 or 37 degrees Celsius (Gunaseelan, 1997).Using lower temperatures for digestion would allow for less energy to be used for heating reactor. Using a temperature of 35 degrees Celsius showed to be only 3% better than a digester operating at 30 degrees Celsius, (Chae et al., 2008) requiring less energy while achieving good digestion. High percentages of methane were also observed at a digestion temperature of 30 degrees (Soboh et al., 2016) showing that high temperatures are not required for high yields of methane during digestion. Mixing samples during digestion allows substrate to move and come in contact with inoculant during digestion. It has been shown that mixing substrate and inoculant does have an effect on digestion. Intense mixing to rapidly move substrate is not necessarily better than mixing contents at a gentle speed (Kaparaju, 2008).

Anaerobic Digestion of Algae

The anaerobic digestion of algae provides additional challenges. Common challenges associated with the digestion of algae include: concentrating algae prior to digestion, high ammonia toxicity due to the high nitrogen content, and the difficulty of breaking apart microalgae leaving it intact as it exits a digester. To make anaerobic digestion of algae feasible with a large scale commercial digester, these issues need to be addressed.

A commonly recognized challenge with the anaerobic digestion of microalgae is the overall low concentration of microalgae after harvesting (Ward et al., 2014). Centrifugation is a common method to concrete microalgae but it is an energy intensive method to do such. An alternate method would be to use a RABR system (Christenson and Sims, 2012) to collect biofilm forming microalgae. Biofilm forming microalgae could provide a way to concentrate the microalgae prior to digestion. Using a biofilm forming microalgae could enhance digester performance due to the high carbon content of extracellular matrix composed of polysaccharides, proteins and other cellular components (Sutherland, 2001). Using concentrated microalgae would increase the volatile solids content for a digester feed improving biomethane yield.

Another challenge with the anaerobic digestion of algae is the high ammonia toxicity the results from the degradation of algae. As proteins for the microalgae are degraded nitrogen starts to accumulate as ammonia causing toxicity to the digester (Sialve et al., 2009). Using microalgae to balance a high carbon wastewater could negate the toxicity by maintaining an appropriate carbon to nitrogen balance. Using microalgae as part of a blended wastewater feed could help to keep anaerobic digesters operating at maximum efficiency resulting in higher methane yields.

Reports of algae not being degraded after digestion (Golueke et al., 1957) have lead to the belief that pre-treatment of microalgae prior digestion is needed. Pre-treatment is a challenge that is often answered by utilizing high energy and high cost solutions. Pre-treatment techniques include: mechanical, ultrasound, microwave, chemical, thermal, and biological treatments (Rodriguez et al., 2015). Mechanical treatments break apart micro algae by grinding or mashing micro algae between two surfaces. Ultrasound treatments use high frequency sound waves to introduce a rapid change in pressure to disrupt cells. Microwave treatment increases the energy around the cells leading to generation of heat breaking apart cells. Thermal treatment disrupts hydrogen bonds weakening the cells. Chemical treatments utilize an acid, base or oxygen to degrade polymers allowing the cell to rupture. Biological methods use bacteria, fungi, or enzymatic activity to degrade cells. Biological methods can be done at lower temperatures but have a disadvantage in requiring longer time (10-14 days) to degrade microalgae (Rodriguez et al., 2015).

Wastewaters

The composition of methane produced from anaerobic digestion of numerous wastewaters and combinations thereof have been examined. Anaerobic digestion of pig manure has shown to produce between 68 and 73 percent methane (Fischer et. al, 2002). Dairy wastewater has shown to produce between 75 and 80 percent methane in a two-phase anaerobic digestion system (Ince, 1998). Municipal wastewater treated in a UASB has shown to produce 69 % methane (Barbosa and Sant'Anna, 1989). Petrochemical wastewater has been shown to produce between 70 and 75 percent methane by

Ramakrishna and Desai, 1997 where Guyot et. al, 1990 showed lower amounts of methane being generated, between 50.2 and 63.1 percent methane. The maximum percent of methane generated from thermal pretreatment of algae was shown to be between 70.6 % and 75.5 percent methane (Marsolek et. al, 2014). Anaerobically digested algae acquired from a municipal pond showed to have a composition between 59 and 61 percent methane (Salerno et. al, 2009). Most of these values fall below the commonly accepted range of methane composition reaching up to 75 percent methane volume per volume.

MATERIALS AND METHODS

A bio-methane potential assay (BMP) was used to monitor biogas production during the treatment of wastewaters using the inoculants describe previously. The content of biogas was analyzed weekly to determine composition of biogas being produced, including methane and carbon dioxide. This analysis shows how effective the treatment could be for bioenergy production by measuring the amount of carbon in the form of methane and the amount of carbon in the form of carbon dioxide. Treatments were carried out for a duration of 60 days or until the system stopped producing biogas. Wastewater organic chemical concentrations prior to and after treatment were measured to determine treatment effectiveness. The ability of the inoculants to decrease organic chemical concentration of wastewater was based on biological oxygen demand (BOD) chemical oxygen demand (COD), total organic carbon (TOC), and volatile solids (VS) concentrations.

It was hypothesized that facultative lagoon sediment would have greater algalytic ability due to its constant co-habitation with microalgae, thus the production of biogas would be improved over anaerobic digester sludge. Anaerobic digester sludge is under a smaller working volume and is constantly flushed with fresh wastewater. This constant flushing would wash out algalytic microbes if a stable population is not maintained by a healthy concentration of microalgae. Comparing the rate of biogas production, the total volume of biogas produced, and the resulting composition of biogas produced would reveal if one microbial community can outperform the other.

Triplicate treatments of each inoculant (FLS and ADS) treating each wastewater (dairy, swine, municipal, and petrochemical) were setup. Triplicate treatments were used to show the average of each parameter measured and the standard deviation for each measurement. To determine if one treatment was statistically significantly better, treatments were compared using single and two factor ANOVAs tables. Treatments were group based on inoculant and wastewater and a significant difference was determined based on a 0.05 alpha level.

Measuring the volume of biogas formed can be accomplished in several ways. The ways to measure biogas include: the volumetric displacement of water in a flask, the use of a manometer system, and capture of gas in a bag or syringe. A manometer system is an effective way to measure biogas production because of ease in measuring volume as water that is displaced and correlating pressure of the system to barometric pressure. As biogas is produced, the water that is displaced can be measured to determine the total volume of the system. By correlating the pressure of the system at the water level on a manometer, calculations can be made to determine the volume of the system at a standard pressure. An example of a simple manometer system is shown in Fig. 3.1. Treating multiple replicates of each wastewater provides a challenge for consistency. To increase consistency in measuring biogas formation, a 36 manometer style system was used to measure the formation of biogas by the displacement of water, as shown in Fig. 3.2. This system increased consistency by allowing more samples to be measured simultaneously and provided increased precision as water levels were better approximated using a reference grid.

The scaled up system included 36 treatment flasks connected to the top of 36 glass burette tubes filled with water. As volume of biogas increased, water was displaced out of the bottom of the treatment burette. The base of all treatment burettes were

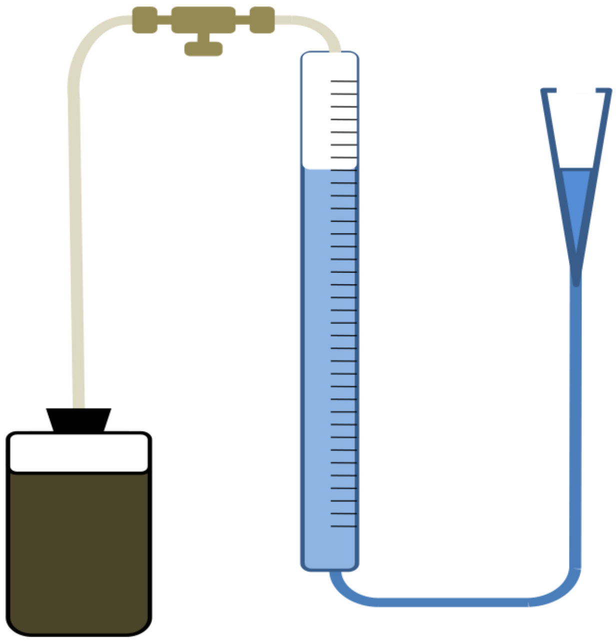

Fig. 3.1 Manometer style biogas measuring system with treatment connected to burette for biogas measurement and pressure equalizing reference.

Fig. 3.2 Image of 36 burette biogas monitoring system. Each burette has septum located at top to remove biogas produced.

connected to a common manifold and storage container for displaced water. To account for pressure of each flask and burette, a reference burette open to the atmosphere was also connected to the manifold parallel to the 36 treatment burettes. Positioning the overflow storage container at the same level as the reference burette provided a constant water level in the reference burette. As gas was produced, water was pushed down the treatment burette providing a measure of total system volume. The difference in height of water between the treatment burette and the reference burette provided a hydraulic head pressure. Combining the hydraulic pressure and atmospheric pressure together gave a total system pressure. With the calculated volume and pressure, the volume of gas was converted to standard pressure (1 atm) and biological standard temperature 25 degrees Celsius.

Volume measurements were taken by comparing the level of water in each treatment burette to a reference grid. Barometric pressures were recorded from National Weather Service station located at the Logan, Utah municipal airport (National Weather Service, 2016). BMP Measurements were taken every few days while production of gas was low, and then taken more frequently at high production times as need to keep volume of biogas below the maximum volume of the system.

Samples during treatment were placed in an insulated box on a shaker table as shown in Fig. 3.3. The shaker table was set at 80 rpm to provide continual mixing of samples. Temperatures of treated wastewaters were maintained at 30 degrees Celsius in an incubator using Campbell Scientific data logger as a control unit. Heat for the box was provided by the heat generated from the shaker table assisted by an incandescent light bulb when needed. When incubator temperature increased above 30 degrees Celsius, a

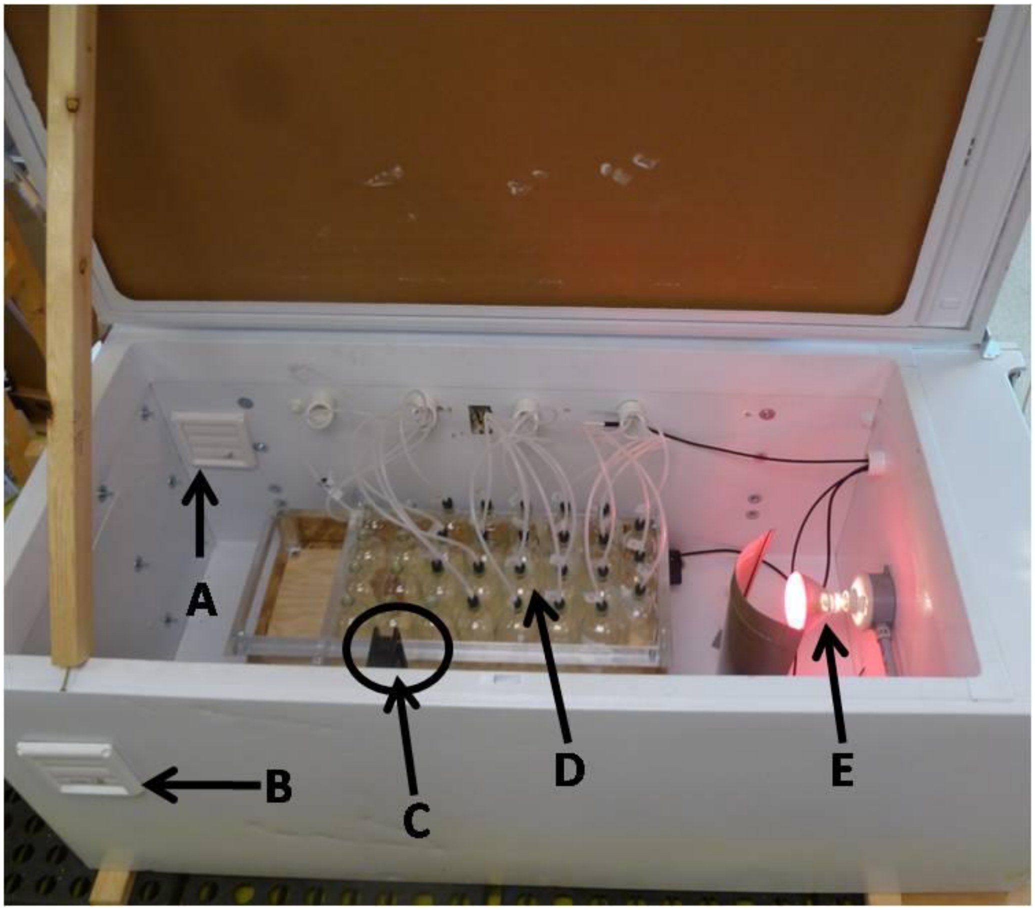

Fig. 3.3 Image of incubator used to maintain 30° Celsius environment with shaker table for mixing of samples during treatment. A) Cold air intake B) Warm air exhaust C) circulation fan to move air within incubator D) 500 ml glass sample bottles filled to 350 ml on shaker table E) Secondary heating element as shaker table provides some heat for the incubator.

fan turned on to move cool air into the insulated box and warm air outside of the box. Cooling fan and heating light bulb were both controlled by data logger that monitored the temperature of the Incubator.

The chosen inoculants for comparison of anaerobic digestion of wastewaters were Facultative Lagoon Sediment (FLS) and Anaerobic Digester Sludge (ADS). FLS was acquired from the City of Logan, Utah Lagoons municipal wastewater treatment plant pond C (Fig. 3.4). Samples were acquired from the bottom of the lagoons using an

Fig. 3.4 Location where Facultative Lagoon Sediment(FLS) was acquired from Logan City Municipal Wastewater Treatment Facility. FLS was removed from the bottom of pond C.

Eckman dredge. This location was chosen for the growth of mixed cultures of microalgae during spring, summer and fall seasons. This growth provides an environment where algalytic microbes could flourish. ADS was acquired from the Central Weber Sewage District anaerobic digester, located in Ogden, Utah. A total of five gallons was acquired from both sources and stored at 4 degrees Celsius. Characteristics of each inoculant is shown in Table 3.1

Four types of wastewater were selected for treatment comparison and for bioenergy production. These wastes were selected due to high volume of wastewater produced and the potential for treatment. The four wastewaters selected were dairy, swine, municipal, and industrial. Dairy wastewater was selected for its high volume of wastewater and potential for methane production. The Utah State University Caine Dairy

Table 3.1
Starting chemical oxygen demand (COD), total organic carbon (TOC), Biological oxygen demand (BOD), volatile solids (VS), and total nitrogen (TN) of inoculants, algae and wastewaters used for conversion to biomethane.

BMP Component	COD mg/l	TOC mg/l	BOD mg/l	VS mg/l	TN mg/l
Facultative Logan Sediment	48800	8730	247*	26200	1190
Anaerobic Digester Sludge	25600	4780	1220*	15500	2930
Microalgae	71300	25000	26400*	43200	6750
Dairy	6440	1810	484*	4100	93
Swine	14100	4020	2720*	8610	1650
Municipal	27700	3560	2580*	1360	1480
Petrochemical	187	27.9*	36*	377	26

* Values were determined from analysis done by Chemtech-Ford Laboratories.

was selected as a source of wastewater comparable to other dairy farms. Dairy wastewater was acquired from a lagoon style treatment pond. Swine wastewater was selected for the same reason as dairy and was acquired from Circle Four Farms, located in Southern Utah. This wastewater was taken from the surface of a lagoon treatment pond. Municipal wastewater was acquired from the South Davis Treatment Plant to represent the treatment of municipal wastewater. Sample was acquired at a location believed to be high in organic carbon. The industrial wastewater selected was petrochemical wastewater and was acquired from a local refining industry located in Woods Cross, Utah. This wastewater sample was taken before the wastewater was allowed to have a final biological treatment prior to discharge to local wastewater treatment plant. The characteristics of each wastewater are shown in Table 3.1. These wastewaters were selected for evaluation of treatment and resource recovery as methane using anaerobic digestion and the added inoculants described above.

Concentrated microalgae was acquired from algal raceways at the Logan, Utah

Facultative Lagoons treatment plant. These microalgae were initially frozen, but were moved to a 4 degrees Celsius refrigerator for storage. Characteristics of microalgae are shown in Table 3.1. Microalgae cultivated at the Logan Lagoons provided a good representation of microalgae that could be used in the feed stream of a large scale digester. Augmenting the wastewater feed stream with microalgae would increase the nitrogen content of the wastewater to provide a 25:1 molar carbon to nitrogen ratio (Soboh et al., 2016).

Balancing the nutrients in the feed wastewaters for treatment was accomplished by adding microalgae and sodium acetate with the wastewater as shown in Table 3.2. Knowing the starting amount of nitrogen in each wastewater, microalgae was added to raise the level of total nitrogen to 165 mg. With the nitrogen level fixed at 165 mg, sodium acetate was used to increase the total content of organic carbon to 3500 mg for each wastewater. The final carbon to nitrogen ratio of each wastewater was set to 25:1 molar ratio for each wastewater feed stream. Inoculants (FLS and ADS) containing 2000 mg of volatile solids were added to each nutrient augmented wastewater. Adding the inoculants to the wastewater feed steam changes the final carbon to nitrogen ratios of the

Table 3.2
Nutrient management showing how carbon and nitrogen were adjusted for different treatments.

C:N 25:1 Molar Ratio	**Wastewater**	**Microalgae**	**Sodium Acetate**	**Total**
Total Organic Carbon	Xc mg	Yc mg	Zc mg	3500 mg
Total Nitrogen	Xn mg	Yn mg	0 mg	165 mg

batch digestion, resulting in a molar ratio of 19:1 and 9:1 for FLS and ADS respectively. This was done to represent a C:N balanced wastewater feed stream being added to an inoculant during digester startup, providing an environment to measure digester startup time and initial biogas production rates. Augmented wastewaters combined with inoculants were made with triplicates for each treatment and were brought to a total volume of 350 ml using double distilled water as needed. Treatments were then mixed on a stir plate to make a homogeneous mixture. The pH was adjusted to 7.0 using drops of 1.0 N hydrochloric acid and 1.0 N sodium hydroxide as needed.

To remove oxygen, treatments were made anaerobic by one of two ways prior to placing samples in the incubator and monitoring the production of biogas. The first method was the use of the Coy anaerobic chamber for a minimum of 24 hours. This would provide a high nitrogen and oxygen free atmosphere to allow oxygen to be removed from samples via passive diffusion. An oxygen free environment is created as hydrogen gas is allowed to combine with free floating oxygen forming water in the presence of a palladium catalyst. As hydrogen gas was consumed to remove oxygen, additional hydrogen gas was added to ensure adequate levels of hydrogen present. The second method used to make the samples anaerobic by bubbling nitrogen through the wastewater while monitoring the dissolved oxygen. Nitrogen was bubbled through samples until a dissolved oxygen probe showed stabilized readings and no further reduction of dissolved oxygen was observed. Prior to attaching treatment flasks to the BMP system, hoses connected to each treatment were flushed with nitrogen. After system flush, the flasks were attached followed by another system flush. Multiple flushing of the system was done as a precaution to remove as much oxygen from the system as possible that may have entered while connecting sample bottles and hoses prior to starting treatment.

The formation of biogas (methane and carbon dioxide) was monitored immediately following the attachment of wastewater samples to the BMP system and continued throughout the duration of the treatment. Weekly samples of the biogas were taken and analyzed using the Agilent 7890B Gas Chromatography with a thermo conductivity detector and a pneumatic sampling valve. Ultra high purity helium was used as a carrier gas set at a constant pressure of 20 PSI. Contents of samples were separated using a HP Gas Pro column. Temperature of column was maintained at 25 degrees Celsius in an isothermal oven. The Analysis of biogas through gas chromatography allowed for separation of nitrogen, methane, and carbon dioxide to determine the percentage of each gas.

The final composition of biogas was determined two ways. The first method involved directly measuring the composition of biogas with gas chromatography as outlined above. Measurements using gas chromatography provided information on the composition of biogas on the specific days measured, but did not completely reflect the amount of biogas being produced. As the volume of the BMP system expanded to account for the biogas generated, the generated gas mixed with the previous composition of biogas. As the BMP system filled with biogas, there was a need to remove biogas from the BMP system to accommodate for generation of more biogas. With biogas being generated, mixed with previous contents, and a portion released, the composition of biogas would constantly be changing until it reached steady state. If the system were to reach steady state, gas chromatography would accurately represent the composition of biogas of the system and the composition of the biogas being generated. This assumption did not seem feasible during times of low biogas production. To compensate, a system of linear equations was developed to determine the average composition of biogas being generated based on the volume of biogas being produced and the initial and final concentrations of biogas. As the percent of biogas being generated may change between gas chromatography measurements, the calculated percent of biogas generated represents an average percentage of methane between weekly gas chromatography measurements.

A standard volume for the formation of biogas was calculated as shown in Equation 1, providing the formation of biogas in reference to standard pressure. The total system volume (TSV) accounted for the volume of flask, tubing to connect to burette to the flask, and the volume of the burette. For increased accuracy, burette volumes were measured according to the treatment burette water level (TBL) in mm, then multiplied by a burette conversion constant (BC) to convert from mm to ml. Pressure of the system included atmospheric pressure (ATM) plus the hydraulic head of water above the treatment burette water level. Hydraulic head is calculated using the difference in height between the treatment burette level (TBL) and the reference burette level (RBL) multiplied by density of water (rho) and the acceleration of gravity (g). Combining the pressure and volume equations lead to Equation 2. This equation consists of the standard volume of gas at a specific time point. The difference between two time points provide the volume of gas produced in that duration of time.

$$Standard\ Volume = \left(\frac{Volume\ of\ System * Pressure\ of\ System}{Standard\ Pressure}\right) \qquad \textbf{Eqn. 1}$$

$$Standard\ Volume = \left(\frac{(TBL * BC + TSV) * \left(ATM + rho * g * (TBL - RBL)\right)}{STDP}\right) \quad \textbf{Eqn. 2}$$

As biogas is produced and burettes are filled, there was a need to evacuate the gas or reset the system to capture more gas. Septum's were used to access gas for evacuation and sampling. Just prior to sampling and gas evacuation, measurements were taken for the final volume and pressure for that gas production window. Gases were removed and measurements were taken for the initial conditions of the next gas production duration. Biogas production was monitored until gas production approached zero or a duration of 60 days was met.

The total volume of biogas was monitored over the duration of treatment time providing a sigmoidal pattern for the cumulative total biogas produced and a left modal pattern for the rate of biogas produced. Treatments were compared for the maximum rate of biogas production, the time that the maximum rate occurred, the average rate of biogas production, the duration of average rate of biogas production, and the time required to initiate biogas production. To compare the time required for the system to produce biogas, a standardized start time was established. This start time was accounted for by the time at which biogas production passed 10 ml/day, just prior to maximum rate of biogas production. An average rate of biogas production and duration the system operated at the average rate was also calculated. Measurements were calculated by determining the slope of the total volume of biogas produced. Some treatments required longer duration to treat wastewaters thereby skewing measurement to have a shallower slope. To standardize the measurement, end points were truncated until a calculated slope had an R^2 value of 0.99 compared to collected data (Fig. 3.5). End points were systematically truncated, truncating the point furthest from the calculated average slope. After the calculated slope matched an R^2 value of 0.99 compared to collected data, the slope of the line was determined to be the average rate as shown in Fig. 3.5. The duration was calculated from the difference in time determined by the time at each end of the calculated slope. The average slope was calculated and used to compare treatments when the R^2 value of 0.99 was calculated and more than two points remained in the average slope.

Hach kits were used to determine the concentrations of total nitrogen (method 10072), TOC (method 10128), and COD (method 8000) following the manufacturer's directions except where noted. When measuring COD after treatment, due to small

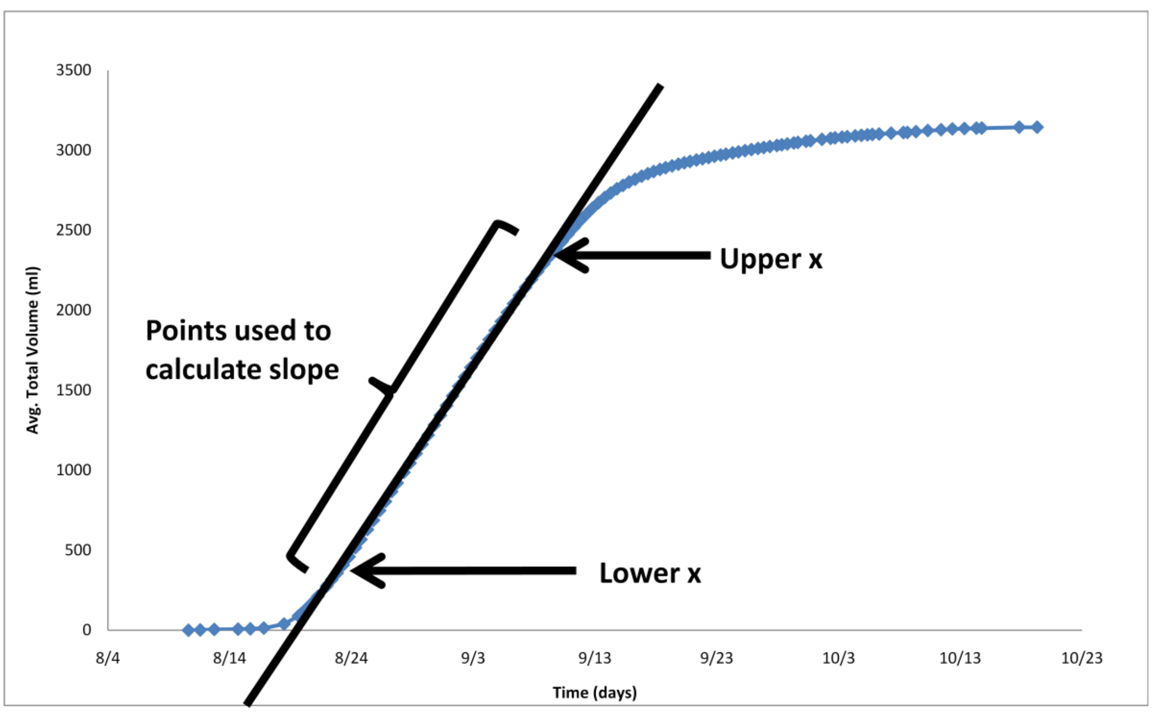

Fig. 3.5 Technique to find average rate of biogas production by narrowing window of points to a specific target value. This window from lower x to upper x represents the duration of the average slope.

volumes of waste, treatments were not blended for homogeneity. Samples were instead well mixed via vigorous shaking. Also due to small sample volumes and limited equipment, TOC sample preparation was changed from using 10 ml of sample on a stir plate, to 1 ml sample mixed in test tubes on a shaker table for the same duration of time. Volatile solids and total solids were measured according to Method 2540 as presented in Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Determination of BOD in samples were performed by Chemtech-Ford Lab, a certified lab located in Sandy, UT.

