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Anaerobic digestion of two feedstocks in a solid state system: Algae and Beef Carcass

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ABSTRACT Solid state anaerobic digestion (AD) technology for the production of biogas is an alternative to more popular liquid state anaerobic digestion systems. In this study, the viability of solid state AD with various feedstocks was evaluated. Two feedstocks were chosen for this study; algae and beef carcass, each mixed with solid beef cattle manure. A bench scale solid state digester unit was used to evaluate the gas production and quality from the feedstock trials. Inoculation strategies were compared in the algae trial with 0%, 10% and 25% inoculum by weight at 40% algae composition and manure making up the remainder. For the beef carcass trial, 25% inoculum was used and carcass content was compared at 5%, 15% and 25% by weight with manure constituting the remainder of the mix. Weekly leachate recirculation was also tested on a subset of each mix. Carcass material produced significantly more biogas with higher methane percentages at all points compared to algae. Effects of weekly leachate recirculation were substantial for most mixes in both trials and were inconsistent between treatments. Inoculum strategies also produced significant results in the algae trial, with 25% inoculum producing the most biogas, while 0% inoculum produced little to no biogas and exhibited a toxic drop in pH and an increase in hydrogen sulphide production. Carcass loading schemes produced inconsistent results, with 5% and 25% carcass concentrations having similar results, while the 15% application produced the least amount of biogas.

Keywords: solid state, anaerobic digestion, dry fermentation, algae, manure, beef carcass, biogas production

INTRODUCTION

Anaerobic digestion (AD), specifically liquid state digestion (L-AD) has been successfully used for decades to convert organic wastes (municipal solid waste (MSW), agricultural, industrial) into biogas. This biological process has been used in developing countries for the production of biogas for cooking and lighting fuel as well as waste treatment and electricity generation. In contrast, solid-state anaerobic digestion (SS-AD) has only recently been implemented in Europe with success since the early 1990's for the treatment of up to 10% of the total organic and MSW in solid state digesters (DeBaere, 2000; Shafer et al., 2006). The common L-AD systems are generally comprised of a 0.5% to 5% solids concentration in the digestion liquor, while SS-AD has a solids content upwards of 15-50%, or an equivalent moisture content of 50-85% (Farneti et al., 1999; Rapport et al., 2008). The applicability of a SS-AD system to cattle feeding operations has the potential to deal with a waste product by producing energy and a fertilizer end-product. In Western Canada, approximately 200 large feedlot operations in Alberta and Saskatchewan alone, could provide enough solid waste to generate roughly 6,000,000 GJ of energy annually (Canfax, 2011; SMA, 2012). To put into context, each home in Saskatchewan requires roughly 100 GJ of energy each year for heating. If all the energy from manure in western Canadian feedlots were converted to energy, that would be enough to heat 60,000 homes. Advantages to using a solid state system in Western Canada include: smaller reactor requirements, lower energy input, decreased water usage, and simple batch systems.

The Canadian climate poses limitations to implementing SS-AD due to temperature fluctuations causing associated vessel sealing issues. These sealing issues can cause a loss of methane to the atmosphere as well as the inability to maintain anaerobic conditions, which is imperative for healthy methanogenesis to occur (Rapport et al., 2008). In order to better assess and implement this technology in a harsher climate, bench-scale and pilot scale studies are needed. The Prairie Agricultural Machinery Institute (PAMI) in Humboldt, Saskatchewan, initiated a pilot scale SS-AD research program focusing their efforts on the implementation and research associated with biogas production from feedlot manure in Western Canadian climates (Agnew et al., 2011). Others have studied various components of the SS-AD system including inoculation strategies (Li et al., 2011a; Li et al., 2011b; Shafer et al., 2006), leachate recirculation (El-Mashad et al., 2006; Kusch et al., 2012) and temperature regimes (Golueke et al., 1957; Kim et al., 2002; Parker et al., 2002) with mixed results. For implementation of this technology in Western Canada, a variety of parameters such as initial inoculum concentration, leachate recirculation strategies and various substrates' biogas potential should be analyzed and best management practices created.