Three controls were used to determine the effects of the inoculant, acetate, and algae during each treatment. The use of distilled water as a control allowed for evaluation of the effects of inoculum and its associated organic matter during the treatment process. A control containing dairy wastewater without acetate was used as one treatment to assess the effects of acetate on the system, and control using dairy wastewater without microalgae was used to assess the effects of algae on the system as another treatment. Table 3.3 shows the contents of each sample and controls. Another control containing algae as the primary substrate for digestion was treated with the three primary controls. The pure algae control had a period of time during maximum biogas production where the biogas could not be collected. Last minute attempts were made to collect biogas by increasing the maximum volume of the system. This attempt resulted in biogas leaking and inaccurate measurements taken. As a result the data collected was not included for comparison, but is listed in the appendix for reference and for future study.

Table 3.3.
Content of each sample treated and controls. Each sample contained wastewater augmented with acetate, algae, and inoculant. Controls were setup the same as the dairy sample but lacked one constituent as noted.

Sample Name	Dairy	Swine	Municipal	Petrochemical	No Acetate	No Algae	Inoculum
Wastewater	Dairy	Swine	Municipal	Petrochemical	Dairy	Dairy	-----
Acetate	Yes	Yes	Yes	Yes	-----	Yes	-----
Algae	Yes	Yes	Yes	Yes	Yes	-----	-----
Inoculant	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Water	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BIOGAS PRODUCTION: VOLUMES AND RATES

Introduction

The volumes and rates of biogas produced were examined to compare the differences between inoculant treatments and individual augmented wastewaters treated, which included dairy, swine, municipal and petrochemical wastewaters. The first comparison was made to assess the ability of each inoculant to digest the four augmented wastewaters. The second comparison was made to assess total volume of biogas produced and the rate of biogas production from each wastewater treated by each inoculant. Comparisons between wastewaters were based on the mean value of each triplicate treatment. Comparisons were also examined by identifying specific differences between inoculants with individual wastewaters. The duration of biomethane potential tests was set to a 60 day time scale. This time duration was chosen because every wastewater sample had passed through its maximum biogas production, and many of the samples had ceased to produce biogas.

Wastewater Cross Comparison of Rates and Volumes

The first comparison was made by examining the average total biogas produced from each wastewater with its respective inoculant treatment. All four augmented wastewaters were successfully digested using facultative lagoon sediment (FLS) (Fig. 4.1). The average volumes produced by FLS were within 107 ml of the overall combined treatment mean of 3,060 ml of total biogas. Anaerobic digester sludge (ADS) was also successful in digesting each augmented wastewater as shown in Fig. 4.2. Anaerobic digester sludge showed a greater deviation as the average value of each triplicate had a

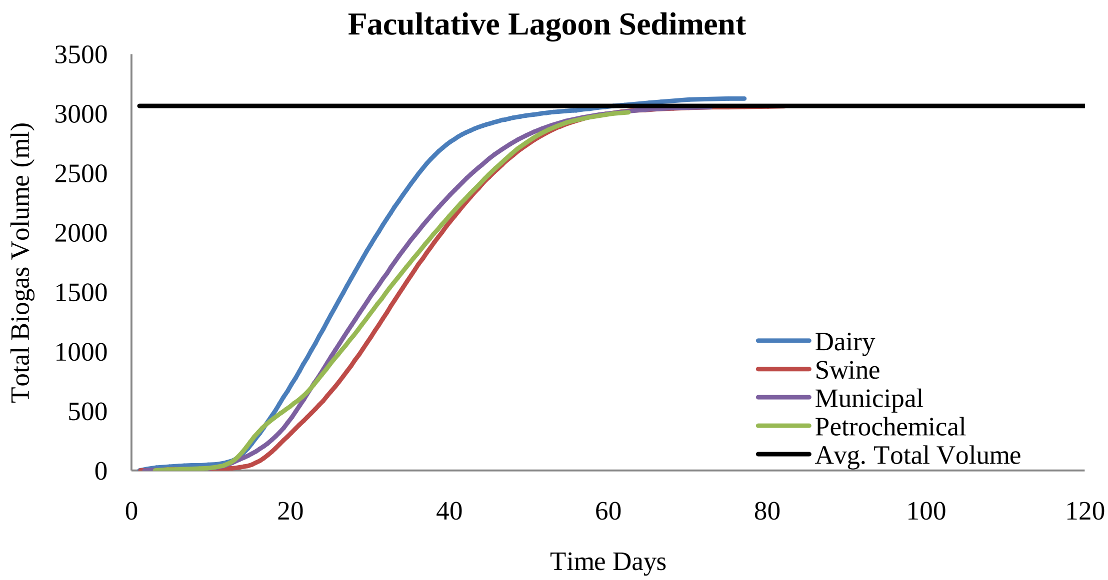

Fig. 4.1 Cumulative average total volume of biogas produced with Facultative Lagoon Sediment (FLS) treatment. Augmented wastewaters included dairy, swine, petrochemical, and municipal. Each plot is an average of 3 replications. Horizontal line is the average total volume produced by all FLS treatments (3060 ml).

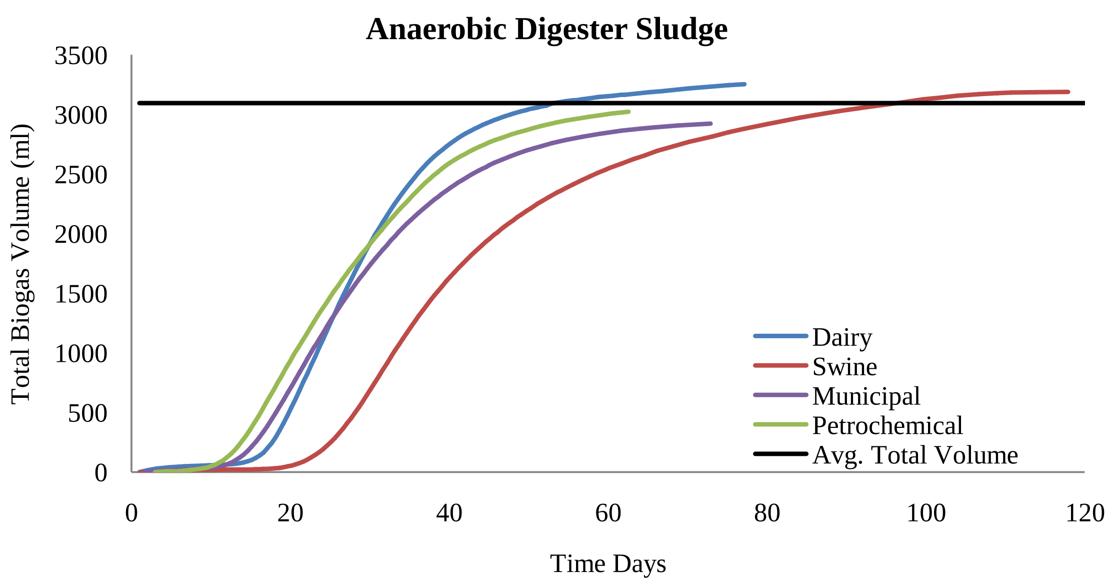

Fig. 4.2 Cumulative average total volume of biogas produced with Anaerobic Digester Sludge (ADS) treatment. Augmented wastewaters included dairy, swine, petrochemical, and municipal. Each plot is an average of 3 replications. Horizontal line is the average total volume produced by all ADS treatments (3100 ml).

deviation of 195 ml from overall combined treatment mean value of 3,100 ml of total biogas. Both treatments showed the ability to digest augmented wastewaters, but neither treatment was significantly greater than the other when each treatment was given a sufficient amount of time to digest the waste. One unusual characteristic observed in Fig. 4.2 was that treating augmented swine wastewater with anaerobic digester sludge produced a curve that lagged more than any other wastewater inoculant combination. Using a 60 day constraint for treatment, a significant difference was found between FLS and ADS. On a 60 day time period, FLS produced a mean value of 3,020 ml of biogas with a deviation 91 ml where ADS produced 2,900 ml of biogas with a deviation of 270 ml. Under this time constraints FLS produced more biogas on average than ADS. With such a small difference measured in total biogas production between treatments the difference in biogas production may not be apparent in a full scale treatment plant.

Some similarities were observed between the average rates of biogas produced by each wastewater. Fig. 4.3 and 4.4 shows the rate of biogas being produced for FLS and ADS, respectively. Each Fig. shows the maximum rate of biogas produced, the time of peak production, and the time of initial biogas production.

Initial production was defined by the first time point prior to maximum production that a rate of biogas greater than 10 ml/day was measured. A significant difference was observed between times of initial biogas production with FLS showing a faster starting time. FLS showed a mean start time at 12.5 ± 1.3 days and ADS mean value of 14.0 ± 4.5 days.

Two rate measurements with corresponding time measurements were determined. Maximum rate of biogas was determined by the peak rate during treatment. An average

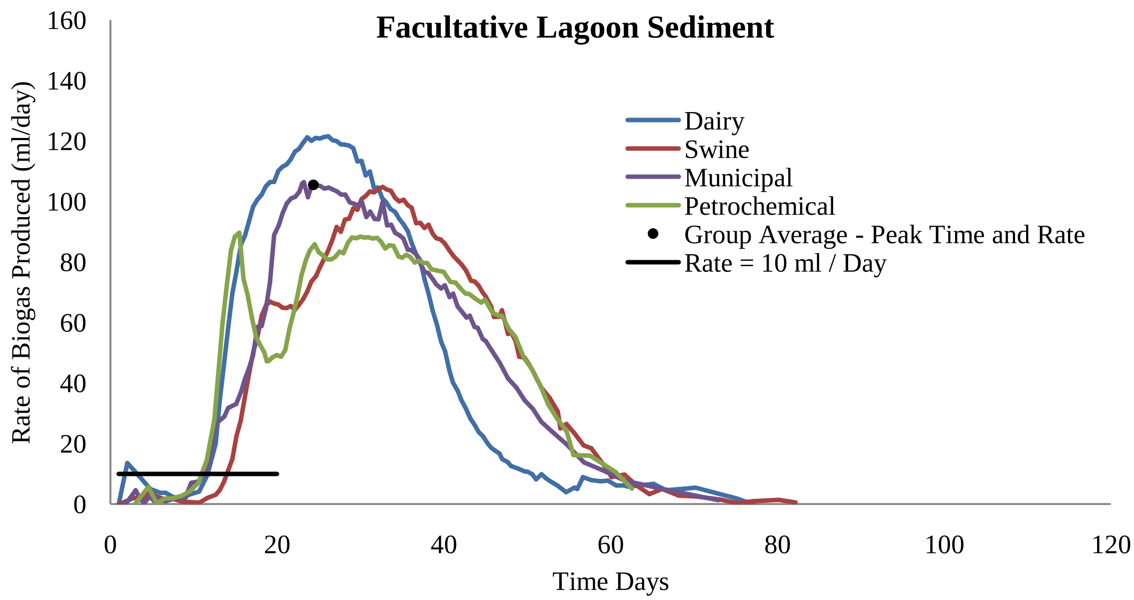

Fig. 4.3 Average rate of total biogas produced by augmented wastewaters during treatment with FLS. Augmented wastewaters included dairy, swine, municipal, and petrochemical. Each plot is an average of 3 replicates. Horizontal line depicts a rate of 10 ml / day for 20 days as reference for start production. Point indicates FLS averaged peak rate and time.

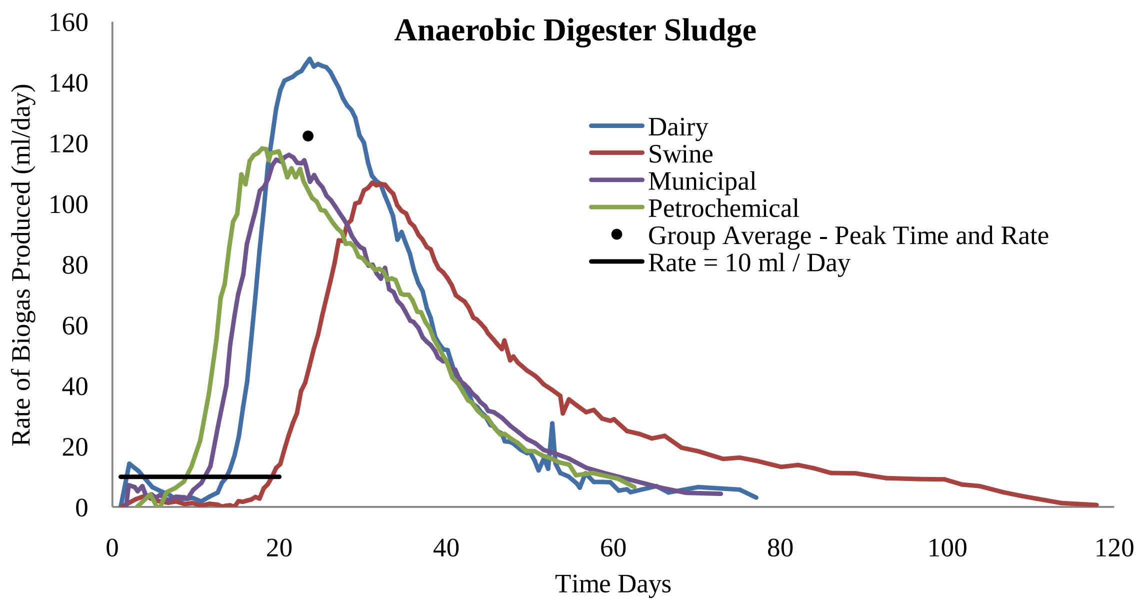

Fig. 4.4 Average rate of total biogas produced by augmented wastewaters during treatment with ADS. Augmented wastewaters included dairy, swine, municipal, and petrochemical. Each plot is an average of 3 replicates. Horizontal line depicts a rate of 10 ml / day for 20 days as reference for start production. Point indicates ADS averaged peak rate and time.

rate was calculated as describe with Fig. 3.5. A significant difference between FLS and ADS was found in the maximum rate of biogas production with ADS out performing FLS. FLS showed a mean peak production of 107 ± 11 ml/day where ADS showed a mean peak production of 124 ± 15 ml/day respectively. The average time that peak production was measured for FLS and ADS was 25.7 ± 0.9 days and 23.8 ± 4.8 days. No significant difference was found between the times when peak biogas production occurred.

FLS showed a calculated average rate at 84 ± 11.5 ml/day while ADS showed a calculated average rate of 96 ± 13.7 ml/day. ADS showed a significantly higher average rate of biogas production. Where FLS had lower rates of biogas production compared to ADS, FLS had a longer time period at which it operated at the calculated average rate. This was first observed in Fig. 4.3 as FLS appears to have a broader base and a steeper decrease in biogas production compared to ADS in Fig. 4.4. This observation was confirmed when the duration of the average rate was calculated to an R^2 value of 0.99 showing that the duration at which FLS operated at the average rate was significantly longer. FLS operated at the average rate for 41.4 ± 6.7 days where ADS only maintained an average rate for 28.9 ± 2.7 days. It is hypothesized that FLS is better able to maintain steady biogas production under changing concentrations of substrate. As change in substrate was not a focus of this study, future work is needed to address this hypothesis.

Treatments reached a common low in biogas production at approximately 60 days, as seen in Fig. 4.3 and 4.4, with one exception. Augmented swine wastewater treated with ADS doesn't reach the same low level of biogas production. Another 40 days were required for digestion. In Fig. 4.3 dairy wastewater also appears to be unique by dropping in rate earlier than the other FLS treated wastewaters.

Dairy wastewater - Rate and Volume

Augmented dairy wastewater was treated well with both FLS and ADS. Fig. 4.5A and 4.5C show total biogas produced for treatment by FLS and ADS, respectively. Both FLS and ADS treatments showed little difference between total biogas production. The average total biogas produced for FLS was 3060 ± 134 ml, and 3150 ± 149 ml for ADS with no statistical difference between treatments for total biogas production. This result can be seen in Fig. 4.5E where both treatments by FLS and ADS overlap at the end of treatment.

Triplicate biogas production rate values are shown in Fig. 4.5B and 4.5D for FLS and ADS respectively. Triplicate values of dairy wastewater treated with FLS and ADS were uniform in pattern providing a good representation for augmented dairy wastewater as shown with the small standard deviation in Fig. 4.5E and 4.5F. FLS showed a significantly smaller time required to reach a biogas production rate of 10 ml/day compared to ADS. FLS reached a production of 10 ml/day at an average time of 12.3 ± 0.6 days where ADS required an average of 14.1 ± 0.0 days.

The maximum rate for augmented dairy wastewater was found to be significantly better with ADS. FLS produced biogas at a rate of 122 ± 6.8 ml/day where ADS produced biogas faster at a rate of 148 ± 1.6 ml/day. ADS not only produced biogas faster, but also produced it sooner. The average FLS peak production time was found to be at 25.6 ± 0.9 days where the time was 23.5 ± 0.3 days by ADS. FLS adjusted to the augmented dairy wastewater quicker by starting to produce biogas faster, but this head start was not enough to reach a maximum rate as fast as ADS.

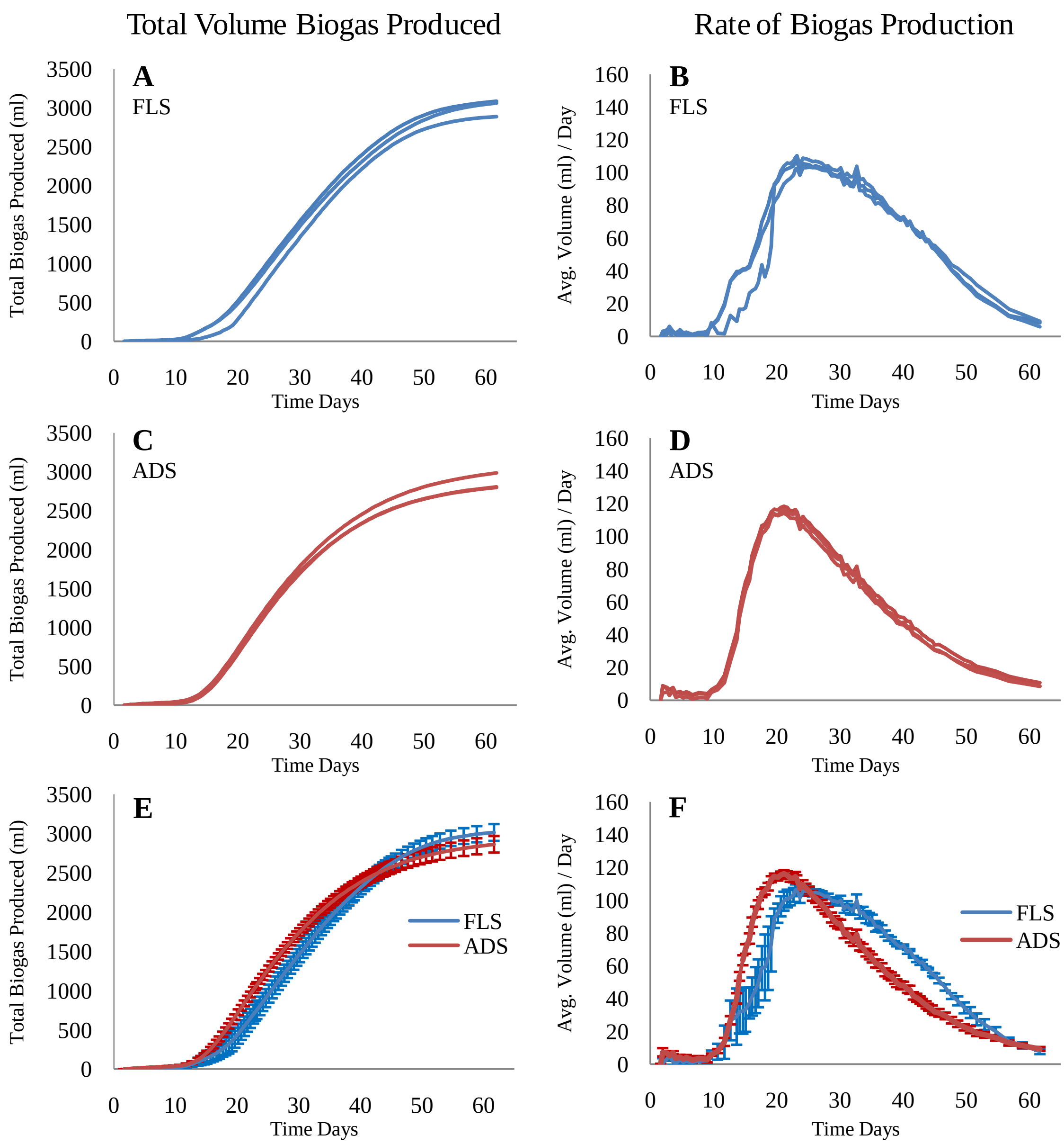

Fig. 4.5 Dairy Wastewater Total volume and rate of biogas produced from Facultative Lagoon Sediment (FLS) and Anaerobic Digester Sludge (ADS) as inoculants for treatment of augmented dairy waste. A) Triplicates of total biogas produced using FLS. B) Triplicates plots with the rate of biogas produced using FLS. C) Triplicates of total biogas produced using ADS. D) Triplicates plots with the rate of biogas produced using ADS. E) Average of total biogas produced from triplicate treatments of FLS and ADS. F) The average rate of biogas produced from triplicate treatments using FLS and ADS as inoculant source. All error bars represent one standard deviation.

The maximum rate of biogas production from dairy wastewater was greatest for treatment with ADS. FLS showed a slower overall average rate of biogas being produced at 100.0 ± 5.6 ml/day compared to ADS that produced 17.6 ml/day faster at 117.6 ± 0.8 ml/day. Although FLS has a slower overall average rate, it makes up for this by maintaining that rate for longer duration of time. FLS was able to maintain an average rate for 33.4 ± 1.0 days where ADS maintained an average rate for 25.9 ± 0.8 days.

Swine wastewater - Rate and Volume

Augmented Swine wastewater was unique in a few aspects. In 60 days, there was a significantly greater volume of biogas produced with FLS. FLS produced a total volume of 3000 ± 13 ml of biogas while ADS produced 2550 ± 159 ml of biogas. The volumes of biogas produced by triplicate treatments with FLS were almost identical as shown by Fig. 4.6A. This similarity between triplicates was not seen in augmented swine wastewater being treated with ADS. One replicate started producing biogas faster than the other two replicates as shown in Fig. 4.6C. This difference accounts for the greater standard deviation in ADS compared to FLS in Fig. 4.6E. Though one treatment by ADS started faster than the other two treatments, overall FLS was still significantly faster at initiating biogas production. FLS reached a production rate of 10 ml/day at day 14.3 ± 0.8 days where ADS took 20.8 ± 2.4 days to reach this value.

The rate of biogas produced with augmented swine wastewater showed a stabilization or slight decrease in rate of biogas production starting day 18 for FLS. This short change in rate at day 18 (Fig. 4.6B) was not seen in treatments by ADS (Fig. 4.6F). This brief stabilization in rate may be caused by a metabolic change in the methanogenic archaea with the FLS treatment.

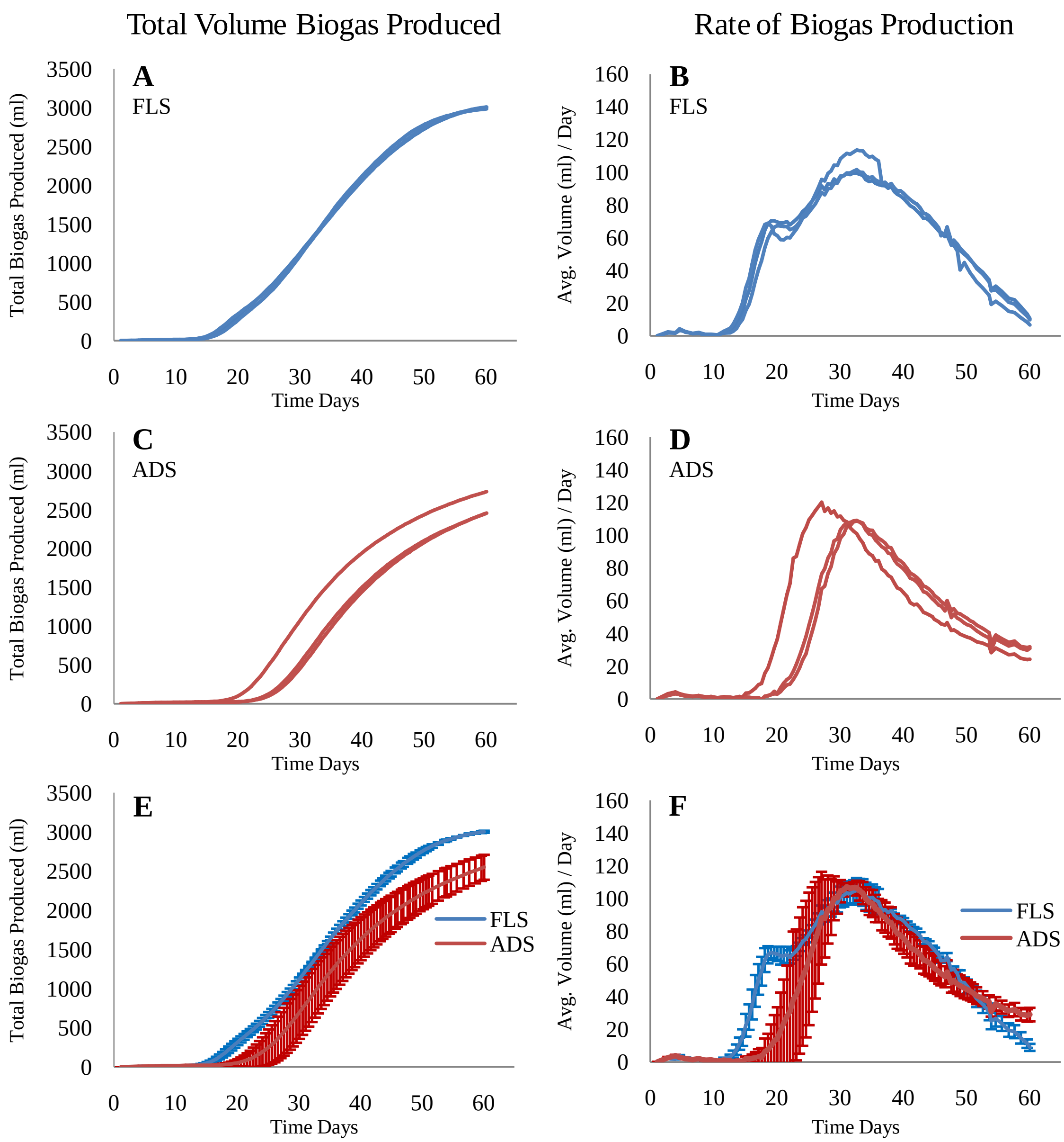

Fig. 4.6 Swine Wastewater Total volume and rate of biogas produced from Facultative Lagoon Sediment (FLS) and Anaerobic Digester Sludge (ADS) as inoculants for treatment of augmented swine waste. A) Triplicates of total biogas produced using FLS. B) Triplicates plots with the rate of biogas produced using FLS. C) Triplicates of total biogas produced using ADS. D) Triplicates plots with the rate of biogas produced using ADS. E) Average of total biogas produced from triplicate treatments of FLS and ADS. F) The average rate of biogas produced from triplicate treatments using FLS and ADS as inoculant source. All error bars represent one standard deviation.

Similar maximum rates observed from FLS and ADS in Fig. 4.6F resulted in no significant difference between treatments with the maximum rate of biogas being produced. FLS showed a maximum rate of 105 ± 7.6 ml/day while ADS showed a maximum rate of 113 ± 6.6 ml/day. Also there was not a significant difference found between the times of maximum rate or the calculated average rate. FLS showed maximum rate at 32.5 ± 0.3 days and the calculated average rate to be 80.0 ± 4.3 ml/day. ADS showed the maximum rate at 30.8 ± 3.2 days and a calculated average rate of 84.6 ± 4.6 ml/day. There was a significant difference found in the duration of the average rate. Like augmented dairy waste, FLS maintained a longer duration of average rate of biogas production of 43.1 ± 6.7 days, while the average rate for ADS lasted 28.3 ± 0.8 days.

Municipal wastewater - Rate and Volume

Little deviation was found in total biogas production from municipal wastewater within triplicate treatments by FLS and ADS as shown Fig. 4.7A and 4.7C. The mean total biogas produced from FLS augmented wastewater (3014 ± 108 ml) was not significantly different from the biogas produced in ADS augmented wastewater (2863 ± 159 ml) (Fig. 4.7E). Fig. 4.7B and 4.7D show the rate of biogas production for FLS and ADS. The rate of biogas production was consistently increasing prior to reaching the maximum rate when treated with ADS, while FLS treatment showed a short stabilization of rate observed at day 15. This small change in rate as shown in Fig. 4.7B is similar to that observed with dairy (Fig. 4.5B) but not as pronounced. Neither FLS nor ADS showed a significant difference in biogas production as measured by a 10 ml/day starting rate. FLS reached 10 ml/day at day 11.7 ± 1.0, while ADS required 11.8 ± 0.0 days, almost identical values between FLS and ADS. The slight difference in rate

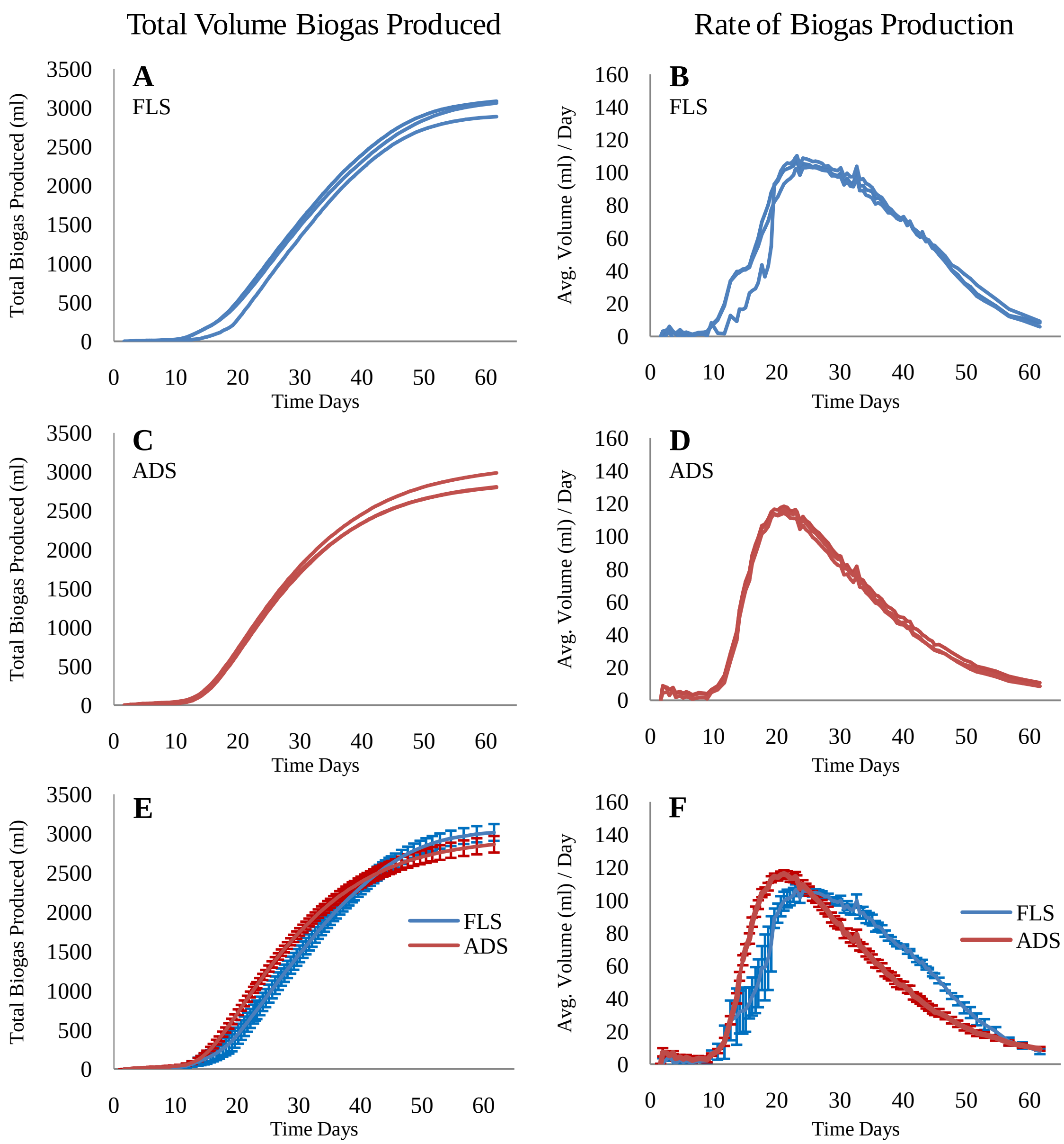

Fig. 4.7 Municipal Wastewater Total volume and rate of biogas produced from Facultative Lagoon Sediment (FLS) and Anaerobic Digester Sludge (ADS) as inoculants for treatment of augmented municipal waste. A) Triplicates of total biogas produced using FLS. B) Triplicates plots with the rate of biogas produced using FLS. C) Triplicates of total biogas produced using ADS. D) Triplicates plots with the rate of biogas produced using ADS. E) Average of total biogas produced from triplicate treatments of FLS and ADS. F) The average rate of biogas produced from triplicate treatments using FLS and ADS as inoculant source. All error bars represent one standard deviation.

of biogas observed in one replicate with FLS may be accounted for by a loose fitting on the biogas collection apparatus. Fittings to collect and store biogas were re-tightened at day 19, resulting in triplicates rates aligning closer together. This possible leak in the system may account for the slightly slower starting time observed with one of the FLS triplicates.

A significant difference was found with the maximum rate of biogas production measured at 107 ± 3.5 ml/day for FLS and 116 ± 2.2 ml/day for ADS. This difference in maximum rate can be seen in Fig. 4.7F as FLS showed a lower maximum value than ADS. The time for FLS to reach the maximum rate took significantly longer than the time for ADS to reach the maximum rate. FLS reaching a maximum rate on day 23.8 ± 1.2 compared to ADS that reached a maximum rate 2.6 days sooner on day 21.8 ± 0.0. The maximum rate of biogas produced with municipal wastewater was larger and quicker when treated with ADS. FLS showed an average rate of 84.6 ± 2.6 ml/day where ADS showed a significantly greater average rate of biogas production at 90.9 ± 1.6 ml/day. As seen with other wastewaters, augmented municipal wastewater treated with FLS maintained the average rate for a duration of time significantly longer than wastewaters treated with ADS. FLS maintained an average rate 10.1 days longer than ADS. FLS maintained an average rate for 39.0 ± 2.6 days while ADS was able to maintain the average rate for 28.9 ± 0.5 days.

Petrochemical wastewater - Rate and Volume

Augmented petrochemical wastewater showed some unique results. Fig. 4.8A and 4.8C show a plot of total biogas produced for FLS and ADS. It was observed that there was little deviation in triplicates as shown in Fig. 4.8A. Analysis of the total

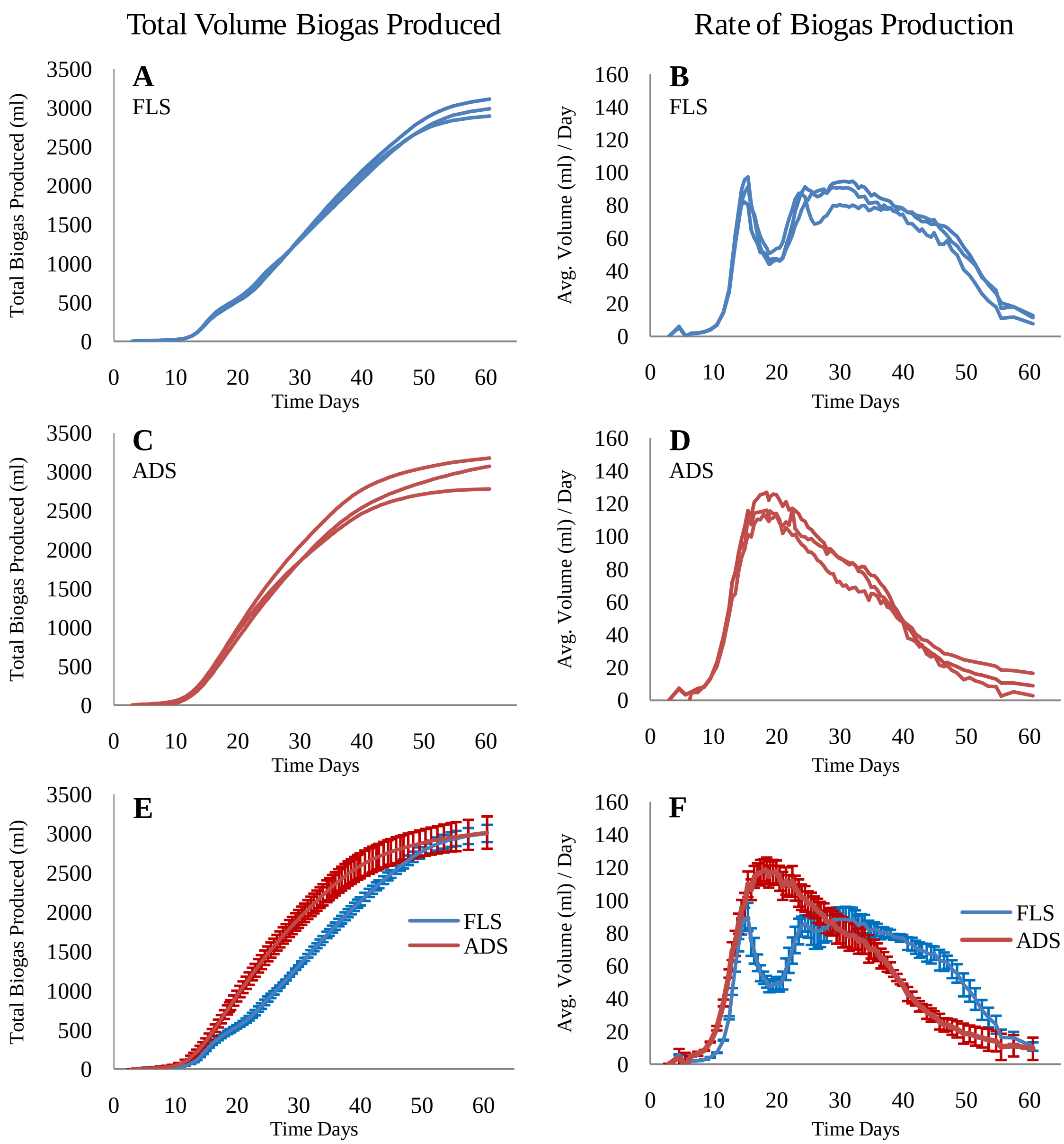

Fig. 4.8 Petrochemical Wastewater Total volume and rate of biogas produced from Facultative Lagoon Sediment (FLS) and Anaerobic Digester Sludge (ADS) as inoculants for treatment of augmented petrochemical waste. A) Triplicates of total biogas produced using FLS. B) Triplicates plots with the rate of biogas produced using FLS. C) Triplicates of total biogas produced using ADS. D) Triplicates plots with the rate of biogas produced using ADS. E) Average of total biogas produced from triplicate treatments of FLS and ADS. F) The average rate of biogas produced from triplicate treatments using FLS and ADS as inoculant source. All error bars represent one standard deviation.

biogas production showed no significant difference between the treatments. This similarity is observed in Fig. 4.8E. The total biogas produced by both treatments was closer than any other augmented wastewater with only a difference of 12 ml in 60 days of measurements. FLS produced 3000 ± 109 ml of biogas in 60 days where ADS produced 3012 ± 206 ml.

Augmented petrochemical wastewater was different than any other augmented wastewater by being the only wastewater treated with ADS having a significant difference in start time as measured by a rate of 10 ml/day. FLS was two days slower at reaching 10 ml/day starting on day 11.5 ± 0.0 where ADS started on day 9.5 ± 0.0.

A difference in treatments was observed with the rate of biogas produced from augmented petrochemical waste. FLS treating augmented dairy wastewater and municipal wastewater each had a time where the rate was constant for a brief time (Fig. 4.6B and 4.7B). Similar to dairy and municipal wastewaters, petrochemical wastewater did not continue to increase in rate of biogas production until a maximum rate was achieved. Petrochemical wastewater was different in that biogas production started to decrease for a time and then increase to a maximum value or near maximum value. This decrease in rate was hypothesized to be a metabolic shift and acclimation. The change in rate observed with augmented petrochemical wastewater being treated with FLS was different in that it was the only wastewater to have a steep decrease in rate of biogas production followed by a steep increase in production as observed in Fig. 4.8B. This decrease is believed to be caused by a metabolic shift. Like all of the other wastewaters compared, ADS showed a greater maximum rate on average being 25.4 ml/day faster. FLS reached a maximum rate of 94.4 ± 3.0 ml/day while ADS reached a rate of 119.8 ±

6.1 ml/day.

The decrease in rate observed with FLS affected the time that the maximum rate occurred. Two of the triplicates treated with FLS reached a maximum rate at day 16, and the third triplicate reached a maximum rate at day 32. This difference resulted in FLS having an average maximum rate on day 21.0 ± 9.6 where ADS was earlier on day 19.8 ± 2.3. With such a large standard deviation found in the rate of biogas production by FLS, the decision was made to calculate times based on maximum rate prior to the temporary drop in biogas production, and after the drop in biogas production. FLS had a maximum rate on day 15.3 ± 0.3 when the maximum rate of biogas occurred prior to the temporary drop in biogas production. After the drop in biogas production, the maximum rate of biogas production was observed on day 28.1 ± 4.3. Both times before and after the temporary decrease in biogas production was significantly different than the times measured with ADS. It is unclear if FLS or ADS has an earlier maximum rate start time due to the inconsistency caused by the temporary decrease in rate of biogas.

The calculated overall average rate of biogas production with augmented petrochemical wastewater was significantly greater in treatments with ADS. FLS inoculated wastewater showed an overall average rate of 71.1 ± 2.7 ml/day where ADS had an overall average rate of 90.9 ± 5.0 ml/day.

While ADS demonstrated a faster rate of biogas production, FLS is able to maintain its average rate over a longer period of time. FLS maintained an average rate of biogas production over 50.0 ± 2.0 days while ADS was only able to maintain this rate for 32.4 ± 2.3 days. FLS augmented petrochemical wastewater maintained the overall average rate longer than any other wastewater being treated with FLS or ADS. This is seen when the plot of total gas produced with FLS treatment has longer duration in the linear region (Fig. 4.8A) than the typical sigmoidal curve observed with the other treatments. The extended duration is also observed in Fig. 4.8F, with FLS maintaining a greater rate of biogas production for a longer period of time than ADS.

Conclusion

FLS showed a quicker start time in biogas production, as well as biogas production for a greater amount of time in all wastewaters. ADS performed better with a higher maximum rate of biogas production and an overall higher average rate. Both inoculants performed equally well in producing the same amount of biogas when given a sufficient amount of time. FLS performed better than ADS treatment with regard to seven wastewater parameters for individual wastewaters where ADS performed better with respect to nine wastewater parameters. The significant differences in individual augmented wastewaters as well as a combined effect can be seen in Fig. 4.9.

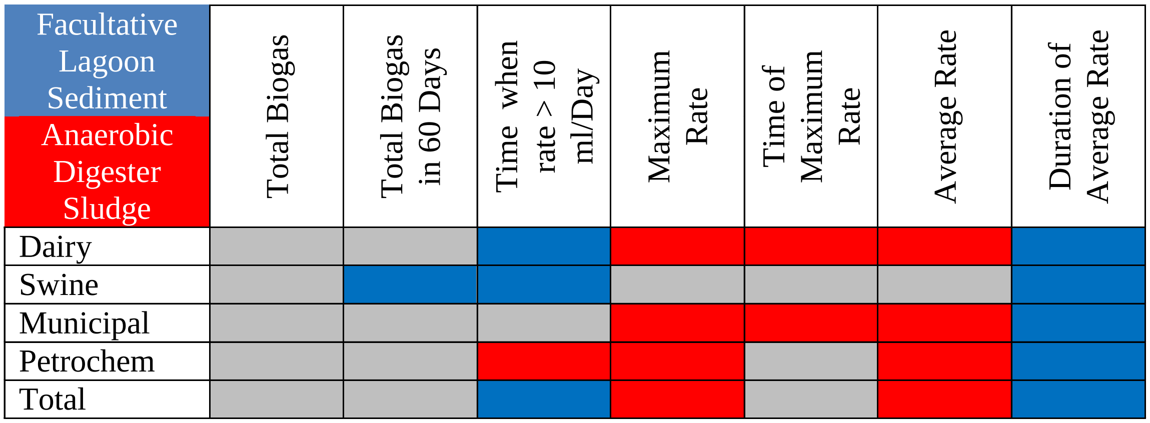

Fig. 4.9 Visual representations of significant rate and volume measurements for treatment of dairy, swine, municipal, and petrochemical wastes by Facultative Lagoon Sediment (FLS) and Anaerobic Digestive Sludge (ADS). Non significant findings are marked in gray, FLS significant findings are marked in blue and ADS significant findings are marked in red. Bottom row (Total) whether significance was found basted on all four wastewaters treated.

YIELD AND COMPOSITION OF BIOGAS WITH CHANGE IN ORGANIC LOADING

Introduction

The yield and composition of biogas were monitored throughout treatment of augmented wastewaters to determine bioenergy (methane) production and treatability. Composition of biogas was measured by gas chromatography and the results were used to calculate average rate of methane being generated as well as percent of methane, carbon dioxide, and nitrogen in the biogas. The amount of methane and carbon dioxide accounts for the total carbon in the biogas. Total carbon in the wastewater was accounted for directly and indirectly through volatile solids (VS), biological oxygen demand (BOD), total organic carbon (TOC) and the chemical oxygen demand (COD) prior to treatment and post treatment. The change of organic chemicals in the wastewater resulting from treatment and the amount of methane produced per change in organic carbon provide twelve parameters for treatment comparison. Each parameter addressed the treatability of each wastewater by facultative lagoon sediment (FLS) and anaerobic digester sludge (ADS). Treatability was also assessed by grouping all augmented wastewaters by treatment and determining if a significant difference exists between combined wastewater treatments groups.

Compositions and Yield

The composition of biogas in each BMP treatment was measured weekly using gas chromatography. Fig. 5.1 shows a bar graph of the maximum percentage of methane observed from each wastewater by each treatment. Each wastewater reached a maximum percentage of methane in the range of 82 to 86% methane. Augmented dairy wastewater was the only wastewater to show a significant difference in maximum percentage of methane measured. FLS produced 84.4 ± 0.8% methane, where ADS produced a maximum value of 82.6 ± 0.4% methane. A significant difference was also found in the combined wastewater group where FLS produced a higher maximum percentage of methane. FLS had a mean value of 85.2 ± 0.8% methane where ADS had a maximum value of 84.2 ± 1.3%. Municipal wastewater showed the highest methane composition at 85.96% methane with FLS.

A bar graph was created to compare the highest average percent of methane generated (Fig. 5.2). Augmented municipal wastewater was the only wastewater to show a significant difference between treatments. FLS showed a maximum percent of methane being generated at 89.2 ± 0.4%, where ADS showed 86.9 ± 0.1% methane. All other treated wastewaters had a higher maximum percentage of methane being produced with FLS, though none were statistically greater than the percentage of methane generated by treatment with ADS. FLS had a significantly higher percentage of methane being produced regardless of the type of wastewater treated. The combined treatment group showed FLS treatment producing a maximum percent of methane being generated at 87.3 ± 1.7% where ADS showed a lower percentage at 85.8 ± 1.5%.

No significant difference was found in the mass of methane produced from each treatment. This is observed when comparing wastewater treatments in Fig. 5.3. FLS treatment produced more methane with municipal and petrochemical wastewaters where ADS produced more methane with dairy and swine wastewaters. While FLS and ADS had instances where each produced more methane than the other, no significant

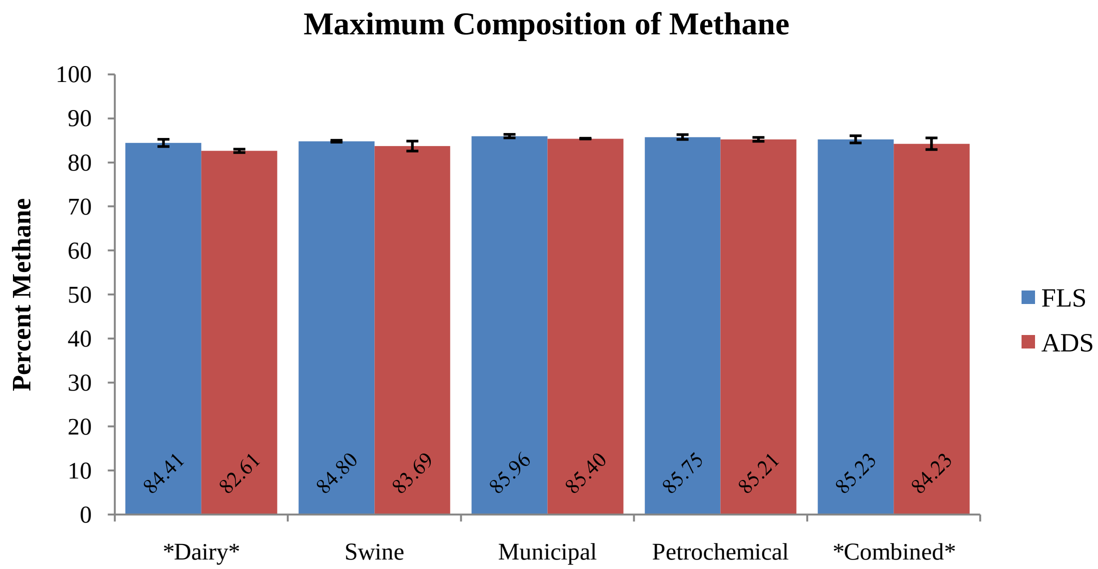

Fig. 5.1 Average maximum composition of biogas measured from triplicate treatments of four augmented wastewaters. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Dairy*) indicate a significant difference between treatments was measured.

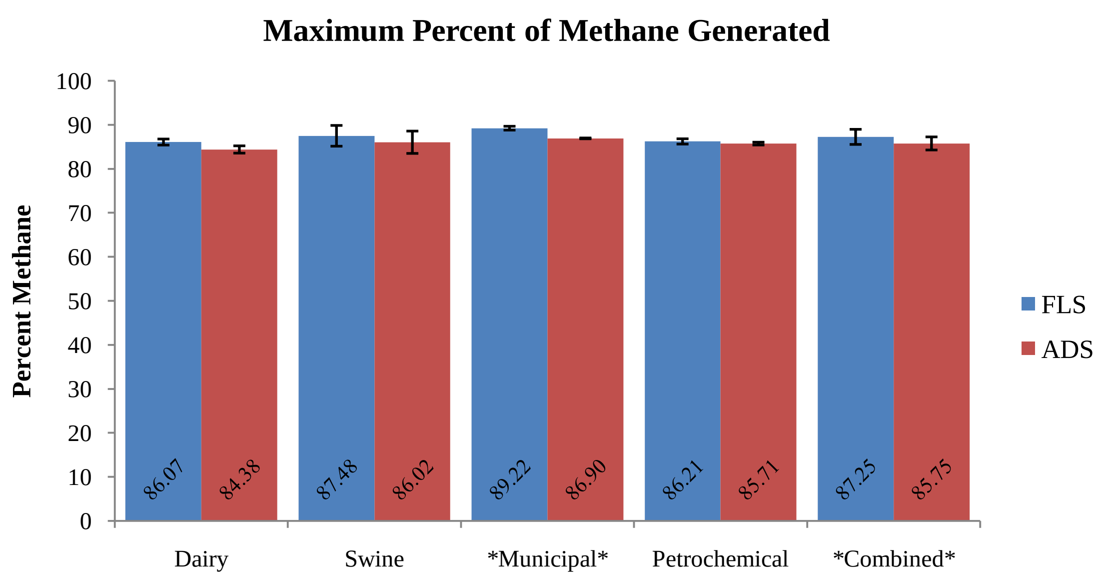

Fig. 5.2 Average values for maximum percent of methane generated from triplicate treatments of four augmented wastewaters. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Municipal*) indicate a significant difference between treatments was measured.

difference was found in the amount of methane produced by either treatment.