For the purpose of this study, two substrates (algae and beef carcass) mixed with beef feedlot manure will be assessed for biogas potential, as well as various inoculation and leachate recirculation tactics under mesophilic (40° C) conditions. Algae has been used for decades in wastewater treatment (Golueke et al., 1957; Muller and Slezak, 2009) and more recently biofuel production (Alcántara et al., 2013; Demirbas, 2010), disposal of excess algae via AD could be a valuable waste disposal option. Some have studied implementation of algae into liquid AD units and found it produced similar biogas results to that of MSW (Alcántara et al., 2013; Golueke et al., 1957; Muller and Slezak, 2009), while others have shown low methane potential (Muller and Slezak, 2009) and coupled process toxicity involving a dangerous buildup of hydrogen sulfide (H_2S) (Cecchi et al., 1996; Haghighatafshar, 2012). On-farm carcass disposal via AD could provide an attractive energy boost to a system as well as alleviating disposal costs associated with pickup and rendering. Many have studied the digestibility of animal carcasses and slaughterhouse waste with some success in the L-AD realm, finding that concentrations of carcass above 5% generally hindered the process due to the buildup of long chain fatty acids and ammonia (Hejnfelt and Angelidaki, 2009; Massé et al., 2008; Massé et al., 2010). It has been shown that ammonia concentrations exceeding 150 mg/L are proven toxic in AD (Speece, 2008). There is a general lack of information about methane yields for carcass material in not only L-AD (Hejnfelt and Angelidaki, 2009; Martin et al.), but a complete lack of information for SS-AD.

METHODS & MATERIALS

Incubator Set-Up

A bench scale SS-AD system constructed at PAMI and the University of Saskatchewan was used to assess biogas production and quality for this study (Gaudet and Fonstad, 2012). The system consists of two incubators with 9 vessels each capable of holding approximately 4-6 kg of substrate (Fig. 1). Each vessel is equipped with a thermocouple, gas line and leachate recirculation line. Gas production was measured with wet tip gas meters (*Wet Tip*) calibrated to tip for every 80 mL of gas produced. A data logging system (*DataTaker D80*) was used to record temperatures via thermocouple (in-vessel, incubator, and room), barometric pressure, and gas tip events. Upon loading, initial substrate weights and headspace volume were recorded upon execution with a sub-sample of each mix sent to ALS Laboratories (Saskatoon, SK) for analysis of moisture content, EC, total carbon by combustion, loss on ignition, total nitrogen, ammonia-N, nitrate-N, pH, total P, K and S by standard methods. Gas samples for each trial were taken daily for the first ten days and then incrementally on Monday, Wednesday, and Friday for the duration of the experiment. A total of 30 mL was purged via syringe from the sample line before a 15 mL gas sample was drawn and placed into a vacutainer for gas chromatography (GC) analysis for percent carbon dioxide (CO_2) and methane (CH_4). At the end of the trial, leachate was evacuated, vessels were weighed and a subsample of each mix was again sent to ALS Laboratories for the same analysis as the initial samples.

Figure 1: Incubator set-up with 9 vessels

Algae Trial

To assess biogas production of algae mixed with feedlot manure, algae was sourced from the St. Lawrence River by Rio Tinto, it was then dried and ground for homogeneity and shipped to the University of Saskatchewan. For the trial, the algae was rewetted with distilled water at a ratio of 2:1 weight of water to algae. To assess inoculum strategies, three percentages of inoculum from a previous beef feedlot AD trial were used; 0, 10% and 25% by weight for mix 1, 2, and 3 respectively, with each containing 40% algae by weight and the remainder made up with beef feedlot manure (Table 1). Six vessels of each mix were loaded with approximately 3.5 to 4 kg of substrate with 3 vessels of each mix receiving leachate recirculation every 7 days. All trials were conducted in triplicate and average values reported. In order to increase the moisture content of the mix and to provide a leachate to recirculate, 2.6 L of water was added to each vessel. At the end of the trial, leachate was evacuated, vessels were weighed and a subsample of each mix was again sent to ALS Laboratories for the same analysis as the initial samples.