The ratio of methane to carbon dioxide (Volume:Volume) was significantly higher for FLS in every treatment observed. Fig. 5.4 shows a comparison of each wastewater treatment and treatment as a combined group. FLS treating augmented dairy wastewater produced 0.92 ml more methane per ml carbon dioxide than ADS. FLS produced a ratio of 7.38 ± 0.1 methane to carbon dioxide when dairy wastewater was treated, where ADS produced a ratio of 6.47 ± 0.2. Augmented swine wastewater showed the largest methane to carbon dioxide ratio of the treated wastewaters. FLS produced a ratio of 10.65 ± 0.1 methane to carbon dioxide where ADS produced a ratio of 9.52 ± 0.3. FLS was able to produce 1.13 more methane per carbon dioxide than ADS. Augmented municipal wastewater showed a methane to carbon dioxide ratio in between dairy and swine wastewaters. FLS showed a ratio of 9.75 ± 0.3 where ADS produced a ratio of 7.65 ± 0.2. Augmented municipal wastewater showed the biggest difference between treatments with FLS producing 2.1 more methane per carbon dioxide compared to ADS. The methane to carbon dioxide ratio with augmented petrochemical wastewater showed that FLS out performed ADS by 1.43 moles of methane per mole of carbon dioxide. This is the second highest difference measured having a greater difference than found with dairy and swine wastewaters. Augmented petrochemical wastewater treated with FLS produced a ratio of 8.82 ± 0.3 where ADS produced a ratio of 7.39 ± 0.4 methane to carbon dioxide. Petrochemical wastewater showed a higher methane to carbon dioxide ratio than dairy wastewater, but showed a lower ratio than swine and municipal wastewaters. FLS showed a significantly greater amount of methane produced per carbon dioxide in each individual wastewater compared to ADS. FLS as a combined

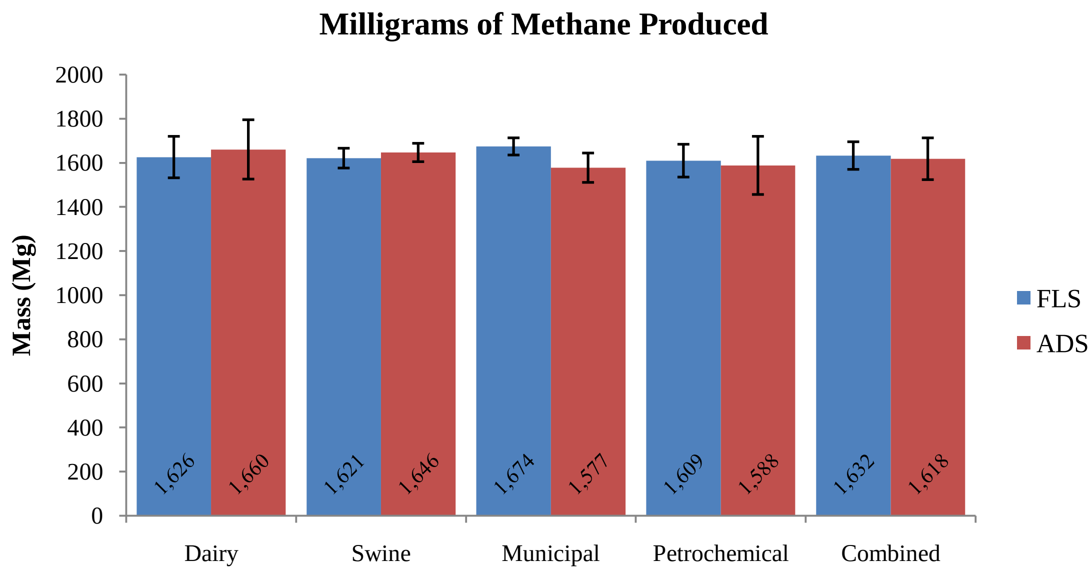

Fig. 5.3 Average values for total methane (mg) produced from triplicate treatments of four augmented wastewaters. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation no significant differences between treatments were measured for any of the augmented wastewaters.

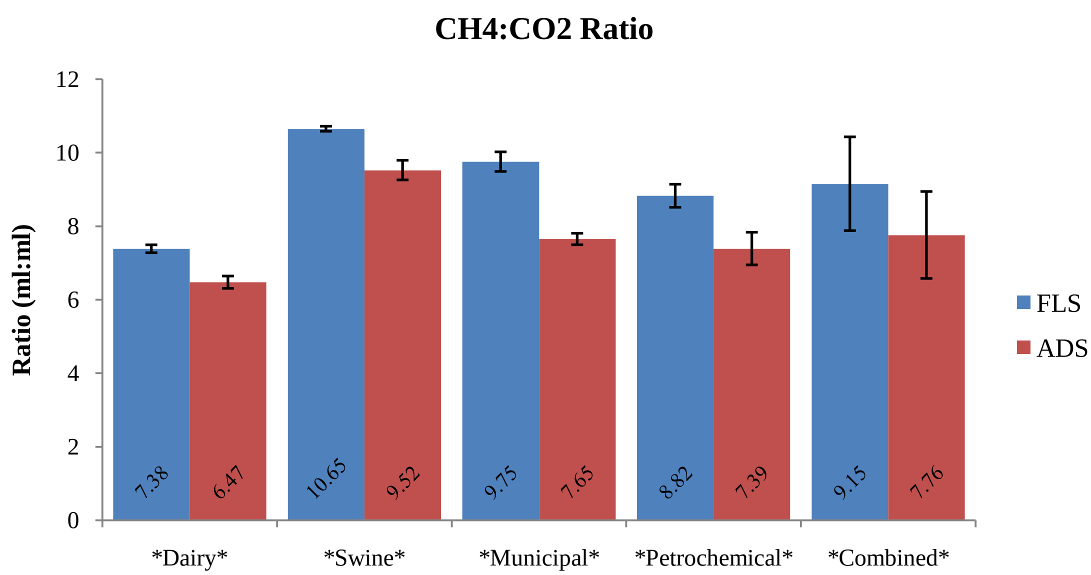

Fig. 5.4 Average values for methane to carbon dioxide ratio (ml:ml) from triplicate treatments of four augmented wastewaters. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Dairy*) indicate a significant difference between treatments was measured

group produced 1.39 more molecules of methane per molecule of carbon dioxide than ADS produced. FLS produced an average ratio of 9.15 ± 1.3 where ADS produced 7.76 ± 1.2 methane to carbon dioxide.

Decreases in Organic Content Measured as VS, BOD, TOC, and COD.

Fig. 5.5 shows a bar graph with a comparison of the change of VS observed with each wastewater and as a combined group. ADS showed a significantly greater reduction in VS with every wastewater treated than FLS. Augmented dairy wastewater treated with FLS showed a decrease in VS of 5.27 ± 0.09 g where ADS showed a decrease in VS by 6.93 ± 0.09 g, a difference of 1.66 g. Augmented swine wastewater showed a greater decrease in VS than dairy wastewater. Augmented swine wastewater showed a decrease in VS by 5.95 ± 0.10 g for treatment by FLS, where ADS showed a decrease of VS by 7.48 ± 0.04 a difference of 1.53 g. The smallest difference shown between FLS and ADS as measured by VS was observed with municipal wastewater. FLS showed a decreased in VS by 6.19 ± 0.29 g where ADS showed a decrease of 6.98 ± 0.05 g, a difference of 0.79 g. Petrochemical wastewater showed the biggest difference in FLS and ADS as measured with the change of VS at 1.97 g. FLS showed a decrease in VS by 4.92 ± 0.07 g where a decrease of 6.89 ± 0.05 g was observed with ADS. FLS as a combined wastewater group showed a decrease in VS by 5.58 g ± 0.55 where ADS showed a greater decrease in VS by 7.07 g ± 0.25.

Where ADS showed a significant change in VS across all treatments, the opposite was found with the change in BOD, with FLS showing a significantly greater change in BOD across all treatments (Fig. 5.6). FLS was 0.16 g better at decreasing BOD than ADS when treating augmented dairy wastewater. FLS demonstrated an average

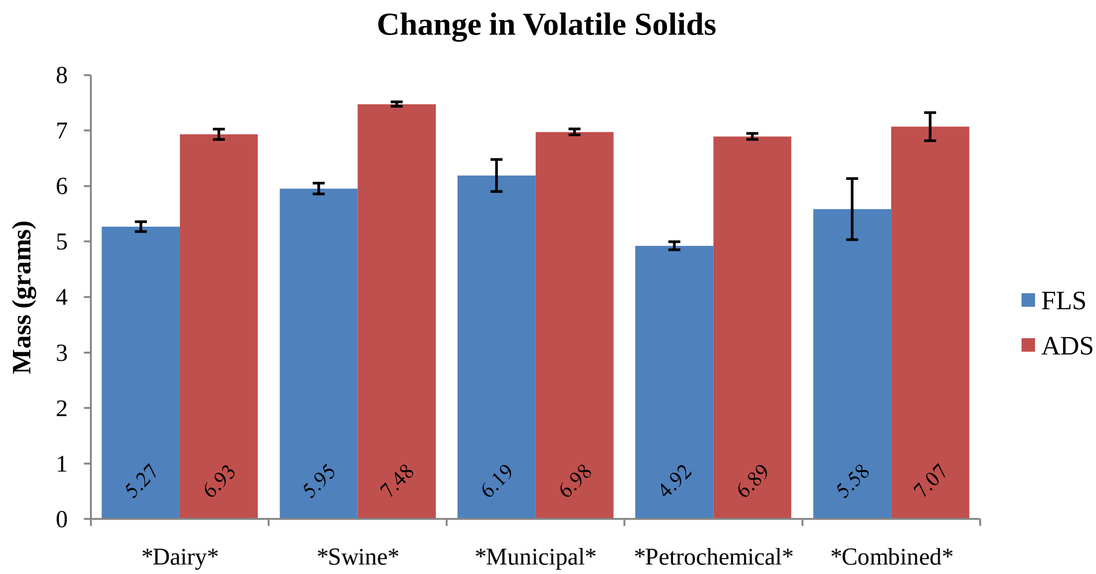

Fig. 5.5 Average change in volatile solids from triplicate treatments of four augmented wastewaters. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Dairy*) indicate a significant difference between treatments was measured.

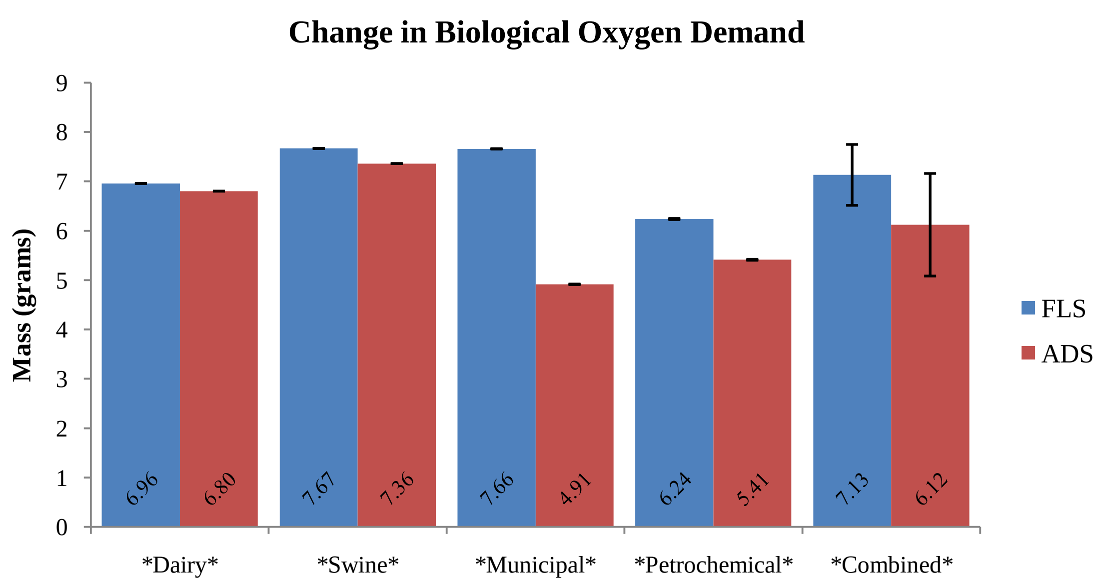

Fig. 5.6 Average change in biological oxygen demand from triplicate treatments of four augmented wastewaters. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Dairy*) indicate a significant difference between treatments was measured.

decrease of 6.96 g where ADS showed a change of 6.80 g. Augmented swine wastewater showed the biggest change in BOD. FLS showed a decrease in BOD by 7.67 g where a decreased of 7.36 g was observed with ADS. Municipal wastewater showed the biggest difference in the change of BOD between treatments. FLS showed a decrease in BOD by 7.66 g where a decrease of 4.91 ± 0.01 g was observed with ADS, a difference of 2.75 g. ADS treating municipal wastewater showed the smallest change in BOD compared to all other treatments. Petrochemical wastewater showed a decrease in BOD by 6.24 g ± 0.01, the smaller change observed with FLS. ADS showed a decrease in BOD with petrochemical wastewater of 5.41 ± 0.01 g. The combined group showed the biggest decrease in BOD by FLS with a change of 7.13 ± 0.62 g where ADS showed a decrease in BOD by 6.12 ± 1.04 g.

Half of the wastewaters treated with FLS showed a significant difference in the amount of TOC reduced, while the other half showed little difference between treatments (Fig. 5.7). Augmented dairy wastewater showed a significant difference in the amount of TOC reduced. The difference between FLS and ADS was observed to be small at 0.05 g. FLS treatment reduced the TOC of dairy wastewater 3.47 ± 0.01 g where ADS showed a decrease in TOC by 3.42 ± 0.03 g. The difference observed between FLS and ADS treating augmented dairy wastewater was small but still statistically significant. Augmented swine wastewater had almost identical values in the change of TOC with 3.6 g. Augmented municipal wastewater was significantly different showing the biggest difference in TOC between treatments. FLS showed decrease in TOC by 3.60 ± 0.03 g where a decrease of 3.05 ± 0.05 g was observed by ADS, a difference of 0.55 g. Augmented petrochemical wastewater showed identical treatments reducing TOC 3.07 g.

FLS was significantly better in treating all augmented wastewaters as a combined group. FLS treatment as a combined group showed a decrease in TOC by 3.43 ± 0.23 g. ADS showed a decrease in TOC by 3.28 ± 0.24 g. Municipal wastewater showed the biggest difference in treatments as shown with the bar graph in Fig. 5.7.

The change in COD fluctuated more from wastewater to wastewater than any other organic parameter measured as shown in Fig. 5.8. ADS showed a decrease in the COD with dairy wastewater being 0.94 g more than FLS, but this difference was a not significant having an alpha value of 0.06. The total change in COD was greater with swine wastewater being treated with ADS compared to FLS. The difference between FLS and ADS treatments as measured by COD was smaller than dairy, with only a difference of 0.24 g. Municipal wastewater was the only wastewater to show a significant difference between treatments as measured by the change in COD. Municipal wastewater was the only wastewater that FLS showed a greater decrease in COD compared to ADS. Municipal wastewater treated with FLS showed a changed of 8.86 ± 0.21 g of COD where ADS showed a change of 7.30 ± 0.36 g. Treatment of municipal wastewater with FLS showed the greatest difference between treatments having a difference of 1.56 g. Petrochemical wastewater was similar to dairy and swine wastewaters showing a bigger decrease in COD with ADS, but the difference between treatments was not statistically significant. FLS and ADS showed similar changes in the decrease of COD as observed with the combined treatment group. Both groups showed a change of about 7.86 g. The effects of the combined wastewaters showed no significant difference in treatments by FLS or ADS.

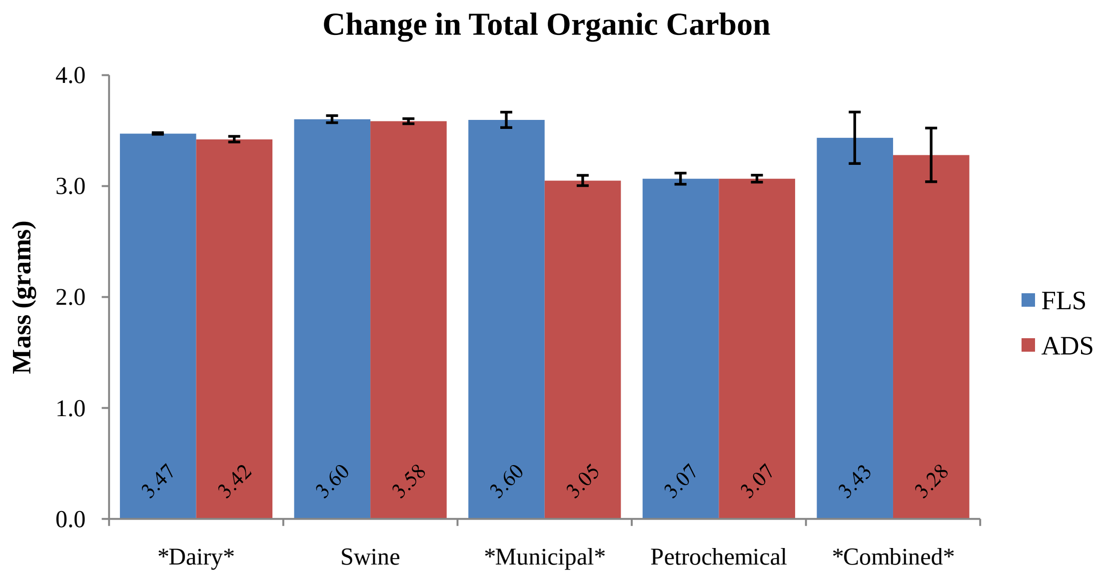

Fig. 5.7 Average change in total organic carbon from triplicate treatments of four augmented wastewaters. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Dairy*) indicate a significant difference between treatments was measured.

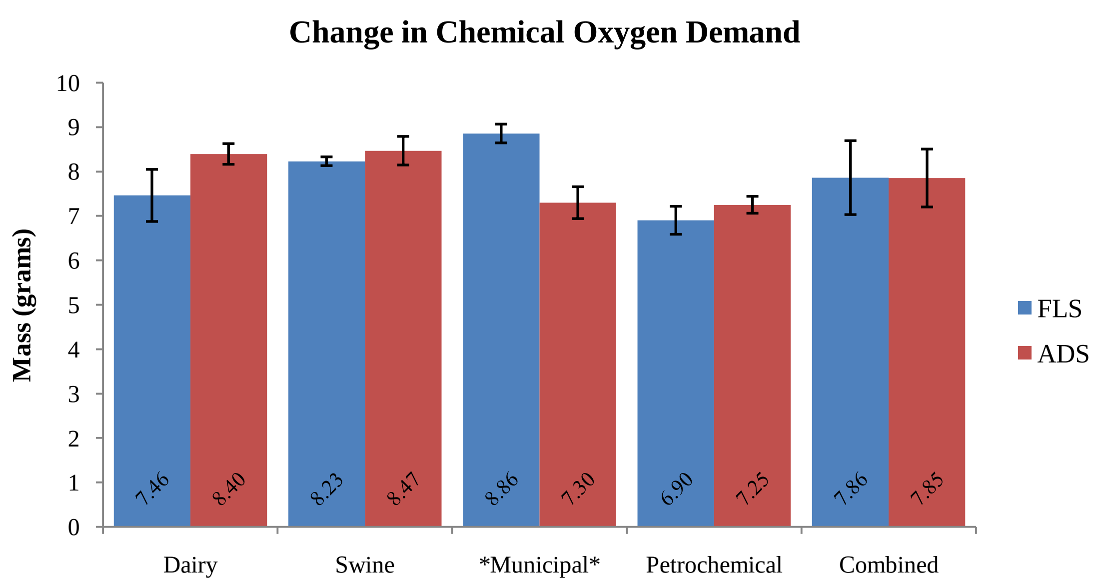

Fig. 5.8 Average change in chemical oxygen demand from triplicate treatments of four augmented wastewaters. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Municipal*) indicate a significant difference between treatments was measured.

Methane Produced from the Decrease in Organic Content Measured as VS, BOD, TOC, and COD

There is greater amount of methane produced per gram of volatile solid (CH_4/VS) observed with FLS than ADS for all wastewaters tested (Fig. 5.9). While ADS showed a better capacity to reduce the amount of VS (Fig. 5.5), FLS displayed a better conversion of VS to methane. Augmented dairy wastewater produced 308 ± 12.7 mg/g CH4/VS when treated with FLS. ADS showed production of methane at 240 ± 21.0 mg/g CH_4/VS, a difference of 68 mg/g. When augmented swine wastewater was treated, a difference of 52 mg/g of CH_4/VS was observed between treatments. FLS showed the ability to produce 272 ± 4.12 mg/g, where ADS showed a production of 220 ± 5.5 mg/g. Municipal wastewater had the smallest difference between FLS and ADS treatment. FLS produced 271 ± 17.2 mg/g CH_4/VS, being 45 mg/g more than ADS at 226 ± 8.4 mg/g. Petrochemical wastewater showed the biggest difference of CH_4/VS between treatments. FLS produced 327 ± 19.9 mg/g where ADS produced 230 ± 17.5 mg/g being 97 mg/g less than FLS. Looking at combined treatment groups, FLS showed a greater amount of CH_4/VS with 295 ± 28.1 mg/g where ADS showed less at 229 ± 14.5 mg/g. It is interesting to note how similar the CH_4/VS is with each wastewater treated with ADS as seen in Fig. 5.9. Where ADS showed very similar values for CH_4/VS, FLS shows a bigger variation of CH_4/VS over all treatments.

Where every wastewater group showed a significant difference between treatments as measured by the change in BOD (Fig. 5.6), only one wastewater showed a statistically significant difference with the amount of methane produced per gram of BOD (CH_4/BOD) (Fig. 5.10). Augmented municipal wastewater treated with FLS

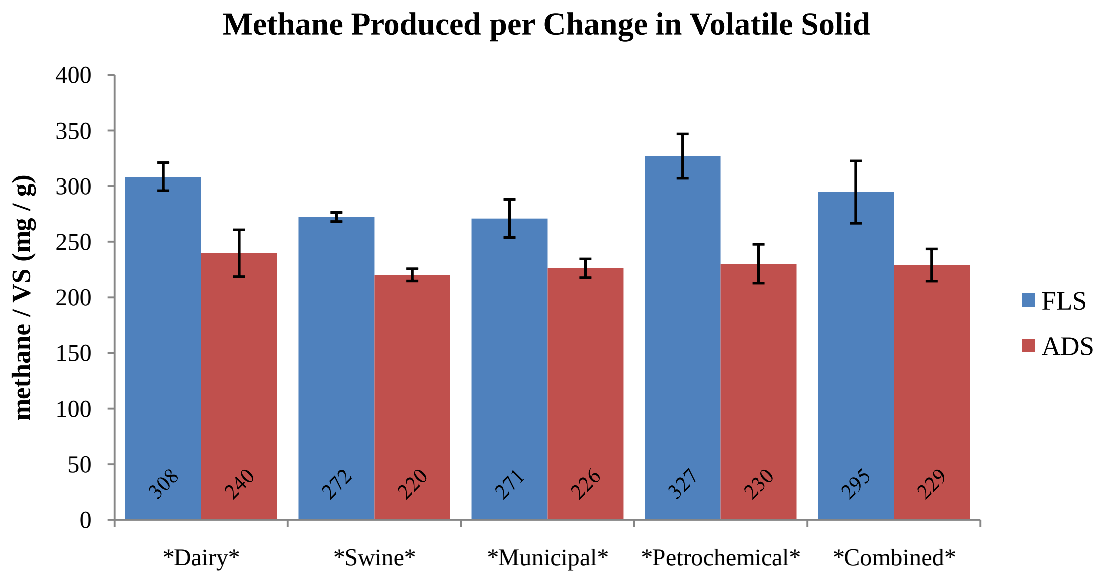

Fig. 5.9 Methane produced per gram of volatile solid for four wastewaters. Averages of triplicate augmented wastewater treatments shown. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Dairy*) indicate a significant difference between treatments was measured.

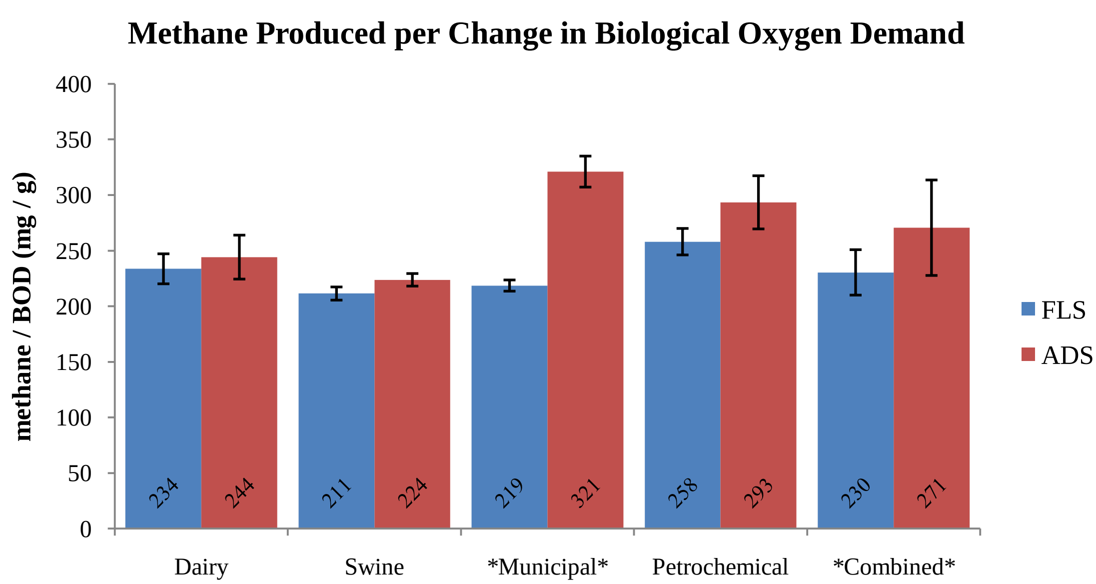

Fig. 5.10 Methane produced per gram of biological oxygen demand for four wastewaters. Averages of triplicate augmented wastewater treatments shown. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Dairy*) indicate a significant difference between treatments was measured.

produced 219 ± 5.0 mg/g, 102 mg/g less CH_4/BOD than ADS. ADS showed 321 ± 14.0 mg/g CH_4/BOD, the most of any treatment. A significant difference was also observed with the amount of CH_4/BOD when treatment groups were combined. FLS produced 230 ± 20.4 mg/g where ADS was able to produce 271 ± 42.9 mg/g CH_4/BOD. While every group in Fig. 5.10 shows a greater production of CH_4/BOD with ADS over FLS, only municipal wastewater was statistically significantly different.

The amount of methane produced per total organic carbon (CH_4/TOC) was similar to CH_4/BOD in that some wastewaters that were significant with the change in TOC were not found to be significant with the amount of methane produced per TOC (Fig. 5.11). Dairy wastewater showed a significant difference between the total amount of TOC removed between FLS and ADS treatments (Fig. 3.7). When comparing the amount of methane produced based on the change in TOC, dairy wastewater was no longer significantly different (Fig. 5.11). Neither was a significant difference found between FLS and ADS when treatments were grouping by inoculants, not by a specific wastewater. The only wastewater to show significantly more methane per TOC was municipal wastewater. Treatment with FLS produced 465 ± 4.2 mg/g where ADS showed a production of 517 ± 21.9 mg/g. Fig. 5.11 shows that all wastewaters demonstrated an insignificant difference in the amount of methane produced per TOC, except municipal wastewater.

Municipal wastewater also showed a significant difference between FLS and ADS treatments when measured as the amount of methane produced per COD (Fig. 5.12). FLS showed 189 ± 7.9 mg of methane per gram of COD, where ADS produced 216 mg/g ± 14.0 methane per COD. Dairy and Petrochemical wastewaters showed a difference in

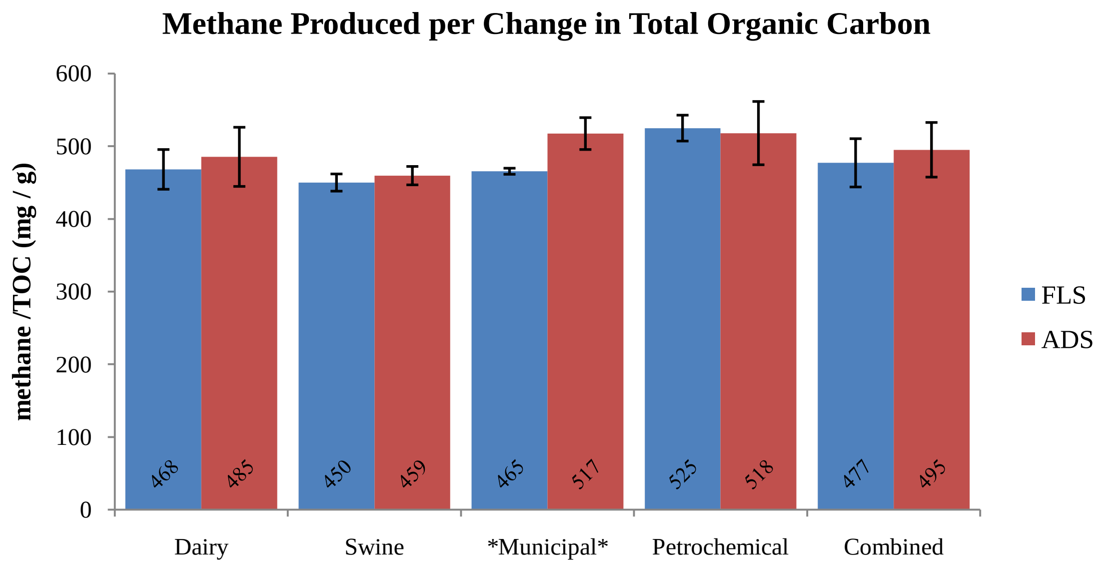

Fig. 5.11 Methane produced per gram of total organic carbon for four wastewaters. Averages of triplicate augmented wastewater treatments shown. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Municipal*) indicate a significant difference between treatments was measured.