Table 1: Algae trial mix ratios by weight

	Mix 1	Mix 2	Mix 3
Inoculum	0%	10%	25%
Algae	40%	40%	40%
Manure	60%	50%	35%

Carcass Trial

To assess biogas production of beef carcass mixed with feedlot manure, a hind-quarter of beef carcass was sourced from Western Beef Development Centre (Lanigan, SK). The hind-quarter was received frozen and macerated into 2.5 cm or less fragments and was kept at 4° C until vessel loading. This piece of carcass contained a majority of muscle tissue with some fatty tissue along with the associated bone, skin and hair. Rumen material was not included. All substrate mixes with carcass material contained 25% inoculum by weight from a previous beef feedlot AD trial. In order to assess the implications of various quantities of carcass material to the system, 5, 15, and 25% of carcass material by weight was added to mix 1, 2, and 3 respectively and the remainder made up with beef feedlot manure (Table 2). Six vessels of each mix were loaded with approximately 3.5 to 4 kg of substrate with 3 vessels of each mix receiving leachate recirculation every 7 days. In order to increase the moisture content of the mix and to provide a leachate to recirculate, 1.5 L of water was added to each vessel. At the end of the trial, leachate was evacuated, vessels were weighed and a subsample of each mix was again sent to ALS Laboratories for the same analysis as the initial samples.

Table 2: Beef carcass trial mix ratios by weight

	Mix 1	Mix 2	Mix 3
Inoculum	25%	25%	25%
Beef carcass	5%	15%	25%
Manure	70%	60%	50%

Gas Analysis

Gas samples were analyzed for methane using a Varian CP-3800 gas chromatograph, while carbon dioxide was assessed using a Varian Micro-GC CP-2003 with a sample time of 30 seconds and a 180 second run time. Carbon dioxide was detected using a thermal conductivity detector (TCD) coupled with a Poraplot U column 10 m in length with a 0.32 mm inner diameter. Column temperature for the TCD was 45 °C with an injector temperature of 55°C with an injection time of 25 ms under 100 kPa. The detection limit for CO_2 measurement with this device is 80 ppm. The detector used for methane analysis was a flame ionization detector (FID) which uses a Poraplot Q8 column that is 3.65 m in length with a 3.175 mm diameter and 2 mm film thickness. Column temperature for the FID was 45 °C with an injector temperature of 55°C with an injection time of 20 ms under 140 kPa. The detection limit for methane on this instrument was 360 ppb. The carrier gas used in all detectors was helium for both CO_2 and CH_4 measurement.

Substrate Analysis

Subsamples of each substrate mix pre and post AD were sent to ALS Laboratories (Saskatoon, SK) for analysis. Moisture content was determined using ASTM D2216-80 (ASTM, 2010) and total carbon, phosphorus, potassium and sulphur by Soil Science Society of America Standards (SSSA, 1997). Ammonia-N and nitrate-N were determined using standards from Canadian Society of Soil Science standards (Carter, 1993) while total N using RMMA A3769 3.3 (Wolf et al., 2005). Test Methods for the Examination of Composting and Compost standards (Leege and Thompson, 2001) were used to determine EC (4. 10-A), loss on ignition to determine volatile solids (VS) (05.07-A) and pH (4.11-A).

Statistical Analysis

Analysis of variance (ANOVA) using Minitab was used to determine significant difference between trials and treatments with a significance level of 0.05. All digestion treatments (mixes) were conducted in triplicate and average values are reported.

RESULTS

Algae Trial

Effect of Inoculum Strategy Under mesophilic conditions, solid-state anaerobic digestion of algae demonstrated that increased inoculum application yielded more biogas at all points during the trial. Between 1 and 2 days, temperature effected gas expansion showed a quick peak and immediate drop of gas production, with a leveling off over a period of approximately 5 days, followed by increasing gas production for the duration of the trial (Fig. 2a). Mix 1 (no inoculum) yielded a very minute amount of biogas, with daily production rates averaging 80 mL per day or less as compared to mix 2 and mix 3 (10, 25% inoculum, respectively) which yielded average gas production values of approximately 4.5 and 7 L per day respectively. Figure 2c aligns this with percent methane concentrations over the same time period. Percent methane was also higher in the increased inoculum mixes than those mixes without inoculum. Cumulative biogas production shows a larger volume of gas was created as more inoculum material was added, with 25% inoculum concentration consistently producing the most biogas. ANOVA tests suggest a significant difference ($p<0.05$) in biogas production between mix 1, mix 2 and mix 3, ($p=0.001$).