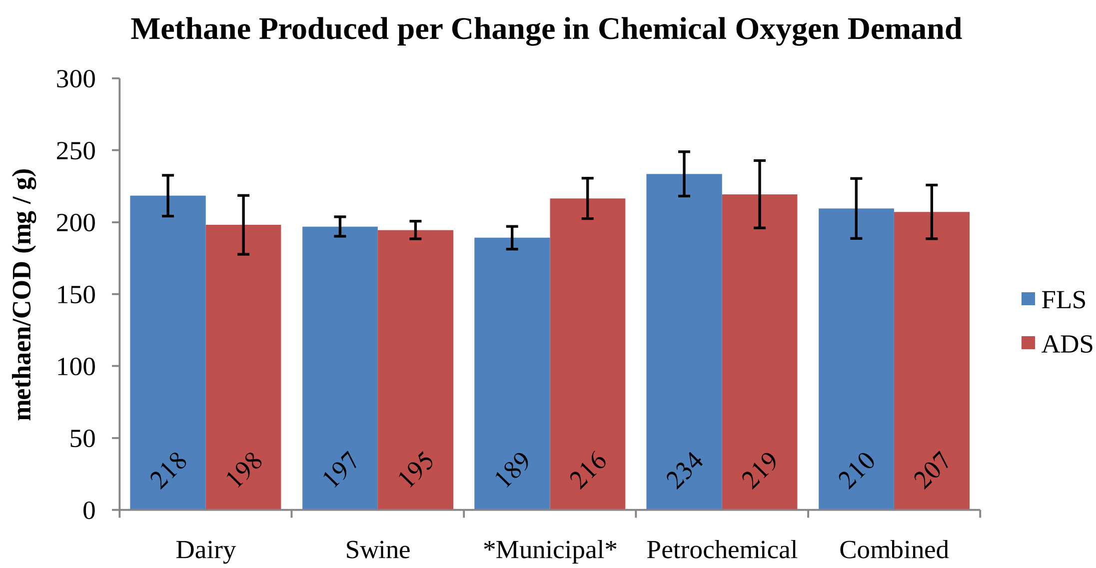

Fig. 5.12 Methane produced per gram of chemical oxygen demand for four wastewaters. Averages of triplicate augmented wastewater treatments shown. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (e.g., *Dairy*) indicate a significant difference between treatments was measured

treatments but the difference in the amount of methane produced per COD was not significantly different. Swine wastewater and the combined treatment group showed nearly identical average values of methane produced per gram of COD as shown in Fig. 5.12.

Conclusion

FLS augmentation showed significantly better treatment of wastewater by outperforming ADS in 6 of the 12 wastewater parameters. This is shown with blue boxes in the combined row of Fig. 5.13. ADS showed significantly better than FLS in two parameters, change in VS and the amount of methane produced per change in biological oxygen demand. Parameters that are better with ADS are shown with the red boxes in

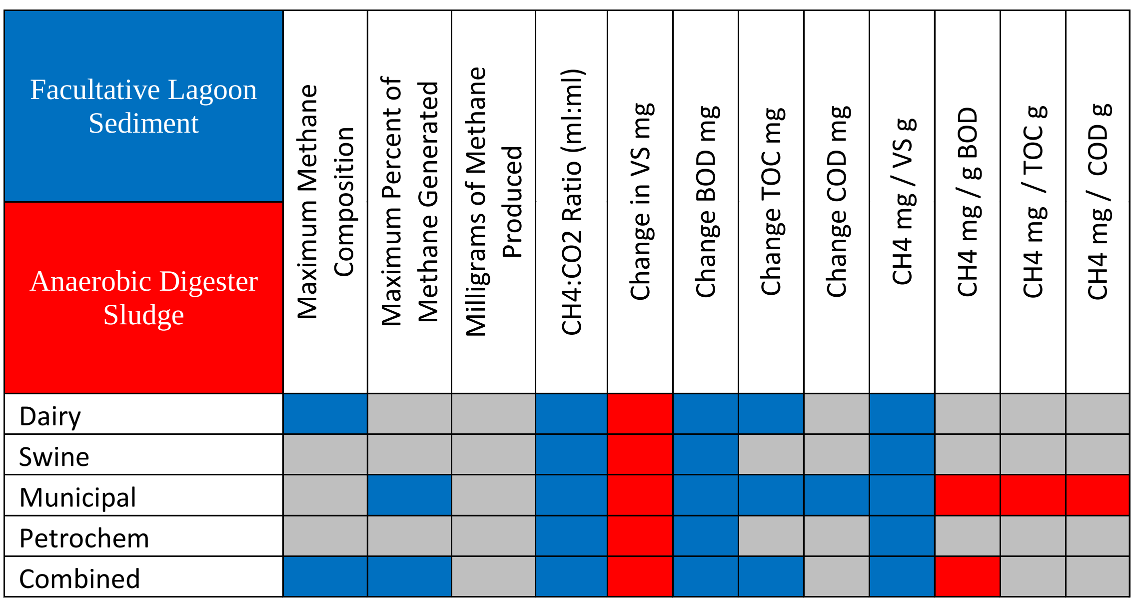

Fig. 5.13 Visual representations of significant Yield and composition parameters found from treatment of dairy, swine, municipal, and petrochemical wastewaters by Facultative Lagoon Sediment (FLS) and Anaerobic Digestive Sludge(ADS). Non significant findings are marked in gray, FLS significant findings are marked in blue and ADS significant findings are marked in red. Bottom row (Combined) show if significance difference was found between treatments based on all wastewaters treated.

the combined row of Fig. 5.13. A total of 48 individual wastewater parameters were measured. Of the 48 measurements, 17 of them treated by FLS were significantly better than the treatment by ADS. Only 7 out of the 48 individual wastewater parameters showed ADS to be significantly better than FLS.

COMPARISON AND EVALUATION OF CONTROLS

Introduction

Three controls were analyzed to determine the influence of the addition of algae, acetate, and inoculant to dairy wastewater. Controls contain the same proportions of dairy wastewater, acetate, algae and inoculant as augmented dairy wastewater previously described. Dairy wastewater augmented with acetate was examined to determine the influence of acetate on dairy wastewater without the aid of algae. Dairy mixed with algae was used to determine the influence of algae on dairy wastewater without the aid of acetate. The third control contained the same amount of inoculant used in each treatment with the addition of distilled water (no algae or acetate). Inoculants treating distilled water show the influence that the additional nutrients found in each inoculant have on treatment. The rates and volumes of biogas were examined for each control as well as the change in organic chemical level as measured by volatile solids (VS), biological oxygen demand (BOD), total organic carbon (TOC), and chemical oxygen demand (COD). The volume of methane produced was compared to the volume of carbon dioxide. The mass of methane was compared to the change in organics (VS, BOD, TOC, COD). These controls showed the impact on anaerobic treatment from the addition of algae, acetate, and inoculants.

Control Rates and Volumes

Dairy and Acetate (No algae)

Dairy augmented with acetate showed a pattern of biogas production similar in shape to augmented dairy wastewater (Fig. 4.5). Facultative lagoon sediment (FLS) augmented with acetate showed a more uniform plot of total volume (Fig. 6.1A) than augmented dairy wastewater (Fig. 4.5A). Replicates of Acetate treated with anaerobic digester sludge (ADS) showed a greater variation in total volume (Fig. 6.1C) than replicates of augmented dairy wastewater (Fig. 4.5C).

As observed in other FLS treatments, dairy wastewater augmented with acetate showed a brief stabilization in rate of biogas production at day 20 (Fig. 6.1B). The stabilization in rate was not observed in treatment with ADS (Fig. 6.1D). Although dairy wastewater augmented with acetate produced more biogas when treated with FLS than ADS treatment, the difference was not significant (Fig. 6.1E). Dairy wastewater augmented with acetate showed a significant larger maximum rate of biogas produced when treated with FLS than when treated with ADS (Fig. 6.17). FLS showed a maximum rate of biogas production at 110.6 ± 2.6 ml/day, significantly greater than ADS, which had a maximum rate of 90.1 ± 6.2 ml/day.

Dairy wastewater augmented with acetate showed a maximum rate of biogas production sooner when treated by FLS than ADS. FLS required 29.5 ± 2.8 days to reach a maximum rate, while ADS required 34.6 ± 1.2 days to reach maximum biogas production. FLS required significantly less time to start biogas production as measured be reaching a rate of 10 ml/day. FLS required 17.4 ± 0.0 days to reach the initial rate, where ADS required 20.5 ± 1.0 days.

Dairy augmented with acetate showed a significantly larger percentage of methane produced than dairy wastewater augmented with algae and acetate. The high concentrations of methane came with a cost. Dairy wastewater augmented with acetate required more time to reach the maximum biogas production rate compared to

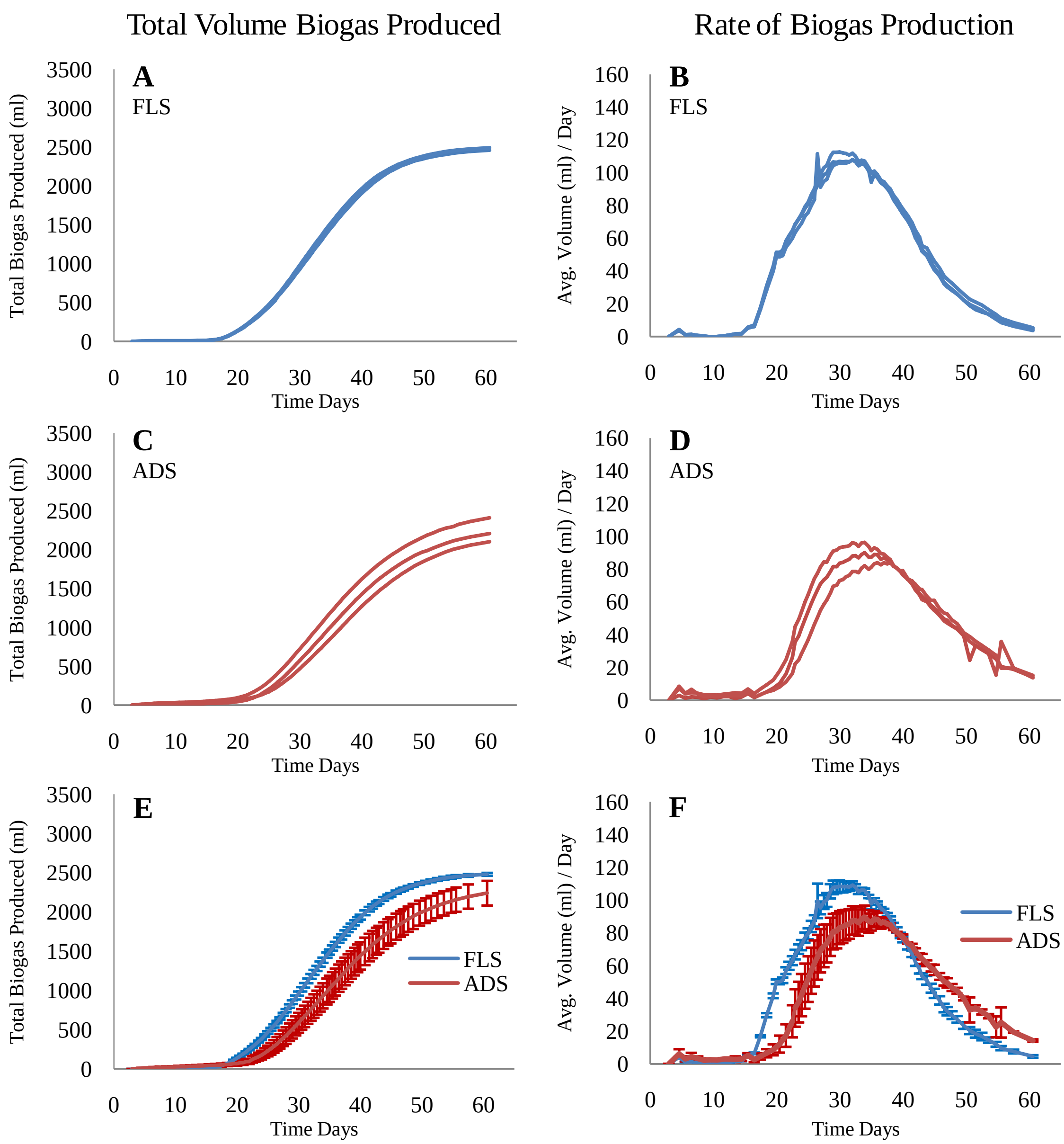

Fig. 6.1 Dairy Acetate (No Algae) Total volume and rate of biogas produced from Facultative Lagoon Sediment (FLS) and Anaerobic Digester Sludge (ADS) as inoculants for treatment of dairy wastewater augmented with acetate. A) Triplicates of total biogas produced using FLS. B) Triplicates of the rate of biogas produced using FLS. C) Triplicates of total biogas produced using ADS. D) Triplicates of the rate of biogas produced using ADS. E) Average of total biogas produced from triplicate treatments of FLS and ADS. F) The average rate of biogas produced from triplicate treatments using FLS and ADS as inoculant source. All error bars represent one standard deviation.

dairy augmented with acetate and algae. When algae was present, the maximum rate at which biogas was produced was sooner than treatment without algae.

Dairy and Algae (No acetate)

Dairy wastewater augmented with algae displayed the shortest duration of biogas production. Both treatments reached a low in biogas production after 35 days (Fig. 6.2F). Treatment of dairy wastewater augmented with algae was terminated prior to reaching day 60 due to plateau of biogas production. Treatment of dairy wastewater augmented with algae exhibited a variation within replicates observed with FLS (Fig. 6.2A) and ADS (Fig. 6.2C). One of the replicates treated with FLS showed little biogas production up to day 15 when it displayed a sudden increase (Fig. 6.2B). When a large deviation in triplicates was observed, fittings to collected biogas were checked and tightened. A loose fitting accounted for the low production of biogas observed. The cause of low biogas production from one replicate (Fig. 6.2C) treated with ADS is unknown. Biogas was produced with the replicate displayed a lower and less stable rate as shown in Fig. 6.2D.

Treatment of dairy wastewater augmented with algae showed similar volumes of biogas produced with FLS and ADS as shown in Fig. 6.2E. Both treatments showed a larger standard deviation in total biogas produced relative to other dairy treatments. The large standard deviation is observed because of one replicate in each treatment did not produce biogas like the others.

Dairy wastewater augmented with algae treated by ADS showed a maximum rate of biogas earlier than treatment by FLS (Fig. 6.2F). The maximum rate observed with ADS was more than 7 days earlier than FLS. This was the only significant difference

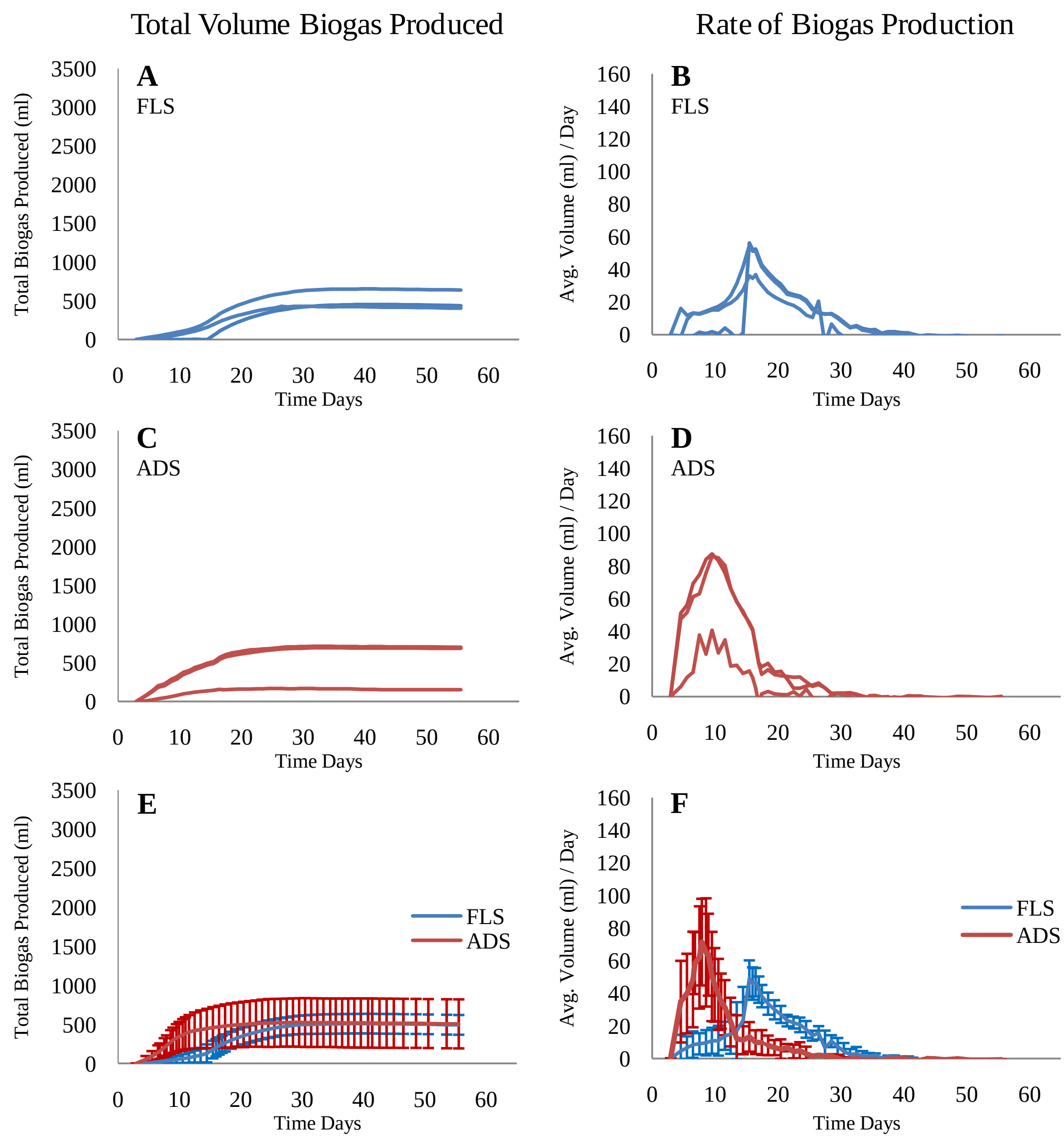

Fig. 6.2 Dairy Algae (No Acetate) Total volume and rate of biogas produced from Facultative Lagoon Sediment (FLS) and Anaerobic Digester Sludge (ADS) as inoculants for treatment of dairy wastewater augmented with algae. A) Triplicates of total biogas produced using FLS. B) Triplicates of the rate of biogas produced using FLS. C) Triplicates of total biogas produced using ADS. D) Triplicates of the rate of biogas produced using ADS. E) Average volume of total biogas produced from triplicate treatments of FLS and ADS. F) The average rate of biogas produced from triplicate treatments using FLS and ADS as inoculant source. All error bars represent one standard deviation.

found when comparing parameters associated with the volume and rate of biogas production.

The average rate of biogas production and duration at which the biogas operated at this rate were calculated and are not included in this study. The two points calculated were found to be adjacent when using an R^2 value of 0.99. Due to the subjectivity of selecting which two points to use as the average rate, this value was omitted.

Dairy wastewater augmented with algae showed an earlier maximum rate than was observed with dairy wastewater augmented with acetate or dairy augmented with acetate and algae. The addition of algae decreased the time required for treatment to reach its maximum biogas production. Both treatments containing algae (dairy wastewater augmented with acetate and algae as well as dairy wastewater augmented with algae) showed a significantly earlier maximum rate of biogas production when compared to treating dairy wastewater augmented with acetate. The two shortest times to reach maximum rate contained the largest and smallest organic loading. Therefore decreasing the time requirement to reach maximum rate was best achieved using algae, rather than increasing the organic chemical loading.

Inoculant Treating Distilled Water (No wastewater, No acetate, No Algae)

Fig. 6.3 shows the production volumes and rates of biogas produced from each inoculant using the same scale for x and y axes as other treatments reported, which shows that little biogas was produced from either inoculant without addition carbon or nitrogen from the wastewater or augmentation. FLS treatment showed the lowest volume of biogas produced (Fig. 6.3A). The final total volume of biogas produced was negative, (-18.1 ± 6.1 ml) revealing that it consumed more gas than produced. This is observed in

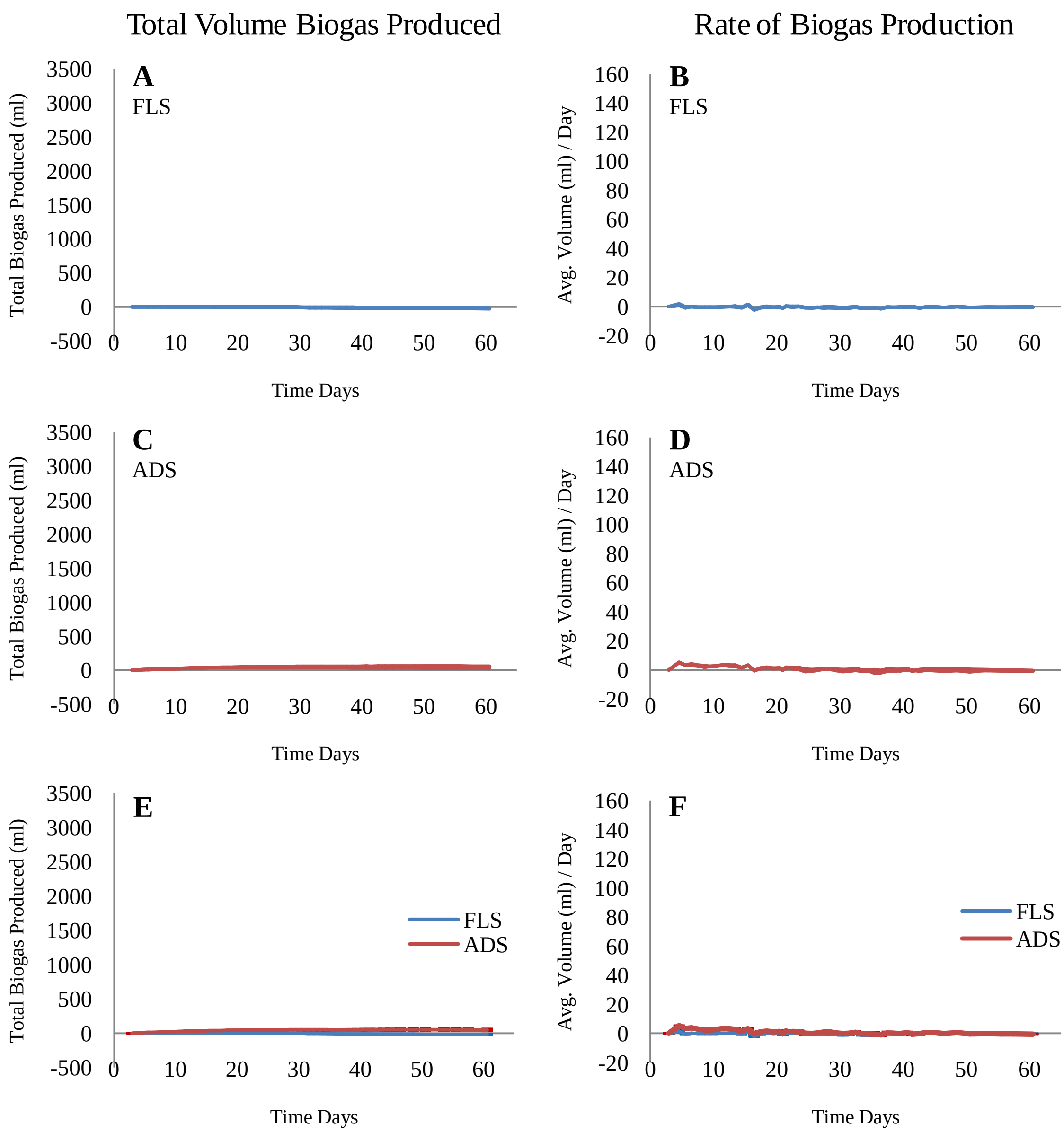

Fig. 6.3 Inoculants Total volume and rate of biogas produced from Facultative Lagoon Sediment (FLS) and Anaerobic Digester Sludge (ADS) as inoculants for treatment of distilled water. A) Triplicates of total biogas produced using FLS. B) Triplicates of the rate of biogas produced using FLS. C) Triplicates of the total biogas produced using ADS. D) Triplicates of the rate of biogas produced using ADS. E) Average of total biogas produced from triplicate treatments of FLS and ADS. F) The average rate of biogas produced from triplicate treatments using FLS and ADS as inoculant source. All error bars represent one standard deviation.

Fig. 6.3B where the rate of biogas production fluctuated between positive and negative showing both production and consumption of biogas. ADS also showed a small amounts of biogas produced (Fig. 6.3C) with at final total volume of 48.8 ± 15.0 ml. A significant difference was shown in the total volume of biogas produced with FLS and ADS, almost undistinguishable in Fig. 6.3E. ADS also showed times of positive and negative biogas production similar to FLS (Fig. 6.3D).

Neither FLS nor ADS treating distilled water showed a time when biogas production reached 10 ml per day as observed with other treatments. Calculation of an average rate and duration of average rate was omitted as the R^2 value of 0.99 was limited to two adjacent points, leaving measurement subjective. Overall inoculants showed baseline values for the total volume of biogas produced (Fig. 6.3E) and rate of biogas production (Fig. 6.3F). The values obtained from volume and rate measurements were often smaller than the standard deviation found in the treatment of other augmented wastewaters suggesting that the contribution of inoculants (FLS or ADS) on volume of biogas produced is negligible.

Composition and Organic Chemicals

A significant difference was observed with the total volume of biogas produced between FLS and ADS treating distilled water. FLS treatment showed a consumption of biogas with a final volume of -18 ± 6 ml and ADS showed a final volume of 49 ± 15 ml. No significant difference was found between FLS and ADS treatment in the total amount of biogas observed with any of the controls containing dairy wastewater. Dairy wastewater augmented with both algae and acetate produced more biogas than dairy wastewater augmented with acetate or algae individually (Fig. 6.4). This was expected because controls augmented with only algae or acetate combined with dairy wastewater contained a lower organic load than dairy wastewater augmented with both acetate and algae.

Combining algae and acetate to attain a molar ratio of 25:1 carbon to nitrogen showed better biogas production than individual augmentations of algae with ratio 8:1 or acetate with a ratio of 181:1. An additional amount of 645 ml of biogas was observed for dairy wastewater containing algae and acetate compared with dairy wastewater augmented with acetate only when treated with FLS (Table 6.1). The volume of biogas produced when dairy was augmented with algae alone was only 514 ml. Therefore combining algae and acetate showed more biogas produced (3128 ml) than the sum of the individual augmented treatments (2997 ml). Treatment by ADS showed similar findings with a larger difference observed in total biogas produced when dairy wastewater was augmented with both algae and acetate (Table 6.1).

Treatment by FLS and ADS showed a significant difference in the maximum percentage of methane when dairy augmented with acetate was treated (Fig. 6.5). FLS

Table 6.1
Volume of total biogas produced from FLS and ADS treating dairy wastewater augmented with algae , acetate, or algae and acetate (left). The additional amount of biogas produced when algae and acetate were combined (Right).

	Dairy wastewater augmented with:			Additional biogas observed due to mixing Algae and Acetate over isolated augmentation.	
	Acetate	Algae	Acetate and Algae	Increase in biogas from Acetate	Increase in biogas from Algae
FLS	2483 ± 19 ml	514 ± 122 ml	3128 ± 152 ml	2614 ± 195 ml	645 ± 153 ml
ADS	2260 ± 159 ml	526 ± 309 ml	3247 ± 206 ml	2721 ± 371 ml	987 ± 260 ml

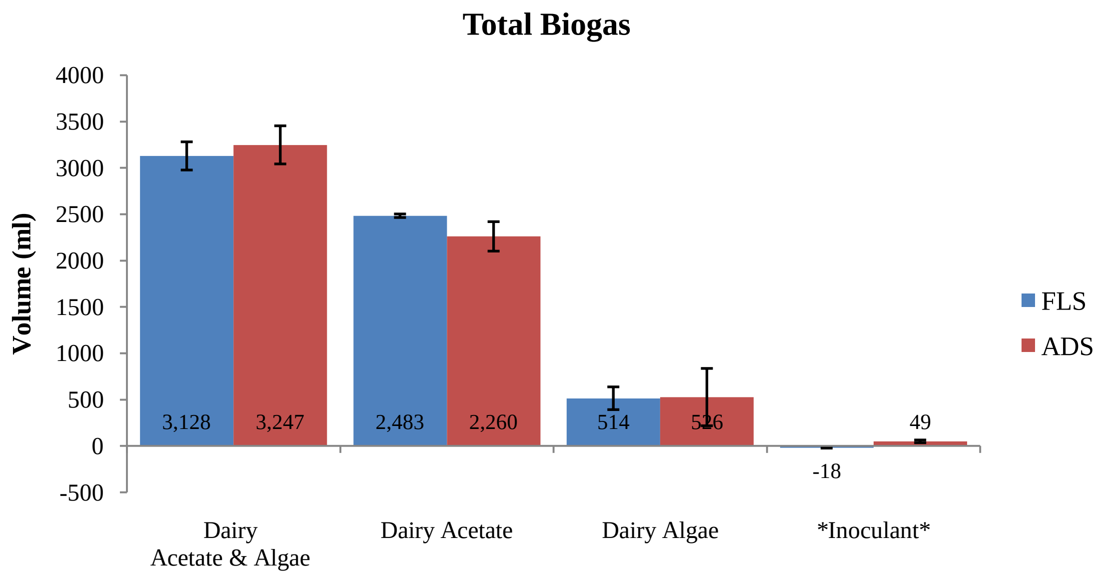

Fig. 6.4 Average total biogas from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around control (*Inoculant*) indicate a significant difference between treatments.

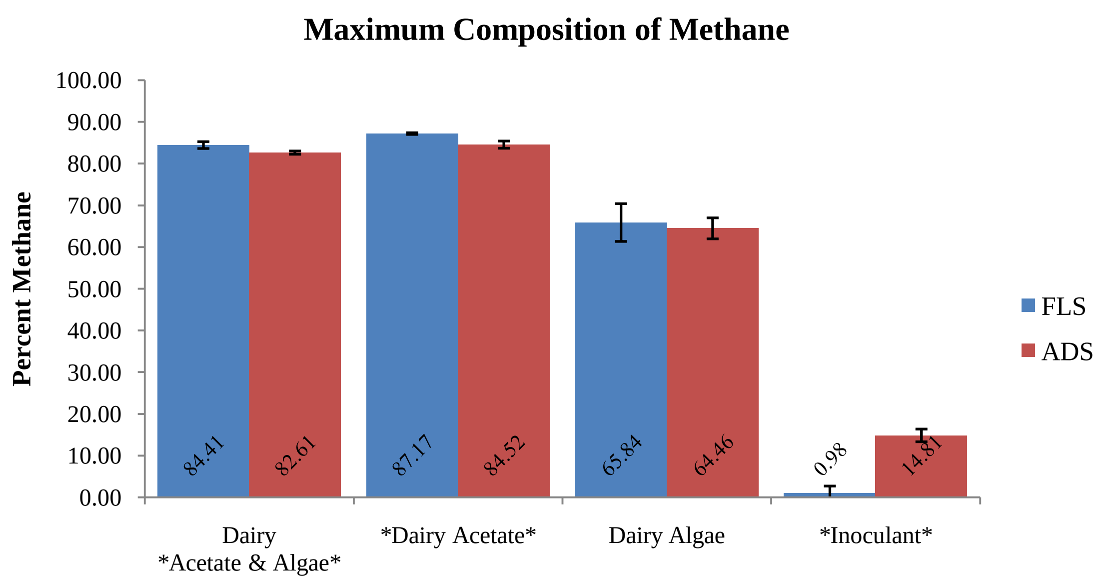

Fig. 6.5 Average maximum composition of biogas measured from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (*Dairy*) indicate a significant difference between treatments.

showed 87.2 ± 0.2% where ADS showed a maximum percentage of methane at 84.5 ± 0.9%. Inoculants treating distilled water showed a significant difference in the amount of methane produced with FLS showing a maximum percentage of methane at 1.0 ± 1.7% and ADS with 14.8 ± 1.5%. The small amount of methane produced with FLS treatment shows some production of biogas where the final total volume showed an overall consumption of gas. Dairy augmented with algae showed no significant difference between FLS and ADS treatments. Both treatments show similar composition of biogas as measured by Mussgnug et al. (2010) when treating algae alone.

Treatment with FLS showed a significantly larger maximum rate of biogas production when treating dairy wastewater augmented with acetate (Fig. 6.6). FLS showed a maximum production of 110.6 ± 2.6 ml/day where ADS showed 90.1 ± 6.2 ml/day. FLS treating deionized water showed a significantly smaller maximum rate of biogas being produced at 1.5 ± 0.5 ml/day where ADS showed a maximum rate of 5.3 ± 0.1 ml/day. Dairy wastewater augmented with acetate was the only group treated where FLS showed a larger maximum rate of biogas production. All other augmented wastewaters and control groups showed a larger rate of biogas production with ADS.

The difference between maximum rate observed with dairy wastewater augmented with acetate and dairy wastewater augmented with acetate and algae was greater with ADS than FLS. Both FLS and ADS treatments received an increase in volatile solids (VS) by 12.9% from the added algae. The additional VS showed an increased maximum rate of biogas production by 11.2 ± 7.28 ml/day with FLS, and an increase of 57.7 ± 6.4 ml/day with ADS.

FLS treating dairy wastewater augmented with acetate showed an earlier time to reach maximum rate than ADS showed (Fig. 6.7). The maximum rate was observed significantly sooner with FLS at 29.5 ± 2.8 days where ADS showed a maximum rate after 34.6 ± 1.2 days. This observation is opposite of what was observed with all other augmented wastewaters and control groups. Dairy wastewater augmented with algae showed a significant difference in time required to attain maximum rate. FLS showed a maximum rate on day 15.8 ± 0.6, taking twice as long as ADS showing a maximum rate on day 7.9 ± 0.0.

A significant difference was observed in the amount of methane produced when dairy wastewater augmented with acetate was treated (Fig. 6.8). Treatment with FLS showed more methane produced (1,348 ± 14 mg) than ADS (1190 ± 90 mg). This is the only instance where a significant difference was observed in the amount of methane produced from treatment of an augmented wastewater (Fig. 6.8 and Fig. 5.3). A significant difference was observed when inoculants treated distilled water. FLS showed 0.2 ± 0.4 mg of methane produced where ADS showed 19.0 ± 3.0 mg of methane produced. FLS treating distilled water showed only one replicate that produced methane, resulting in a low average amount of methane produced. Methane produced from inoculants treating distilled water would come from organic matter with the inoculants portion of each treatment. Dairy wastewater augmented with algae showed little difference between FLS and ADS treatments.

ADS showed a greater methane to carbon dioxide ratio than FLS showed when treating distilled water. This is reflected due to the lack of methane produced with FLS treating distilled water. FLS showed a ratio of 0.10 ± 0.18 ml:ml where ADS showed a ratio of 2.65 ± 0.26 ml:ml. This was the only instance where ADS showed greater

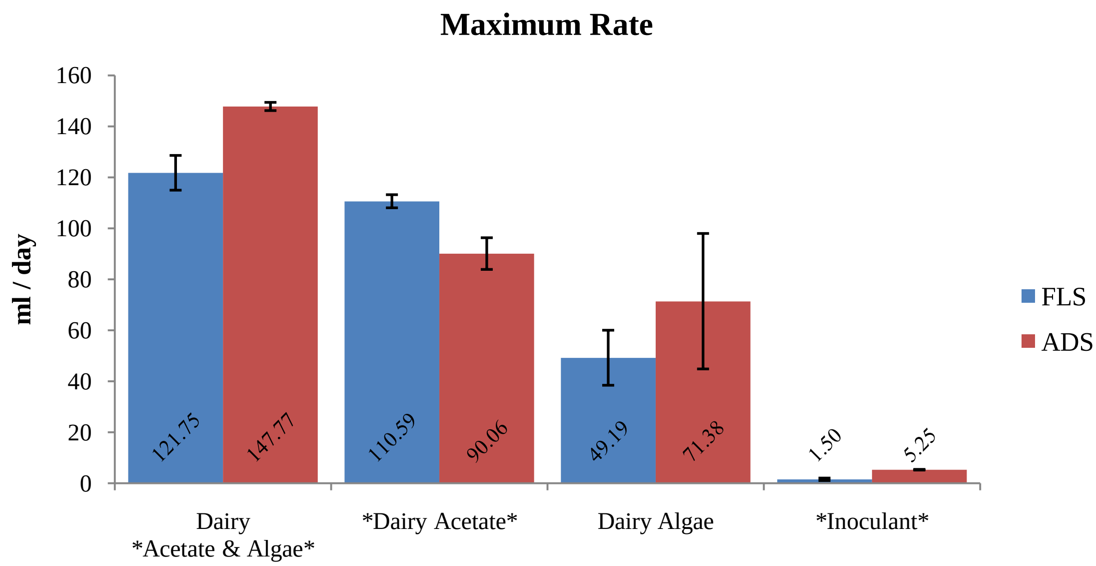

Fig. 6.6 Average maximum rate of biogas produced as measured from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (*Dairy*) indicate a significant difference between treatments.

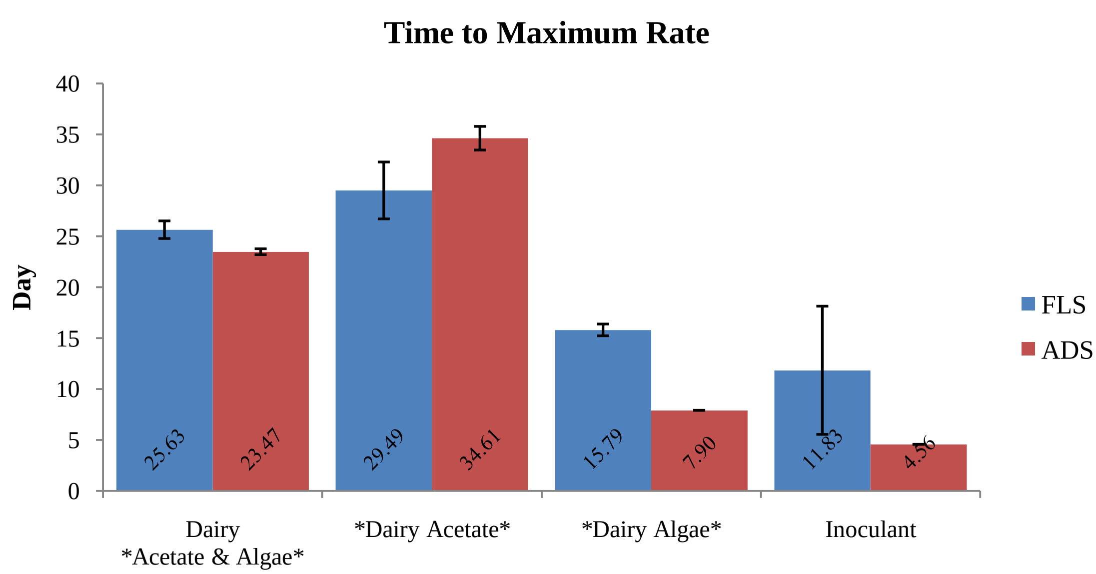

Fig. 6.7 Average time required to reach maximum rate of biogas production as measured from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (*Dairy*) indicate a significant difference between treatments.

methane to carbon dioxide ratio (Fig. 6.9) presumably from the lack of biogas produced with FLS. Dairy augmented with acetate and dairy augmented with algae both showed greater methane to carbon dioxide ratio with FLS but neither were significant at an 0.05 alpha level. Dairy wastewater augmented with algae contain a carbon to nitrogen ratio of 8:1 and dairy wastewater augmented with acetate contain a ratio of 181:1, both not at the optimal wastewater feed ratio of 25:1. Having a wastewater feed ratio of 25:1 carbon to nitrogen showed FLS to be significantly better at converting organics to methane. When the feed ratio was not operating at 25:1 carbon to nitrogen as seen with the dairy wastewater augmented with acetate (181:1) or algae (8:1) individually, the significance between FLS and ADS was not observed. ADS was better at producing methane over carbon dioxide only when inoculants were treating distilled water, an instance where FLS showed an overall consumption of gas.

ADS showed a larger decrease in VS in each control (Fig. 6.10), as was shown with each augmented wastewater (Fig. 5.5). Each control showed a significant difference between FLS and ADS treatments. FLS treating dairy wastewater augmented with acetate showed a decrease in VS by 3.96 ± 0.13 g where ADS showed a decrease of 5.77 ± 0.13 g. FLS treating dairy wastewater augmented with algae showed an increase in VS by 0.19 ± 0.05 g where ADS showed a decrease of 1.17 ± 0.11 g. FLS treating distilled water showed an increase in VS by 0.31 ± 0.04 g where ADS showed a decrease of 0.36 ± 0.02 g. Both instances of an increase in VS is considered to be caused by experimental error.

FLS treating dairy wastewater augmented with acetate showed a greater amount of methane produced per change in VS than ADS showed (Fig. 6.11). FLS showed

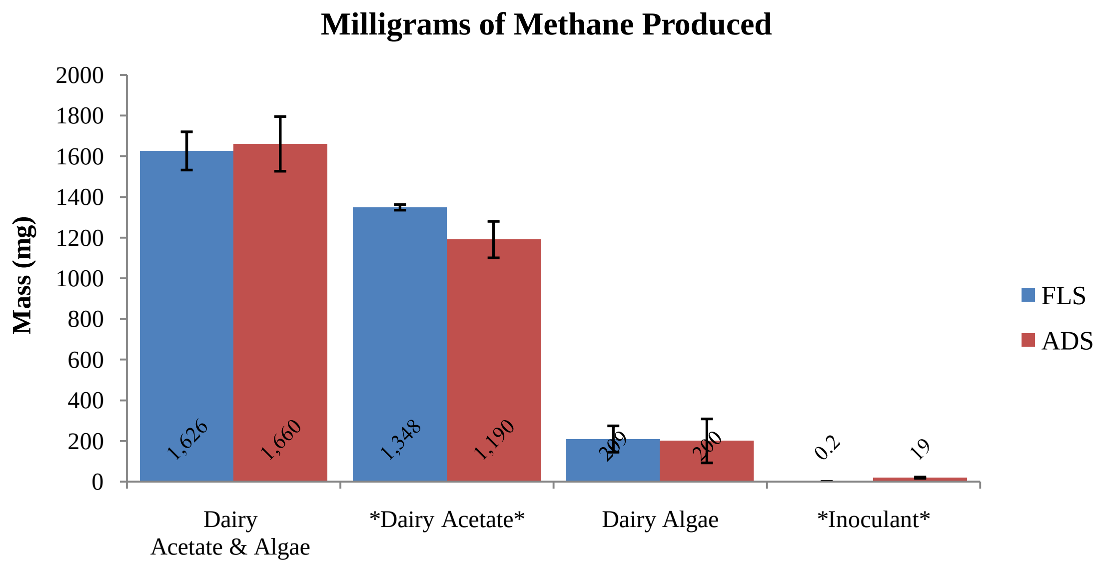

Fig. 6.8 Average values for total methane (mg) produced from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around control (*Dairy Acetate*) indicate a significant difference between treatments.

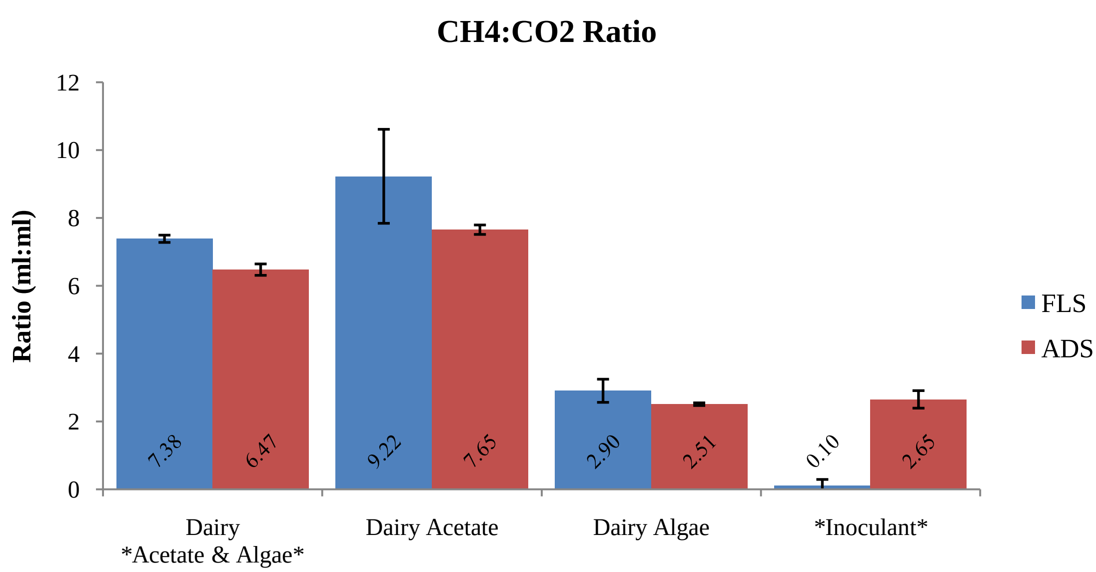

Fig. 6.9 Average values for methane to carbon dioxide ratio (ml:ml) from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (*Dairy*) indicate a significant difference between treatments.

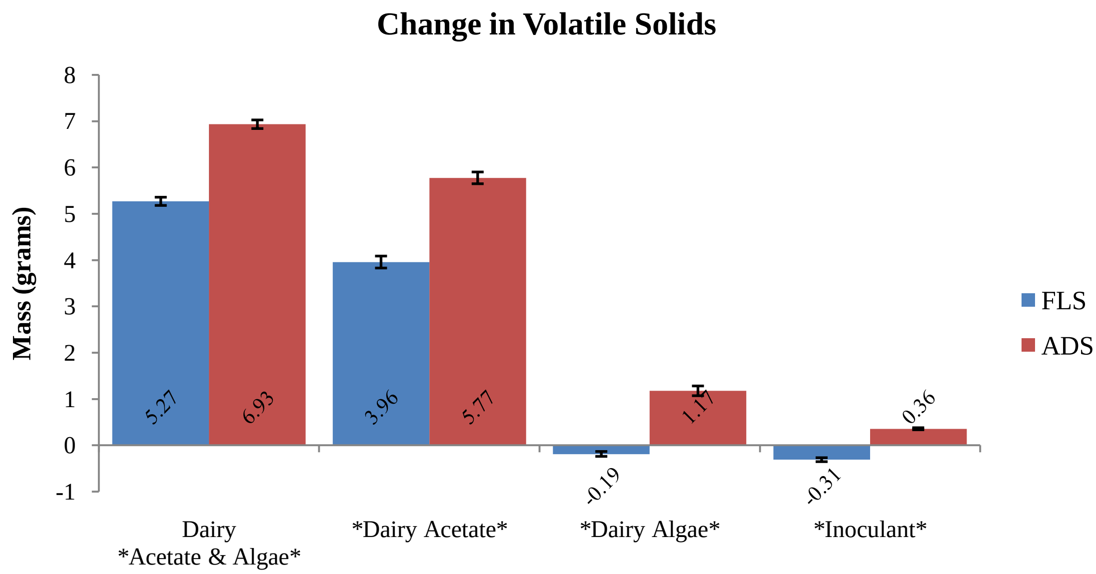

Fig. 6.10 Average change in volatile solids from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (*Dairy*) indicate a significant difference between treatments.

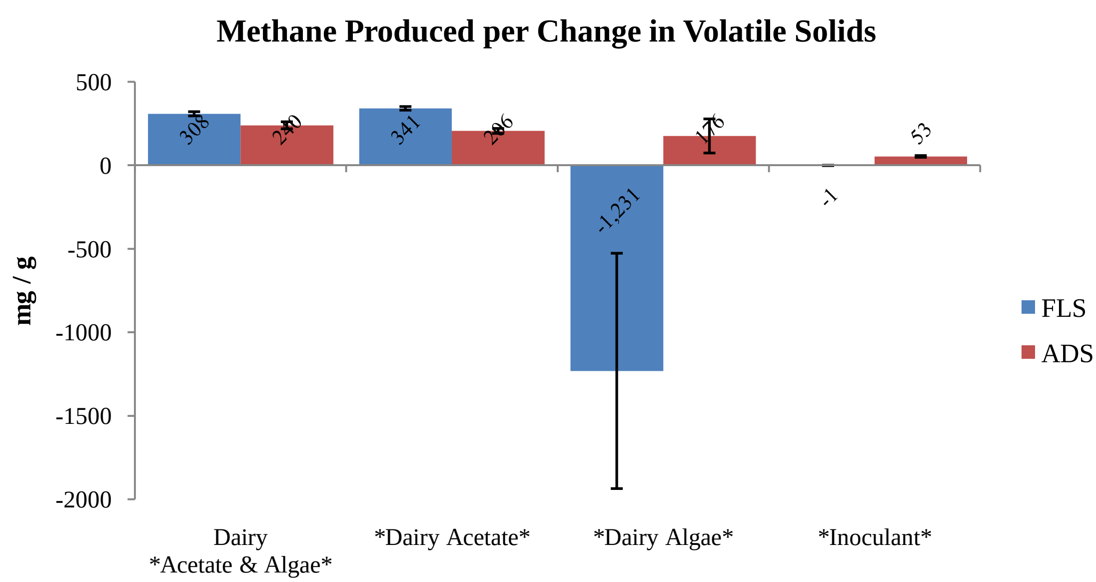

Fig. 6.11 Methane produced per gram of volatile solid for dairy wastewater. Averages of triplicate control treatments shown. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (*Dairy*) indicate a significant difference between treatments.

341 ± 11 mg/g methane per VS where ADS showed 206 ± 15 mg/g methane produced VS. FLS showed a negative amount of methane produced per gram of VS when dairy wastewater augmented with algae was treated, as well as when distilled water was treated (Fig. 6.11). As both showed an increase in VS, the amount of methane produced is represented as negative amount of methane per VS.

A significant difference in the change of biological oxygen demand (BOD) was observed for each control treated by FLS and ADS (Fig. 6.12). FLS showed a greater decrease in BOD when treating dairy wastewater augmented with algae as well as dairy wastewater augmented with acetate. FLS showed a decrease in BOD of 5.98 ± 0.01g when dairy augmented with acetate was treated, where ADS showed a decrease of 4.29 ± 0.01 g. When dairy augmented with algae was treated, FLS showed a decrease in BOD of 0.65 ± 0.00 g, and 0.64 ± 0.00 g when treated by ADS. Inoculants treating distilled water showed an increase in BOD with both FLS and ADS treatments. FLS treating distilled water showed a smaller increase in BOD than ADS showed. FLS showed an increase in BOD of 0.11 ± 0.00 g where ADS showed an increase of 0.21 ± 0.00 g.

ADS showed a significantly greater amount of methane produced per change in BOD when dairy augmented with acetate was treated (Fig. 6.13). FLS showed 226 ± 2 mg/g methane per change in BOD, where ADS showed 277 ± 21 mg/g methane per change in BOD. Treatments where algae was implemented to augment wastewater showed no significant difference between FLS and ADS treatment as shown in Fig. 6.13. Inoculants treating distilled water also showed a significant difference between treatments. FLS showed a -2.2 ± 3.8 mg/g methane per change in BOD, where ADS

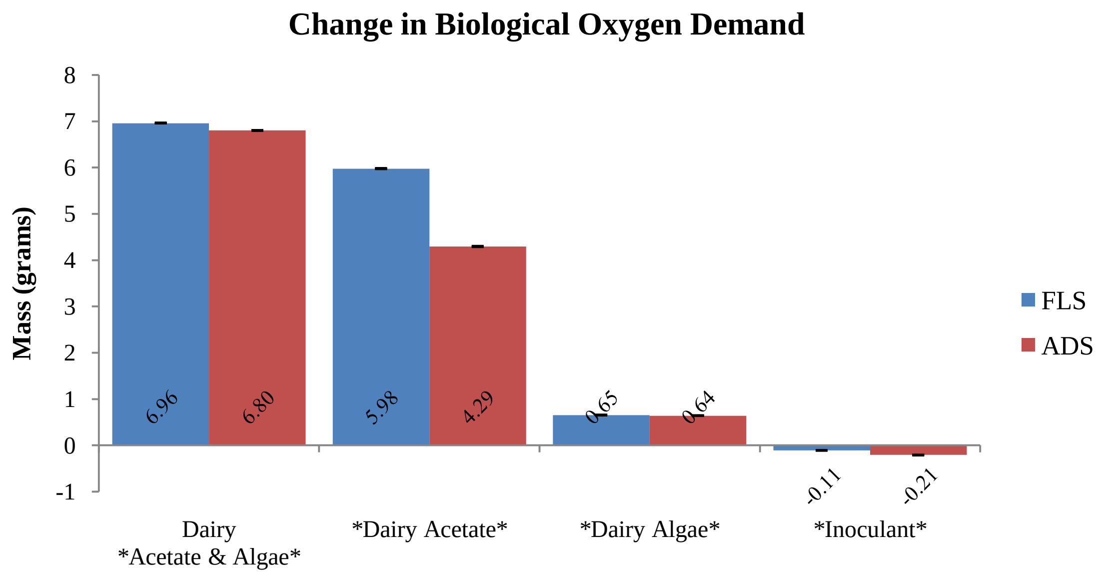

Fig. 6.12 Average change in biological oxygen demand from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (*Dairy*) indicate a significant difference between treatments.