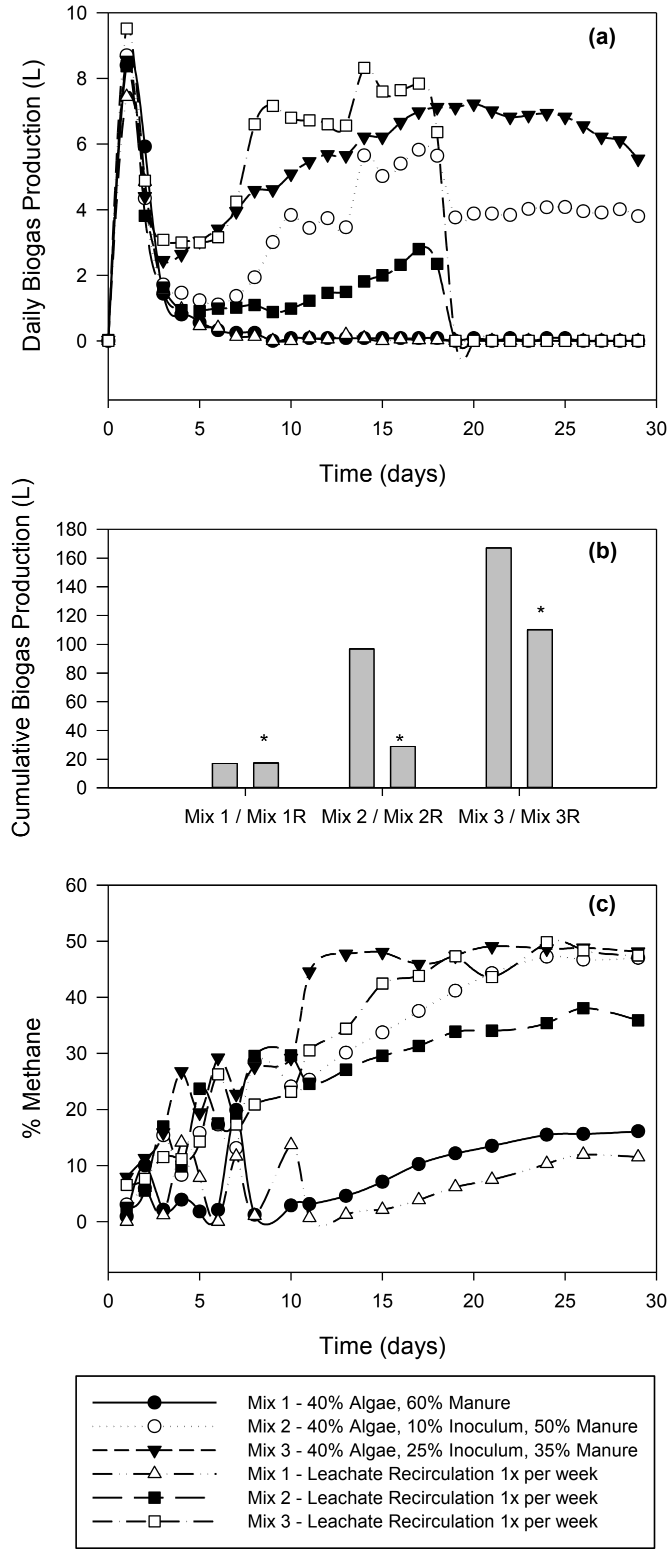
The effect of inoculum strategy on volatile solids produced inconsistent results, with 15% inoculum having the greatest reduction in VS followed by no inoculum and 25% inoculum (Table 3). It could be said that due to mix 3 containing 25% predigested material, there was less volatile solids to begin with and the conversion process may have been hindered by this, although on the contrary, those with higher inoculum concentrations produced a larger quantity of biogas. Mix 1 and 3 that did not receive recirculation had similar reductions in total nitrogen, carbon and sulphur, with mix 2 producing around half the reduction of each. Hydrogen sulfide (H_2S) concentrations reached approximately 100 ppm in some of the vessels throughout the trial. High concentrations of sulphur in algae can easily account for the development of dangerous levels H_2S in the system (Haghighatafshar, 2012). Mix 1 and mix 2 showed a reduction in pH from the beginning to the end of the trial, the pH of mix 3 increased slightly from 7.59 to 8.48. The reduction of pH for mix 1 in particular, reached toxic levels dropping from 6.72 to 5.72, demonstrating that the lack of inoculum material and drop in pH to a level lower than required for methanogenesis, caused AD of algae to fail (Cioabla et al., 2012).

Table 3: Algae trial % reduction results, all values adjusted for initial and final moisture content where appropriate (R indicates recirculated treatments)

ALGAE		Total N	Total S	Total C	Total P	Total K	VS %		pH initial	pH final
% Change	Mix 1	46	70	57	39	78	8.9	pH Units	6.72	5.72
	Mix 2	27	70	32	7	30	12.4		7.62	6.35
	Mix 3	51	74	66	64	64	6.3		7.59	8.48
	Mix 1R	18	47	38	31	43	9		6.72	5.58
	Mix 2R	31	31	31	49	24	4.5		7.62	7.12
	Mix 3R	47	60	50	59	52	3.1		7.59	8.42

Effect of Leachate Recirculation Leachate recirculation for the algae trial resulted in increased biogas production over the 30 days, but decreased methane concentration compared to the non-recirculated sets. Those with higher inoculum concentration that were recirculated also produced more biogas to those that were non-recirculated, with the highest inoculum concentration producing the most biogas compared to other mixes. Due to a glitch in the data logging system during this trial, accurate results for gas tips in the recirculated vessels after day 20, are not available. Figure 2b, highlights the total biogas production and if recirculated data was extrapolated for the missing dataset, it would show that the recirculated mixes produced more biogas. All mixes showed a 3 to 9 percent reduction in volatile solids, with mix 1 having the highest percent reduction and mix 3, the lowest (table 3). Non-recirculated mixes, in general, performed better with respect to total methane production (Table 4). Total nitrogen, sulphur and carbon showed the greatest reduction on the uncirculated sets, with 25% inoculum (mix 3) having the highest reduction of all elements, similarly to the recirculated version of the same mix. The pH changes for each mix receiving recirculation were similar to those that did not receive recirculation. Mix 1 and mix 2 showed a reduction in pH from the beginning to the end of the trial, the pH of mix 3 increased slightly from 7.59 to 8.42. The reduction of pH for mix 1 in particular, reached toxic levels dropping from 6.72 to 5.58, demonstrating that the lack of inoculum material as well as weekly recirculation in this case caused AD to fail. The observed differences were confirmed by the ANOVA test showing significant difference between all recirculated mixes ($p=0.037$). When comparing non-recirculated to recirculated of the same mix, no significant difference was observed except for mix 2 for the first 15 days of the trial, due to data logging error, significance determination is not accurate.

It was observed that after approximately 7 days into the trial, many vessels no longer had leachate, or had a small quantity in the bottom to recirculate. Upon inspection at the end of the trial, most of the leachate had pooled at the top of the vessel, probably due to swelling of the rewetted algae. Due to the effect of leachate recirculation causing this pooling, the majority of the leachate ended up in the final post-AD sample sent to ALS Laboratories for analysis. Due to this, weighing of the vessels prior to removing the cap provided little information about the water pooled at the top, therefore, a complete water balance was not possible



*Complete data set unavailable due to data logging error

Figure 2: Algae AD trial, (a) daily biogas production (L), (b) Biogas yield, (c) % methane produced

Table 4: Total methane produced (L/kg VS) with data extrapolation for recirculated mixes with missing data

	Total Methane Production (L/kg VS)	
	Non-recirc	Recirc
Mix 1	0.8	0.5
Mix 2	35.2	14.9
Mix 3	70.1	55.8