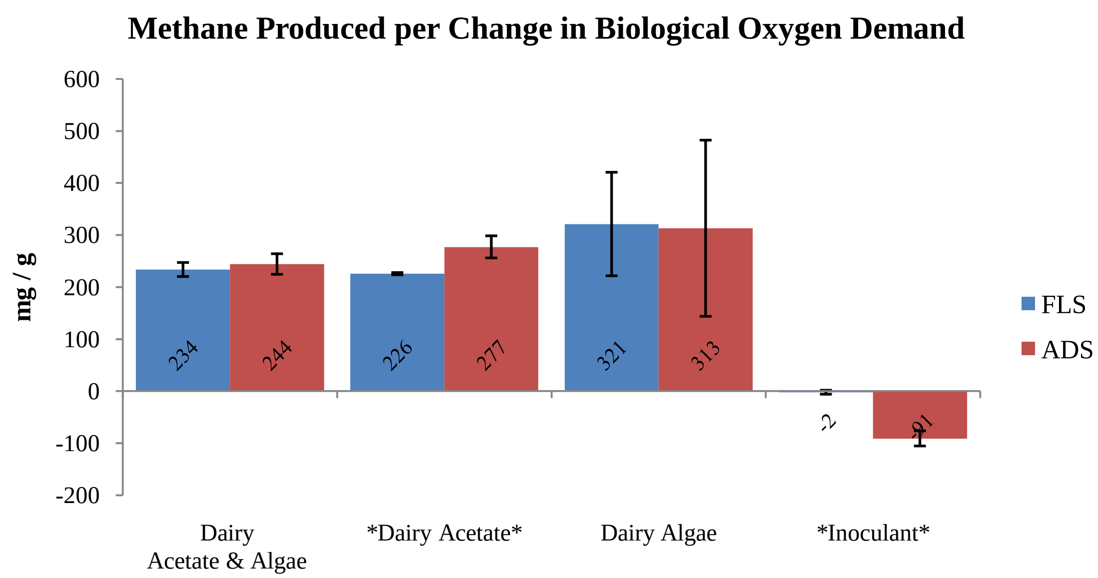

Fig. 6.13 Methane produced per gram of biological oxygen demand for dairy wastewater. Averages of triplicate control treatments shown. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around control (*Dairy Acetate*) indicate a significant difference between treatments.

showed - 91 ± 14.5 mg/g. The standard deviation with FLS treatment is greater than the amount of methane produced per change in BOD, as two of the replicates showed no biogas produced. A significant difference was measured with the change in total organic carbon (TOC) observed with dairy wastewater augmented with acetate (Fig. 6.14). FLS showed a greater decrease in TOC with a change of 2.58 ± 0.08 g where ADS showed a change in TOC of 2.37 ± 0.05 g. Inoculants treating distilled water also showed a significant difference with the change in TOC. FLS showed a greater decrease in TOC than ADS showing a decrease in TOC of 0.32 ± 0.02 g and 0.23 ± 0.01 g respectively.

Dairy wastewater augmented with acetate and dairy wastewater augmented with algae both showed no significant difference in the amount of methane produced per change in TOC (Fig. 6.15). Inoculants treating distilled water showed a significant difference with FLS showing 0.7 ± 1.2 mg/g methane per change in TOC where ADS showed 84.4 ± 16.1 mg/g methane per change in TOC.

All controls treated with FLS and ADS showed a significant difference with the change in chemical oxygen demand as shown in Fig. 6.16. FLS showed a greater change in COD when treating dairy wastewater augmented with acetate. FLS showed a decrease in COD of 6.43 ± 0.06 g where a decrease of 6.00 ± 0.08 g was observed with ADS. ADS showed a greater decrease in dairy wastewater augmented with algae than FLS. FLS showed a decrease in COD by 1.35 ± 0.41 g where ADS showed a greater decrease with 2.12 ± 0.06 g. Inoculants treating distilled water showed results similar to dairy treating algae, with ADS showing a greater decrease in COD (0.03 ± g) than FLS (0.50 ± g). Augmented dairy wastewater showed no significant difference in the change

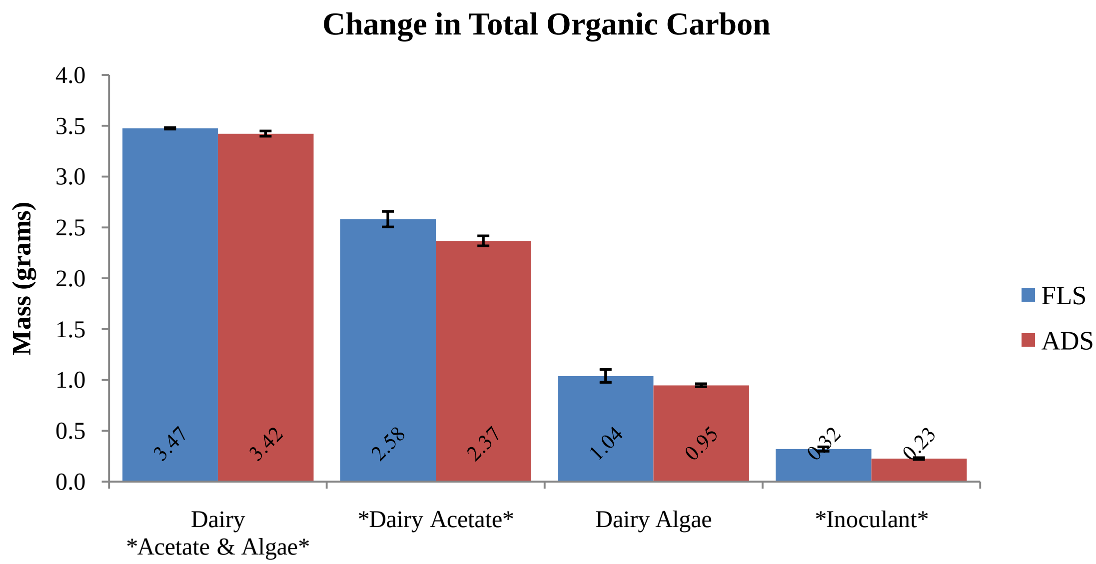

Fig. 6.14 Average change in total organic carbon from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around wastewater (*Dairy*) indicate a significant difference between treatment.

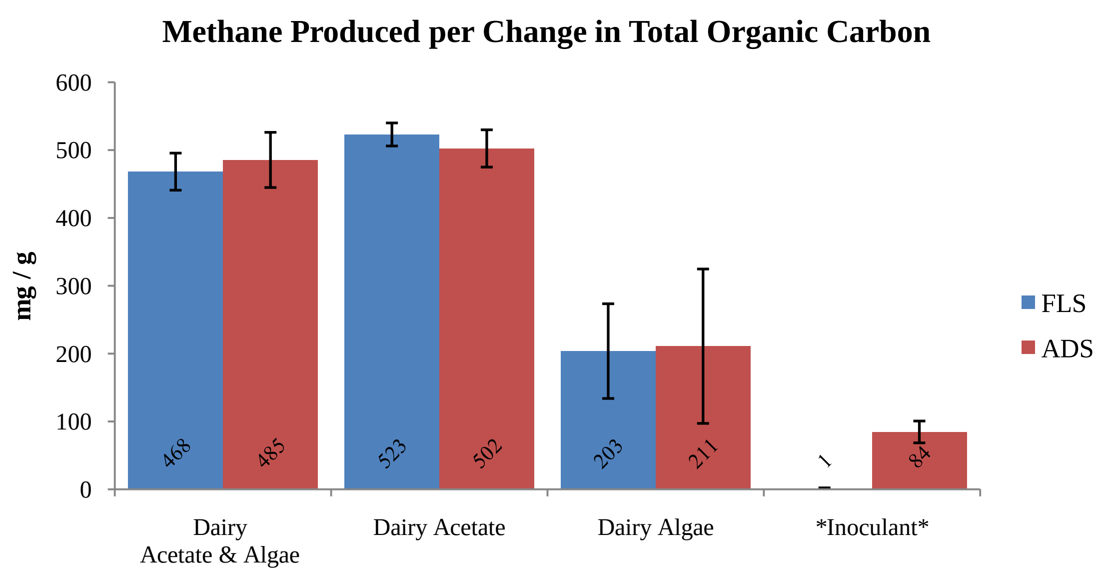

Fig. 6.15 Methane produced per gram of total organic carbon for dairy wastewater. Averages of triplicate control treatments shown. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around control (*Inoculant*) indicate a significant difference between treatments.

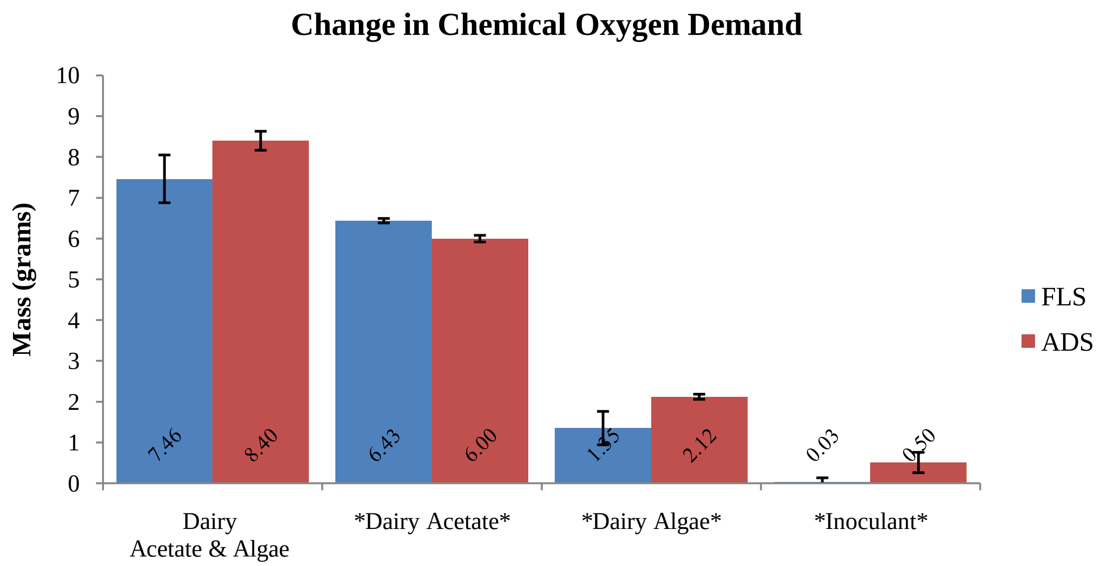

Fig. 6.16 Average change in chemical oxygen demand from triplicate control treatments for dairy wastewater. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around control (*Dairy Acetate*) indicate a significant difference between treatment.

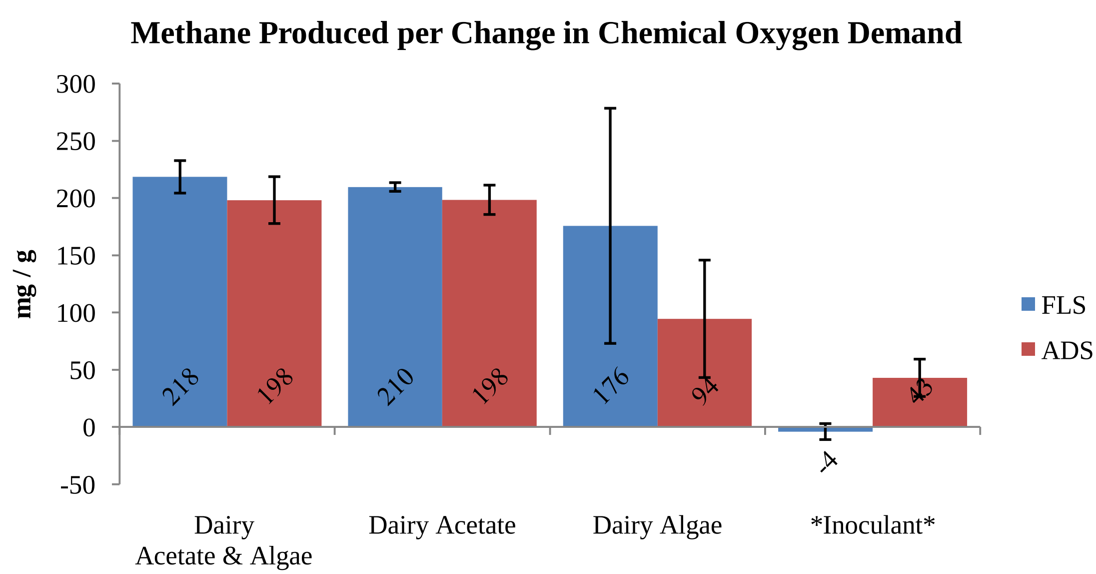

Fig. 6.17 Methane produced per gram of chemical oxygen demand for dairy wastewater. Averages of triplicate control treatments shown. Facultative lagoon sediment (FLS) is shown in blue and anaerobic digester sludge (ADS) is shown in red. Error bars show one standard deviation and asterisks around control (*Inoculant*) indicate a significant difference between treatments.

in COD between FLS and ADS treatments. When dairy wastewater was at a ratio of 181:1 carbon to nitrogen with the absence of algae, treatment by FLS was better at decreasing COD. When acetate was absent and the feed wastewater was at a ratio of 8:1 ADS was better at decreasing COD. When carbon levels where high as seen with dairy augmented with acetate, FLS was significantly better at decreasing COD. With the addition of algae to dairy wastewater, nitrogen levels are higher and carbon structures more complex than acetate are introduced showing ADS to be better at decreasing COD.

No significant difference was found in amount of methane produced per change in COD with dairy was augmented with acetate or dairy was augmented with algae (Fig. 6.17). These results show that a significant difference in the change in COD doesn't reflect a significant difference in the amount of methane being produced from the change in COD.

Inoculants treating distilled water showed a significant difference in the amount of methane produced per change in COD. FLS showed a native amount of methane produced, while ADS was positive. One replicate of the FLS treating distilled water showed a negative change in COD. This replicate was also the only replicate to produce methane, making the average value -4.0 ± 7.0 mg/g. ADS showed 42.9 ± 16.3 mg/g methane produced per change in COD.

Conclusion

Augmenting digester wastewater feed with algae and acetate to a carbon to nitrogen balance of 25:1 showed to be critical for maximum biogas production. The combination of algae and acetate and wastewater with the carbon to nitrogen ratio of 25:1 showed to produce more biogas than the sum of the biogas produced from wastewater augmented with algae at a ratio of 8:1 and wastewater augmented with acetate at a ratio of 181:1.

CONCLUSION ON THE HYPOTHESIS AND OBJECTIVES

It was hypothesized that facultative lagoon sediment (FLS) contains a greater ability to digest wastewater augmented with algae compared to anaerobic digester sludge (ADS). After comparing the treatments by each inoculant it was determined there was not enough evidence to accept this hypothesis. Both inoculants showed high percentages of methane being generated and similar amounts of biogas produced. ADS showed higher rates of biogas production when compared to FLS. This would be advantageous for fast digestion of wastewater with high yields of biogas. A higher methane to carbon dioxide ratio was observed with treatment of wastewaters by FLS. The high methane to carbon dioxide ratio would result in a greater flux of carbon from the organic wastewater to be converted to bioenergy in the form of methane.

The objective of this study was to determine if bioaugmentation with two inoculants, FLS or ADS, demonstrated an advantage in anaerobic digestion of four wastewaters for the production of biomethane. The first comparison was to assess the capacity of each inoculant to digest each of the four selected wastewaters. Each augmented wastewater was successfully digested by both inoculant treatments. Successful digestion of wastewaters by both inoculants show that either inoculant would be feasible for full scale treatment.

The second comparison was made between inoculants treating each wastewater with regard to biogas production as well as the change in organic chemical concentration of each wastewater. Biogas production was started faster with FLS and maintained the average rate of production longer than ADS. ADS showed faster rates of biogas production, and a higher overall average rate. Both inoculants performed equally well in producing the same amount of biogas when given a sufficient amount of time.

ADS showed significantly better than FLS with a greater change in volatile solids and produced more methane per change in biological oxygen demand. FLS showed to be better at reducing biological oxygen demand, total organic carbon and produced more methane per change in volatile solids. Knowing which inoculant is best at reducing biological oxygen demand, total organic carbon, comical oxygen demand, and volatile solids allows a wastewater treatment plant operator to select the best inoculant for treatment of wastewater.

Balancing the wastewater feed ratio to 25:1 carbon to nitrogen (molar ratio) showed to be important in maximizing biogas production. The combination of algae and acetate and wastewater with the carbon to nitrogen ratio of 25:1 showed to produce more biogas than the sum of the biogas produced from wastewater augmented with algae at a ratio of 8:1 and wastewater augmented with acetate at a ratio of 181:1. The proper augmentation of a treatment plants wastewater feed stream with algae to a ratio of 25:1 carbon to nitrogen could increase overall biogas production, creating valuable renewable energy for the plant.

ENGINEERING SIGNIFICANCE

Implementation of anaerobic digestion on a full scale treatment plant would need to be very different from the setup of this research study. It would be impractical to use a batch system when trying to treat wastewater by converting organic material to bioenergy in the form of methane. Using a batch system would require lengthy time periods and large storage of wastewater during the digestion process. If large scale treatment required as much time as shown in this study, treatment plants would need a minimum of 60 digesters, each with a single day's volume of wastewater. As time, volume and other requirements are impractical for full scale batch systems, adjustments will be required for scale up of practices implemented. A continuous flow system is the better choice for treatment of wastewater over the batch system outlined above. Treatment of wastewater by an up flow anaerobic sludge blanket reactor (UASB) or similar continuous flow system would be a better choice for full scale system. These systems allow for wastewater to enter an environment with an established community of microbes and have the complex carbons converted to methane using a shorter hydraulic retention time. This section is intended to bridge the gap between full scale continues flow reactor, and the batch system outlined for this study.

The simplest environmental parameter to apply from the batch reactor to a continuous flow systems is temperature. The temperature of this study was chosen to be 30 degrees Celsius over the more popular temperature of 35 degrees Celsius. Reducing the temperature of a continuous flow reactor would reduce the energy required to treat wastewater. The reduction of energy provides a cost saving alternative to treatment.

Mixing of substrate and microbial community is another factor to consider in scale up of anaerobic digestion. Continuous mixing in a large treatment plant would come at additional cost to plant operations. In this study the samples were mixed at a rate of 80 RPM to help substrate come in contact with microbes. This was implemented to ensure each sample would reach complete digestion by reducing dead zones where no microbial action takes place. In contrast to a batch system, a continuous flow system would allow for the flow of the wastewater through the system aiding in mixing of substrate and microbes. If additional mixing is required implementation of intermittent mixing paddles at a slow speed could be implemented to ensure no dead zones exist in continuous flow system.

Balancing the nutrient requirements required for anaerobic digestion can be a challenging aspect when a full scale treatment plant is considered. Maintaining a wastewater carbon to nitrogen feed ratio of 25:1 can be accomplished in several ways. In this study, sodium acetate was used to increase carbon content of wastewater. It is impractical to use commercial available sodium acetate to increase the carbon content of the wastewater. A more practical approach would be to use another carbon source such as mixed paper (Soboh et al., 2016; Yen and Brune, 2007), blend in food waste, or other high carbon wastes based on local availability. These wastes could be used at a reduced cost for digestion to supplement sodium acetate used in this study.

During the initial startup of a continuous flow anaerobic digester system, microbial communities may not have the density required to digest the wastewater. It may be decided that the digester be feed wastewater and allow the microbes to acclimate before starting the digester as a continues flow system. During this time period, the continuous flow system would resemble the batch systems shown in this study.

Parameters such as startup time, and a scaled maximum rate could be used as a baseline to determine the appropriate time to start the continuous flow system. Starting continuous flow to early could lead to washout of microbes and undigested wastewater resulting with a suboptimal digester. Utilizing the startup times and maximum rate of production determined in this study could allow for plant operators have a better understanding of the digester during startup conditions and be able to make plans for when continuous feed operation could be started.

RECOMMENDATIONS FOR FUTURE WORK

This project has answered many questions, and started some new ones as well. With each wastewater having its own unique carbon to nitrogen ratio the need for augmentation is apparent. In this project acetate and algae were used to augment wastewaters to produce a final wastewater carbon to nitrogen ratio of 25:1. Using this ratio carbon and nitrogen were added to the system. When full scale digesters are being augmented with algae and other blended wastes the question arises how much carbon can be added before it becomes detrimental to the system. The question of the lower bounds in the amount of carbon was also unaddressed, causing question of what concentrations of carbon and nitrogen are required to sustain an anaerobic microbial population.

Other questions that have been left unanswered include the change in rate observed with Facultative Lagoon sediment during initial biogas production (Fig. 4.3). This was observed with three augmented wastewaters (swine, municipal and petrochemical) and at least two controls (Dairy augmented with acetate, as well as algae digested alone). It was hypothesized that the change in biogas formation was caused by change in metabolic pathway, however further study is needed to identify if it is indeed a pathway causing the change in biogas formation and identify the metabolite(s) associated with the change in rate.

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APPENDICES

APPENDIX A: Supplementary Data

BMP Sample number	Inoculant	Treatment or control	Waste	Max Rate	Max Rate time	First Time Rate > 10ml / day just prior to peak	Max average Rate by R2	Avg rate window Days	total gas	total biogas 60 ish
5	FLS	T	Dairy	122.14	26.13	12.65	100.40	33.06	3132.12	3074.41
6	FLS	T	Dairy	128.37	24.63	11.64	105.44	32.52	3278.34	3191.02
7	FLS	T	Dairy	114.75	26.13	12.65	94.18	34.54	2974.41	2924.65
11	FLS	T	Swine	99.46	32.16	14.14	77.59	45.12	3089.92	3008.11
12	FLS	T	Swine	113.57	32.67	15.13	84.97	39.01	3036.80	2985.99
13	FLS	T	Swine	101.49	32.67	13.64	77.58	45.12	3058.99	3010.08
17	FLS	T	Municipal	107.05	23.00	10.66	84.94	40.01	3135.32	3088.25
18	FLS	T	Municipal	110.10	23.20	12.68	87.01	36.00	2913.13	2890.65
19	FLS	T	Municipal	103.20	25.17	11.75	81.83	40.98	3113.71	3063.00
800	FLS	T	Petrochemical	94.71	32.00	11.57	73.92	50.00	3132.92	3115.23
900	FLS	T	Petrochemical	91.33	15.46	11.57	68.63	51.99	3001.83	2991.95
1000	FLS	T	Petrochemical	97.28	15.46	11.57	70.70	48.01	2899.56	2897.08
8	ADS	T	Dairy	148.31	23.13	14.08	117.06	26.51	3329.33	3210.51
9	ADS	T	Dairy	149.05	23.64	14.08	118.64	26.06	3399.59	3263.64
10	ADS	T	Dairy	145.96	23.64	14.08	117.34	25.03	3013.46	2983.70
14	ADS	T	Swine	108.74	32.67	22.63	81.77	28.98	3141.09	2457.81
15	ADS	T	Swine	120.23	27.13	18.14	89.85	27.37	3291.58	2732.90
16	ADS	T	Swine	109.01	32.67	21.64	82.22	28.57	3137.86	2456.04
20	ADS	T	Municipal	113.98	21.17	11.75	89.43	28.37	2859.64	2805.48
21	ADS	T	Municipal	118.39	21.17	11.75	92.67	29.45	3057.97	2985.41
22	ADS	T	Municipal	116.08	21.17	11.75	90.56	28.98	2854.95	2798.88
1100	ADS	T	Petrochemical	116.00	18.44	9.51	87.56	29.94	2781.96	2781.96
1200	ADS	T	Petrochemical	126.83	18.44	9.51	96.59	32.87	3189.08	3178.55
1300	ADS	T	Petrochemical	116.63	22.48	9.51	88.50	34.46	3102.54	3074.21