Beef Carcass Trial

Effect of Carcass Loading Strategy Solid-state anaerobic digestion of beef carcass showed unexpected results. Similar to literature, a loading rate of only 5% carcass material (mix 1) produced the highest daily biogas, but, 25% carcass material (mix 3) wasn't significantly lower in production compared to mix 1. Similar to the algae trial, between days 1 and 2 rapid gas expansion due to temperature occurred, followed by a decline for 4-6 days followed by a fairly steady increase in daily gas production for the remainder of the trial (Fig. 3a). Mix 2 (15% carcass) averaged approximately 4.5 L of gas produced per day and was the lowest daily producer; mix 1 produced an average of 7 L per day. Percent methane concentrations (Fig 3c), reached a maximum of approximately 65% for non-circulated mixes 1, 2, and 3; approximately 15-20% more methane that similar algae mixes. Cumulative biogas production demonstrates mix 1 produced the most biogas over the course of the trial, and mix 2, the least, while mix 3 is nearly similar to mix 1 (Fig 3b), ANOVA test and Tukey method confirmed a significant difference among the three loading strategies ($p=0.059$), with mix 1 being significantly different than mix 3. The carcass trial in general produced nearly double the biogas compared to the algae trial.

Percent reduction in volatile solids somewhat contradicts biogas production results, with the lowest biogas producer (mix 2) having the greatest percent reduction in volatile solids (Table 5). There was nearly a ten-fold difference in the percent reduction of volatile solids when comparing mix 3 to mix 2 and mix 1. Reduction of nitrogen, sulphur and carbon did not produce similar results across the three mixes that did not have leachate recirculation. Mix 1 in general, showed the greatest percent reduction for all. Unlike the algae trial, pH increased from beginning to end with all mixes initially around 7.5 and ending at 8.4. This range of pH is consistent to produce biogas but production could be better optimized by keeping the pH in the range for methanogenesis (6.5 to 8) (Cioabla et al., 2012).

Table 5: Beef carcass trial % reduction results, all values adjusted for initial and final moisture content where appropriate

CARCASS		Total N	Total S	Total C	Total P	Total K	VS %		pH initial	pH final
% Change	Mix 1	39	58	63	34	64	24.5	pH Units	7.65	8.36
	Mix 2	9	25	39	8	24	26		7.23	8.41
	Mix 3	5	38	35	23	41	2.9		7.93	8.53
	Mix 1R	25	27	4	58	34	37.9		7.65	8.52
	Mix 2R	56	15	7	13	9	19.6		7.23	7.85
	Mix 3R	38	72	63	56	73	5.8		7.93	8.48

Effect of Leachate Recirculation The carcass trial exhibited the opposite effect due to leachate recirculation than the algae trial. For mixes 1 and 2, leachate recirculation resulted in less daily biogas (Fig. 3a) but similar methane concentrations comparing all mixes (Fig. 3c). Results for leachate recirculation with respect to methane concentration produced very similar results, with only mix 2 outlying the group. Figure 3b demonstrates the total biogas production for the trial; for two of the three cases, leachate recirculation hindered the production of biogas in mix 1 and 2, while for mix 3, biogas production was slightly increased. The percent reduction in volatile solids was greater for mix 1 and 3 in the leachate recirculated mixes, 25 and 38 percent respectively, than the non-recirculated, but for mix 2, percent reduction was less at 20% and 26% for the recirculated versus non-recirculated set (Table 5). Total methane production was the highest for the recirculated mix 3 (Table 6). The non-recirculated mixes had the highest reduction of carbon and sulphur, while the mixes receiving recirculation had the highest reduction in total nitrogen. The effect on pH was consistent with the non-recirculated mixes, pH generally increased from approximately 7.5 to 8.5. There were no leachate pooling effects throughout this trial as compared to the algae trial. Significant differences were not observed by the ANOVA test for all three mixes receiving recirculation ($p=0.456$), but comparing each mix to its recirculated trial, significant differences were observed for mix 1 and 2, but not for mix 3.