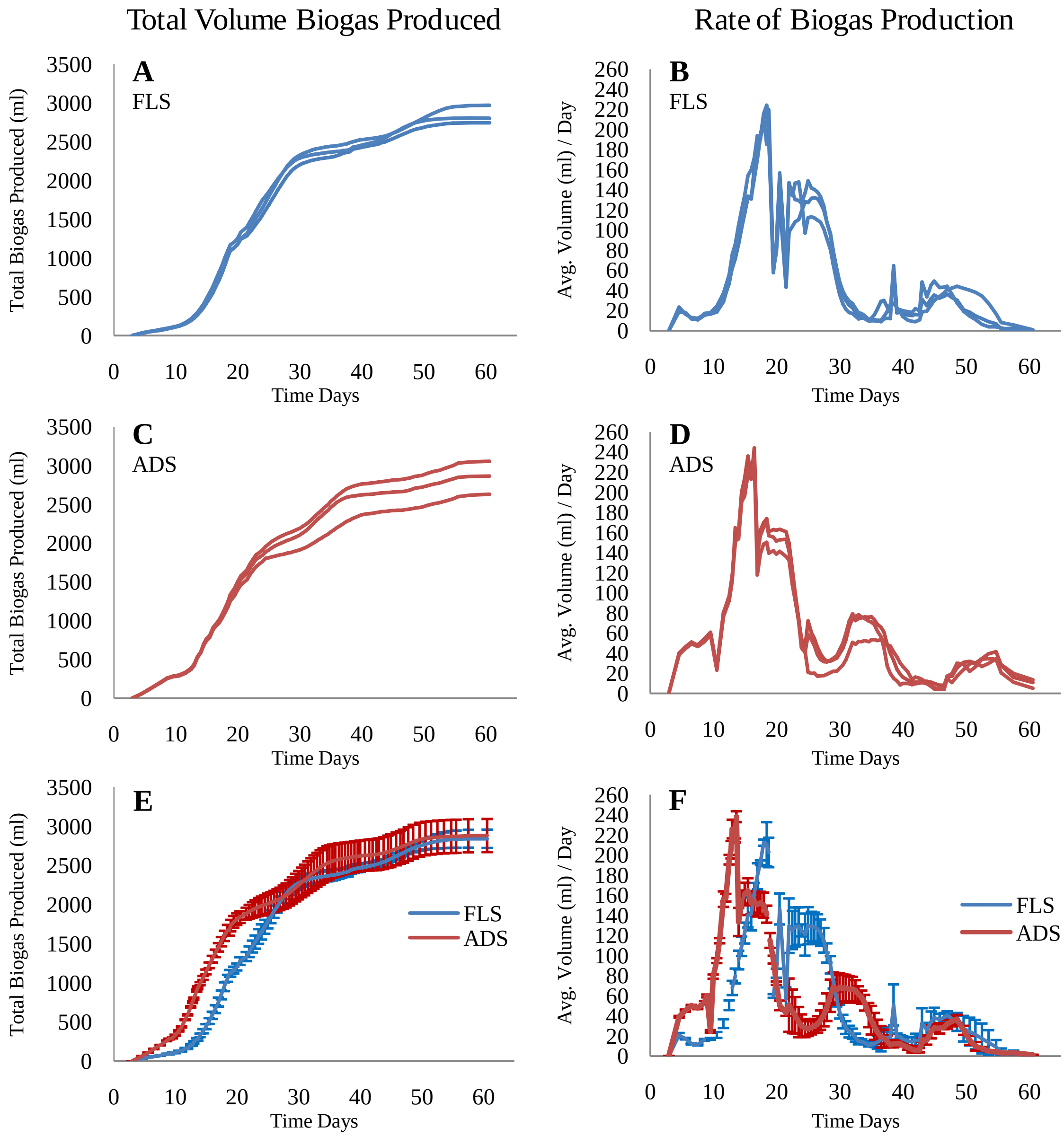
BMP Sample number	Total change in N ml	Total change in Methane ml	Total change in CO2 ml	ml CH4: ml CO2	Max % methane	Total mg of carbon in biogas	total mg CH4	change TOC mg	Change COD mg	Change BOD mg
5	300.84	2492	332	7.50	85.14	1386.48	1634.83	3465.10	6965.57	6958.10
6	290.07	2614	358	7.30	84.56	1459.05	1714.73	3476.13	8107.97	6959.51
7	319.43	2328	317	7.34	83.53	1298.65	1527.38	3479.22	7308.88	6958.42
11	303.14	2538	238	10.67	84.89	1362.80	1665.09	3594.30	8242.25	7665.03
12	322.30	2473	231	10.70	84.95	1327.32	1622.03	3637.50	8126.44	7668.23
13	320.72	2401	227	10.57	84.55	1290.44	1575.33	3575.09	8324.00	7669.79
17	292.89	2600	266	9.78	86.10	1407.03	1705.74	3627.21	8618.13	7664.24
18	187.64	2486	262	9.47	85.50	1349.04	1630.55	3516.37	8926.89	7658.00
19	313.26	2569	257	10.00	86.26	1387.40	1685.45	3644.58	9021.46	7654.56
800	282.53	2570	281	9.16	86.33	1399.36	1685.80	3123.37	7052.46	6232.19
900	276.48	2447	279	8.78	85.23	1337.97	1605.12	3030.76	6539.49	6251.22
1000	281.70	2343	275	8.53	85.70	1285.21	1537.24	3043.56	7112.65	6229.38
8	320.25	2607	401	6.50	82.97	1476.57	1709.94	3392.35	8503.85	6799.82
9	305.05	2687	406	6.63	82.66	1518.43	1762.97	3435.06	8127.73	6801.69
10	347.66	2299	365	6.29	82.20	1307.94	1508.12	3438.18	8553.71	6800.68
14	408.28	2482	257	9.67	84.02	1344.30	1627.97	3563.02	8106.13	7359.34
15	433.49	2582	280	9.21	82.43	1405.35	1694.08	3580.58	8722.28	7359.49
16	423.41	2465	255	9.68	84.63	1335.19	1617.18	3608.52	8575.13	7359.34
20	252.78	2326	297	7.82	85.45	1287.79	1525.73	3083.67	7617.66	4920.19
21	226.70	2519	331	7.61	85.44	1399.17	1652.48	3068.25	7366.56	4907.09
22	186.89	2369	315	7.51	85.30	1317.71	1554.04	2996.92	6910.33	4912.86
1100	288.57	2189	305	7.19	84.75	1223.99	1435.76	3067.82	7460.62	5401.78
1200	291.87	2539	359	7.08	85.27	1422.35	1665.45	3096.64	7201.45	5422.61
1300	247.68	2534	321	7.90	85.63	1401.55	1662.33	3033.79	7089.88	5409.04

BMP Sample number	CH4 mg/ TOC g	CH4 mg / COD g	CH4 mg / g BOD	CH4 mg / g VS	Total 60 change in Methane ml	Total 60 change in CO2 ml	mg CH4 60 days	Max average rate of biogas generated %
5	471.80	234.70	234.95	326966.94	2472	327.41	1621.96	86.80
6	493.29	211.49	246.39	285788.93	2580	351.62	1692.81	85.98
7	439.00	208.98	219.50	218197.21	2317	313.70	1520.33	85.44
11	463.26	202.02	217.23	151371.38	2512	234.97	1647.91	88.87
12	445.92	199.60	211.53	135168.91	2471	229.79	1621.31	88.83
13	440.64	189.25	205.39	121179.31	2402	226.37	1575.56	84.76
17	470.26	197.92	222.56	100337.61	2579	262.49	1691.84	88.76
18	463.70	182.66	212.92	90586.14	2488	261.52	1632.47	89.64
19	462.45	186.83	220.19	88707.98	2546	253.87	1669.99	89.26
800	539.74	239.04	270.50	2107.25	2544	278.36	1668.96	86.81
900	529.61	245.45	256.77	1783.46	2424	276.71	1590.10	85.60
1000	505.08	216.13	246.77	1537.24	2330	273.05	1528.67	86.21
8	504.06	201.08	251.47	213742.03	2538	389.71	1665.25	83.95
9	513.23	216.91	259.20	195885.63	2605	391.64	1709.26	85.34
10	438.64	176.31	221.76	150812.25	2301	362.24	1509.28	83.86
14	456.91	200.83	221.21	116283.50	2014	204.04	1321.15	84.14
15	473.13	194.22	230.19	112938.71	2214	238.29	1452.20	88.91
16	448.16	188.59	219.75	101073.71	1991	203.44	1305.84	85.02
20	494.78	200.29	310.09	76286.26	2293	292.94	1504.40	87.00
21	538.57	224.32	336.75	78689.36	2471	325.15	1620.93	86.80
22	518.54	224.89	316.32	70637.98	2334	310.68	1531.22	86.91
1100	468.01	192.44	265.79	1305.23	2186	303.77	1434.00	85.49
1200	537.82	231.27	307.13	1387.87	2523	356.28	1655.19	85.57
1300	547.94	234.47	307.32	1278.72	2498	316.96	1638.44	86.08

BMP Sample number(Controls)	Inoculant	Treatment or control	Waste	Max Rate	Max Rate time	Max average Rate by R2	Avg rate window Days	total gas	total biogas 60 ish
200	FLS	C	FLS	1.02	15.46				
300	FLS	C	FLS	1.46	15.46				
400	FLS	C	FLS	2.01	4.56				
1400	FLS	C	Big Algae	222.48	18.44	114.09	21.53	2746.71	2746.71
1500	FLS	C	Big Algae	211.26	17.94	122.67	20.54	2806.26	2806.26
1600	FLS	C	Big Algae	224.19	18.44	121.04	22.05	2970.28	2970.28
2000	FLS	C	Dairy Acetate	107.68	32.00	86.05	30.54	2461.68	2458.88
2100	FLS	C	Dairy Acetate	111.53	26.48	86.35	30.33	2498.52	2493.58
2200	FLS	C	Dairy Acetate	112.56	29.98	88.96	29.55	2488.97	2486.18
2600	FLS	C	Dairy Algae	56.17	15.46	38.31	4.49	454.47	454.47
2700	FLS	C	Dairy Algae	36.76	16.46	25.09	14.91	431.90	431.90
2800	FLS	C	Dairy Algae	54.64	15.46	38.81	10.94	655.05	655.05
500	ADS	C	ADS	5.25	4.56				
600	ADS	C	ADS	5.11	4.56				
700	ADS	C	ADS	5.39	4.56				
1700	ADS	C	Big Algae	237.11	13.63	142.11	11.89	2665.46	2665.46
1800	ADS	C	Big Algae	243.87	13.63	152.99	12.90	3089.54	3088.54
1900	ADS	C	Big Algae	233.17	13.63	151.17	11.89	2890.01	2890.01
2300	ADS	C	Dairy Acetate	96.29	33.94	77.61	31.11	2432.33	2409.65
2400	ADS	C	Dairy Acetate	90.03	33.94	72.02	32.01	2228.54	2207.80
2500	ADS	C	Dairy Acetate	83.86	35.95	67.18	32.60	2119.20	2100.39
2900	ADS	C	Dairy Algae	85.96	7.90		1.04	695.33	695.33
3000	ADS	C	Dairy Algae	87.47	7.90		1.18	714.38	714.38
3100	ADS	C	Dairy Algae	40.70	7.90		1.95	169.58	169.58

BMP Sample number(Controls)	Total change in N ml	Total change in Methane ml	Total change in CO2 ml	ml CH4: ml CO2	Max % methane	Total mg of carbon in biogas	total mg CH4	change TOC mg	Change COD mg	Change BOD mg
200	-28.70	1	3	0.31	2.93	2.26	0.72	338.23	-59.73	-109.23
300	-21.10	0	3	0.00	0.00	1.56	0.00	296.23	17.27	-109.23
400	-15.65	0	3	0.00	0.00	1.70	0.00	324.23	136.27	-109.23
1400	339.83	1620	785	2.06	64.25	1180.50	1062.75	2329.27	3638.00	
1500	319.35	1689	798	2.12	64.38	1220.89	1107.79	2406.27	2966.00	
1600	291.12	1826	852	2.14	66.18	1314.63	1197.61	2182.27	2514.50	
2000	228.28	2031	202	10.06	87.10	1096.46	1332.69	2570.83	6495.90	5971.43
2100	227.00	2063	271	7.62	87.02	1145.56	1353.28	2661.22	6386.95	5974.47
2200	210.22	2071	207	9.99	87.40	1118.72	1358.86	2508.96	6421.89	5981.41
2600	-35.96	359	115	3.11	67.52	232.71	235.29	973.81	1429.37	650.73
2700	119.00	206	82	2.51	60.72	141.50	135.17	1099.81	1712.87	650.73
2800	122.65	391	127	3.09	69.29	253.93	256.27	1042.06	904.37	650.73
500	15.17	30	11	2.70	15.05	20.10	19.59	220.30	768.67	-208.40
600	14.29	33	11	2.88	16.20	21.81	21.62	221.35	474.67	-208.40
700	-2.44	24	10	2.37	13.19	16.70	15.69	236.05	271.67	-208.40
1700	253.60	1620	792	2.05	65.81	1183.96	1062.69	2510.93	5103.40	
1800	335.20	1854	900	2.06	66.12	1352.21	1216.46	2157.43	5260.90	
1900	354.23	1714	821	2.09	66.59	1244.69	1124.64	2237.93	4399.90	
2300	222.48	1953	257	7.61	85.22	1084.89	1281.38	2414.23	6088.60	4286.54
2400	181.73	1807	240	7.53	84.79	1004.85	1185.38	2370.52	5937.04	4295.98
2500	223.75	1680	215	7.80	83.55	930.54	1102.21	2316.10	5959.91	4297.86
2900	122.16	401	157	2.55	66.19	274.01	262.99	934.98	2054.77	638.56
3000	137.43	399	161	2.47	65.61	274.80	261.43	962.98	2180.77	638.56
3100	-14.67	114	46	2.50	61.57	78.62	75.00	943.73	2117.77	638.56

BMP Sample number(Controls)	CH4 mg/ TOC g	CH4 mg / COD g	CH4 mg / g BOD	CH4 mg / g VS	Total 60 change in Methane ml	Total 60 change in CO2 ml	mg CH4 60 days	Max average rate of biogas generated %
200	2.14	-12.09	-6.61	3.61	4	3.63	2.34	0.00
300	0.00	0.00	0.00	0.00	0	3.12	0.00	0.00
400	0.00	0.00	0.00	0.00	0	3.26	0.00	0.00
1400	456.26	292.13		759.11	1597	781.66	1047.52	64.51
1500	460.38	373.50		738.53	1668	800.96	1094.47	66.07
1600	548.79	476.28		748.51	1827	852.58	1198.24	66.99
2000	518.39	205.16	223.18	666.34	2025	201.31	1328.38	87.17
2100	508.52	211.88	226.51	644.42	2055	308.86	1348.28	87.11
2200	541.60	211.60	227.18	617.66	2066	206.94	1355.66	87.48
2600	241.62	164.61	361.58	90.50	359	115.35	235.29	76.63
2700	122.90	78.92	207.72	50.06	206	82.18	135.17	75.09
2800	245.92	283.37	393.82	91.52	391	126.60	256.27	75.24
500	88.91	25.48	-93.99	39.17	30	10.67	19.50	64.06
600	97.69	45.56	-103.76	36.04	33	11.11	21.65	67.93
700	66.48	57.76	-75.30	22.42	24	8.99	15.50	62.97
1700	423.23	208.23		625.11	1619	790.99	1062.24	66.40
1800	563.84	231.23		675.81	1854	899.39	1216.33	66.60
1900	502.54	255.61		591.92	1714	820.52	1124.65	67.51
2300	530.76	210.46	298.93	557.12	1922	253.09	1260.63	85.58
2400	500.05	199.66	275.93	493.91	1777	236.86	1166.07	85.49
2500	475.89	184.94	256.46	440.88	1651	212.21	1083.27	85.10
2900	281.28	127.99	411.85	90.69	401	157.24	262.99	70.80
3000	271.48	119.88	409.41	87.14	399	161.23	261.43	69.60
3100	79.47	35.41	117.45	24.19	114	45.82	75.00	85.54



Unused Control - Algae added to reach 3500 mg carbon (No Acetate or Dairy) Total volume and rate of biogas produced from Facultative Lagoon Sediment (FLS) and Anaerobic Digester Sludge (ADS) as inoculants for treatment of algae. Control was not used because of biogas leaking during days 18 through 22 for FLS and days 14 through 22 for treatment with ADS. A) Triplicates of total biogas produced using FLS. B) Triplicates of the rate of biogas produced using FLS. C) Triplicates of total biogas produced using ADS. D) Triplicates of the rate of biogas produced using ADS. E) Average volume of total biogas produced from triplicate treatments of FLS and ADS. F) The average rate of biogas produced from triplicate treatments using FLS and ADS as inoculant source. All error bars represent one standard deviation.

APPENDIX B: Python Code

The following code was used to calculate the concentrations of biogas produced on a linear relationship between gas chromatography measurements.

```
"""
Created on Wed Jun 08 20:01:27 2016

@author: JJP
"""

#Code for calculating rates across whole linear slope while removing points.
        # row then column


#http://stackoverflow.com/questions/1810743/how-to-set-the-current-working-directory-
in-python

import os
import numpy as np
os.chdir('D:\Composition_Rate')

#http://stackoverflow.com/questions/14676265/how-to-read-text-file-into-a-list-or-array-
with-python

from numpy import loadtxt
#measuredpoints = np.genfromtxt('short.txt', delimiter=',', dtype=None)
data_files_in =
['Dairy567.csv','Swine111213.csv','S_Davis171819.csv','Petrochem8910.csv','Inoculant2
34.csv','DairyAcetate202122.csv','DairyAlgae262728.csv','BigAlgae141516.csv','Dairy89
10.csv','Swine141516.csv','S_Davis202122.csv','Petrochem111213.csv','Inoculant567.csv'
,'DairyAcetate232425.csv','DairyAlgae293031.csv','BigAlgae171819.csv']
data_files_in_con =
['Dairy567Con.csv','Swine111213Con.csv','S_Davis171819Con.csv','Petrochem8910Con.
csv','Inoculant234Con.csv','DairyAcetate202122Con.csv','DairyAlgae262728Con.csv','Bi
gAlgae141516Con.csv',
'Dairy8910Con.csv','Swine141516Con.csv','S_Davis202122Con.csv','Petrochem111213C
on.csv','Inoculant567Con.csv','DairyAcetate232425Con.csv','DairyAlgae293031Con.csv',
'BigAlgae171819Con.csv']
data_files_out_concalc =
['Dairy567Out.csv','Swine111213Out.csv','S_Davis171819Out.csv','Petrochem8910Out.c
sv','Inoculant234Out.csv','DairyAcetate202122Out.csv','DairyAlgae262728Out.csv','Big
Algae141516Out.csv',
'Dairy8910Out.csv','Swine141516Out.csv','S_Davis202122Out.csv','Petrochem111213Ou
```

```
t.csv','Inoculant567Out.csv','DairyAcetate232425Out.csv','DairyAlgae293031Out.csv','BigAlgae171819Out.csv']
data_files_out_concalc_each =
['Dairy567Out_volumes.csv','Swine111213Out_volumes.csv','S_Davis171819Out_volumes.csv','Petrochem8910Out_volumes.csv','Inoculant234Out_volumes.csv','DairyAcetate202122Out_volumes.csv','DairyAlgae262728Out_volumes.csv','BigAlgae141516Out_volumes.csv',
'Dairy8910Out_volumes.csv','Swine141516Out_volumes.csv','S_Davis202122Out_volumes.csv','Petrochem111213Out_volumes.csv','Inoculant567Out_volumes.csv','DairyAcetate232425Out_volumes.csv','DairyAlgae293031Out_volumes.csv','BigAlgae171819Out_volumes.csv']
headersconcalc = 'time, Nitrogen, Methane, Carbon Dioxide, Nitrogen, Methane, Carbon Dioxide, Nitrogen, Methane, Carbon Dioxide'

    # for each iteration of treatment
for data in range(0,16):
    measuredVolumes = loadtxt(data_files_in[data], comments="#", delimiter=",", unpack=False)
    concentration = loadtxt(data_files_in_con[data], comments="#", delimiter=",", unpack=False)
    con_matrix = np.zeros([concentration.shape[0],10]) #matrix the in gas compostion for each treament
    volumes_of_each = np.zeros([measuredVolumes.shape[0]+1,10])
    # for each iteration of treatment
    for y in range(1,10,3):
        #sets up size of matrix to hold equations
        diagonal = 0
        eqnlength = concentration.shape[0] + measuredVolumes.shape[0] + 1
        eqns = np.zeros([eqnlength,eqnlength])
        #puts start and final volumes in on diagonal
        while diagonal < measuredVolumes.shape[0]:
            #eqns[diagonal,0] = measuredVolumes[diagonal-1,3]
            eqns[diagonal,diagonal] = measuredVolumes[diagonal,y]
            eqns[diagonal,diagonal+1] = -measuredVolumes[diagonal,y+1]
            diagonal = diagonal + 1
        eqns[diagonal,0] = 1
        # adds chance of volumes and 1 in eqns for concentration values.
        volume_column = diagonal + 1
        concentration_row = 0

        for x in range(0,diagonal):
            if measuredVolumes[x,0] != concentration[concentration_row,0]:
                eqns[x,volume_column] = measuredVolumes[x,y+2] # puts volumes in in the eqns columns
```

```
            if measuredVolumes[x,0] == concentration[concentration_row,0]:
                eqns[x,volume_column] = measuredVolumes[x,y+2]
                eqns[volume_column,x+1] = 1
                volume_column = volume_column + 1
                concentration_row = concentration_row + 1
        #eqns[volume_column-1,diagonal-1] = 1 #adds duplicate value on the bottom.

        #creates answer values for linear solving
        ansn = np.zeros([eqnlength,1])
        ansn[measuredVolumes.shape[0],0] = 100
        anso = np.zeros([eqnlength,1])
        anso[measuredVolumes.shape[0],0] = 0
        ansh = np.zeros([eqnlength,1])
        ansh[measuredVolumes.shape[0],0] = 0
        for x in range(1,concentration.shape[0]+1):
            ansn[measuredVolumes.shape[0] + x,0 ] = concentration[x-1,y]
            ansh[measuredVolumes.shape[0] + x,0 ] = concentration[x-1,y+1]
            anso[measuredVolumes.shape[0] + x,0 ] = concentration[x-1,y+2]

        #np.savetxt("eqns.csv", eqns, delimiter=",")
        n = np.linalg.solve(eqns, ansn)
        h = np.linalg.solve(eqns, ansh)
        o = np.linalg.solve(eqns, anso)
        if y == 1:
            firstout = np.zeros([eqnlength,10])
            for x in range(0,measuredVolumes.shape[0]):
                firstout[x+1,0] = measuredVolumes[x,0] # time for each individual
concentration
                volumes_of_each[x+1,0] = measuredVolumes[x,0] #individual time for matrix
to calculate volumes of each gas produced.  trying to solve for total volume methane
produced
            for x in range(measuredVolumes.shape[0]+1,eqnlength):
                firstout[x,0] = concentration[x-measuredVolumes.shape[0]-1,0] # puts the time
spot on calculated concentration of biogas produced.
        for x in range(0,eqnlength):
            firstout[x,y+0] = n[x,0]
            firstout[x,y+1] = h[x,0]
            firstout[x,y+2] = o[x,0]

    np.savetxt(data_files_out_concalc[data], firstout, fmt='%.9f',
delimiter=",",header=headersconcalc)
#    np.savetxt('eqns.csv', eqns, fmt='%.9f', delimiter=",")
#    np.savetxt('ansh.csv', ansh, fmt='%.9f', delimiter=",")
#    np.savetxt('ansn.csv', ansn, fmt='%.9f', delimiter=",")
    #setup concentration multiplier
```

```
    for row in range(0,concentration.shape[0]):
        for column in range(0,10):
            con_matrix[row,column] = firstout[row+diagonal+1,column]
    current_con_in = 0
    for time in range(0,measuredVolumes.shape[0]): #while time < con_matrix[-1,0]:
        for y in range(1,10,3):
            volumes_of_each[time+1,y+0] = measuredVolumes[time-
1,y+2]*con_matrix[current_con_in,y+0] / 100. #nitrogen
            volumes_of_each[time+1,y+1] = measuredVolumes[time-
1,y+2]*con_matrix[current_con_in,y+1] / 100. #meth
            volumes_of_each[time+1,y+2] = measuredVolumes[time-
1,y+2]*con_matrix[current_con_in,y+2] / 100. #Co2
        if volumes_of_each[time,0] == con_matrix[current_con_in,0]:
            current_con_in = current_con_in + 1

    np.savetxt(data_files_out_concalc_each[data], volumes_of_each, fmt='%.9f',
delimiter=",",header=headersconcalc)
```

The following code was used to calculate the average rate and the duration of average rate as shown in Fig. 3.5

```
"""
@author: JJP
"""

#Code for calculating rates across whole linear slope while removing points.
#http://stackoverflow.com/questions/1810743/how-to-set-the-current-working-directory-
in-python

import os
import numpy as np
os.chdir('D:\RateCode')

#http://stackoverflow.com/questions/14676265/how-to-read-text-file-into-a-list-or-array-
with-python

from numpy import loadtxt
#from numpy import genfromtxt
#measuredpoints = np.genfromtxt('short.txt', delimiter=',', dtype=None)
xy = loadtxt("Dairy5_10.csv", comments="#", delimiter=",", unpack=False)
#print measuredpoints
#http://docs.scipy.org/doc/numpy/reference/generated/numpy.insert.html
#measuredpoints = np.insert(measuredpoints, 2, 0,axis=1)
#print len(measuredpoints)
```

```
#print measuredpoints

#setup output array
ratearray = np.zeros([len(xy)+1,36])

#setup x, and x^2 for calculations
linearvalues = np.zeros([len(xy),3])
for w in range(0,len(xy)):
        linearvalues[w,0] = xy[w,0]
        linearvalues[w,2] = xy[w,0]*xy[w,0]
#put y values in linear values and then repeats for each treatment.

for j in range(4,20,3):
    for a in range(0,len(xy)):
        linearvalues[a,1] = xy[a,j]
    r2 = 0
    outarraycounter = 1

    k = int(2.*j-8.)

    #ratearray = ([['Left point','Right point', 'slope','intercept','R2']])

    toprow = 0
    bottomrow = (len(xy))-1
#   ratearray[0,k+0] = int(1)
#   ratearray[0,k+1] = int(2)
#   ratearray[0,k+2] = int(3)
#   ratearray[0,k+3] = int(4)
#   ratearray[0,k+4] = int(5)

    #print ratearray
    sumx2 = 0
    sumx = 0
    sumxy = 0
    sumy = 0
    n = 0
    SSE = 0
    SST = 0

    while r2 < 1:
        for i in range(toprow,bottomrow+1):
            #print measuredpoints[i,0]
            sumx2 = linearvalues[i,2] + sumx2
            sumx = linearvalues[i,0] + sumx
            sumxy = linearvalues[i,1]*linearvalues[i,0] + sumxy
```

```
        sumy = linearvalues[i,1] + sumy
    n = bottomrow-toprow+1
    #print n

    eqns = np.array([[sumx2,sumx],[sumx,n]])
    solsn = np.array([sumxy,sumy])
    slopepoint = np.linalg.solve(eqns, solsn)
    #print slopepoint
    for i in range(toprow,bottomrow):
        SSE = ((linearvalues[i,1]-
(slopepoint[0]*linearvalues[i,0]+slopepoint[1]))**2)+SSE
        SST = ((linearvalues[i,1]-sumy/n)**2)+SST
    r2 = 1 - SSE/SST
    #print r2
    #nextline = [[measuredpoints[toprow,0],measuredpoints[bottomrow-
1,0],slopepoint[0],slopepoint[1],r2]]
    #print nextline
    #ratearry = np.concatenate((ratearray, nextline), axis=0)
    ratearray[outarraycounter,k+3] = linearvalues[toprow,0] # left point
    ratearray[outarraycounter,k+4] = linearvalues[bottomrow,0] #Right point
    ratearray[outarraycounter,k+2] = linearvalues[bottomrow,0] - linearvalues[toprow,0]
#time frame of aveverage values
    ratearray[outarraycounter,k+1] = slopepoint[0] #slope
    ratearray[outarraycounter,k+5] = slopepoint[1] # y Intercept
    ratearray[outarraycounter,k+0] = r2 # R^2
    #calculates the degree to which point is farther away.
    rtop = (linearvalues[toprow,0]*slopepoint[0]+slopepoint[1]-
linearvalues[toprow,1])**2
    rbottom = (linearvalues[bottomrow,0]*slopepoint[0]+slopepoint[1]-
linearvalues[bottomrow,1])**2

    if rtop > rbottom :
        toprow = toprow  + 1
        #print "top Gone"
    else:
        bottomrow = bottomrow - 1
        #print "bottom gone"

    sumx2 = 0
    sumx = 0
    sumxy = 0
    sumy = 0
    n = 0
    SSE = 0
    SST = 0
```

```
        outarraycounter = outarraycounter + 1

        #print slopepoint
        #print r2
    #print ratearray
headers = 'R2, slope, Avg rate window, Left point, Right point, Intercept, R2, slope, Avg rate window, Left point, Right point, Intercept, R2, slope, Avg rate window, Left point, Right point, Intercept, R2, slope, Avg rate window, Left point, Right point, Intercept, R2, slope, Avg rate window, Left point, Right point, Intercept, R2, slope, Avg rate window, Left point, Right point, Intercept'

np.savetxt("Dairy5_10Out.csv", ratearray, fmt='%.9f', delimiter=",",header=headers)
```