Table 6: Total methane produced for carcass trial (L/kg VS)

	Total Methane Production (L/kg VS)	
	Non-recirc	Recirc
Mix 1	173	147
Mix 2	90	44
Mix 3	161	187

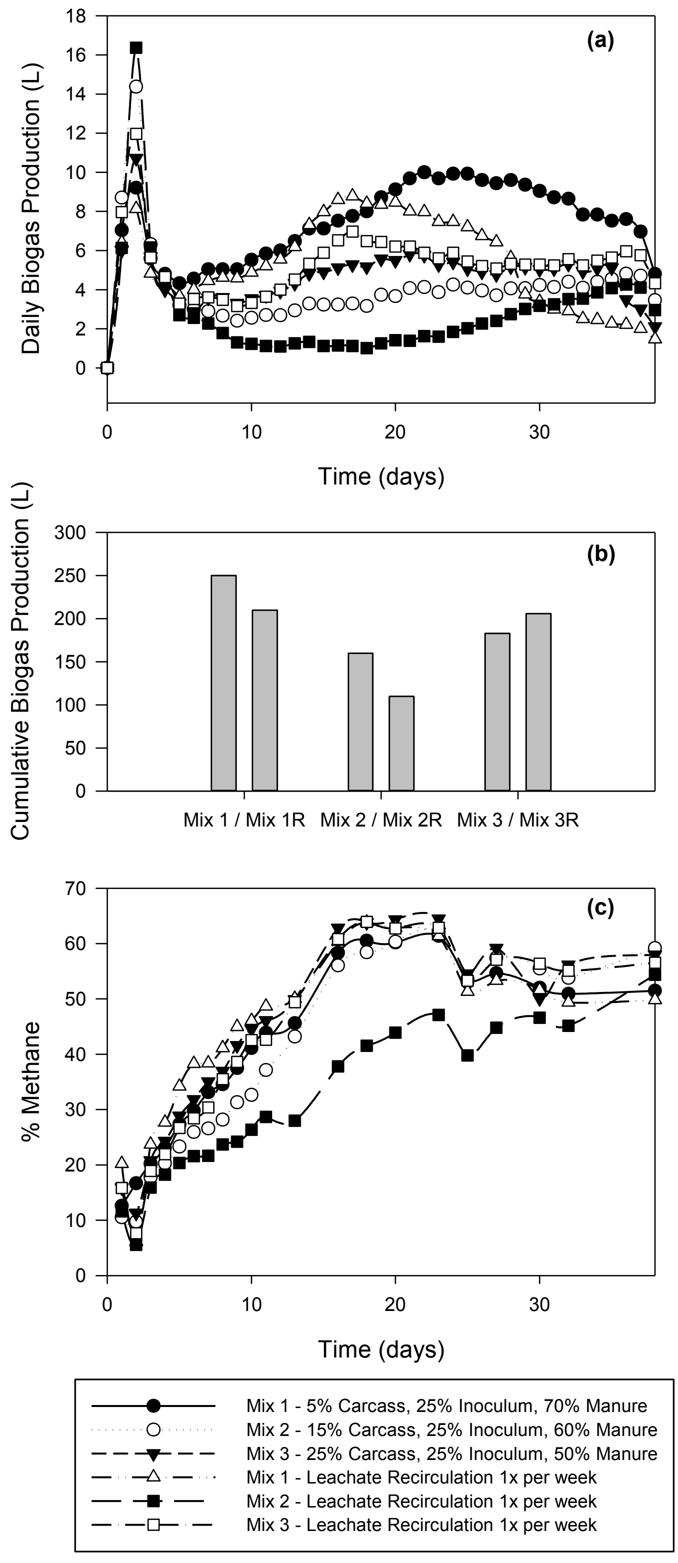


Figure 3: Carcass AD trial, (a) daily biogas production (L), (b) Biogas yield, (c) % methane produced

Comparison between Algae, Beef Carcass and Manure (Gaudet et al., 2013) Trials

In order to determine a feedstock's ability to be a useful tool for anaerobic digestion, it is important to compare similar data sets. Gaudet (2013), examined the effects of various recirculation strategies on beef feedlot manure mixed with 25% inoculum. As a whole, AD of carcass material produced the largest quantity of biogas and methane per kg of VS for both non and weekly leachate recirculation (Table 7) similar to results shown by others for mostly LS-AD (Government of Alberta 2011; Steffen et al., 1998). Methane concentrations as a whole over the duration of the trials were approximately 39%, 49% and 52% for algae, carcass and manure respectively. Of the three trials, carcass material shows the most capacity for higher energy values than the others.

Table 7: Comparison of liters of biogas produced per kg of VS (*Manure from (Gaudet et al., 2013)*) with 25% inoculum

	Algae		**Carcass**		**Manure**	
	Biogas (L/kgVS)	Methane (L/kgVS)	Biogas (L/kgVS)	Methane (L/kgVS)	Biogas (L/kgVS)	Methane (L/kgVS)
Non-recirculated	179	70	328	161	220	115
Recirculated	N/A	N/A	372	187	176	90

Although the carcass trial produced the highest quantity of biogas, it did not have the highest daily production rate. Manure mixed only with inoculum produced approximately 10 L of biogas daily at around 10 days (Fig 4). The problem with digesting only manure is how quickly the daily biogas production drops; after about 6 days of peak production, the daily rate drops considerably. Similarly, the algae trials exhibited the same scenario as manure. This was not the case for the carcass material both with leachate circulated and non-circulated. The carcass material established an extended steady rise in daily biogas production and to a certain degree, maintained the peak rate for the duration of the trial. It is predicted at the current rate shown below, the carcass trial could see a die off somewhere between 40 and 50 days instead of 15 to 30 days like the algae and manure mixes. In general, carcass material could be a good addition to an anaerobic digester to provide a steadier and more consistent biogas production rate.

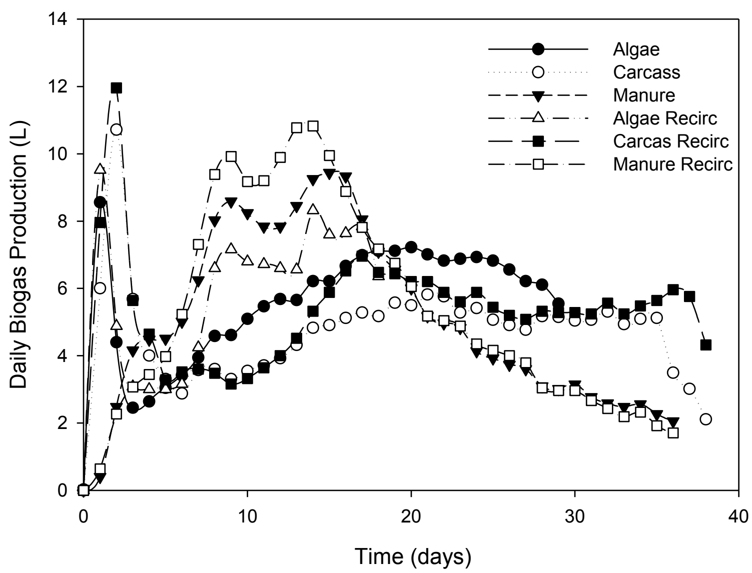


Figure 4: Daily biogas production comparing algae, carcass and manure (Gaudet et al., 2013)

CONCLUSION Solid state anaerobic digestion of various feedstocks such as algae and carcass material, when compared to SS-AD of manure, produces significantly different results. For this trial, carcass material produced significantly more biogas with higher methane percentages at all points compared to algae. Effects of weekly leachate recirculation were substantial for most mixes in both the algae trial and carcass trial. Weekly leachate recirculation for the carcass trial produced inconsistent results compared to algae and manure, with less production of biogas for the 15% carcass mix and slightly more biogas for the remaining mixes, significant differences in biogas production were only observed in the 5% and 15% mix. Inoculum strategies also produced significant results in the algae trial, with 25% inoculum producing the most biogas, while 0% inoculum produced little to no biogas and exhibited a toxic drop in pH and an increase in H_2S production. Carcass loading schemes produced inconsistent results, with 5% and 25% carcass concentrations having similar results, while the 15% application producing the least amount of biogas. When comparing results of these two trials to those from a previous manure trial, significant differences in biogas production were observed, with manure and algae production rates peaking early and dropping off quickly, while carcass production rates peaked a few days later and remained fairly constant throughout the trial. On a liters of gas or methane per kilogram of VS, the carcass mixes had the highest production rate. This suggests that carcass material had the capability to produce consistently more biogas per weight than algae or just manure.

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