Impact of Organic Loading Rates on Variable-Temperature Biodigesters

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Abstract

Anaerobic digestion has been studied under variable temperature conditions, but not to the extremes that occur in temperate climates. This experiment and analysis examine the performance of several organic loading rates under varying temperature conditions in order to make recommendations for improved management strategies for variable temperature digesters in temperate climates. Eight lab-scale digesters paired into four treatments were studied to assess the effects of temperature and organic loading rate (OLR) on digester performance when temperature was varied to simulate an annual cycle of a temperate climate (27°C to 10°C). Four loading rates were studied: high $(1.3 \text{ kg VS } (\text{m}^3 \text{day})^{-1})$, medium (0.8), low (0.3), and inoculum (0.19). The HRT of these treatments ranged from 43 days (high loading rate) to 188 days (low loading rate). The digesters were subject to a temperature schedule designed to mimic the seasonal cycles of a temperate climate. After the beginning of the experiment at 27°C, the temperature was gradually decreased to 10°C before returning to 27°C. The performance of all treatments declined as temperature decreased. The digester with the lowest loading rate recovered from its low performance once the temperature started increasing, but the digesters with the high and medium loading rates remained sour, with low pH and biogas production.

Dedication

This document is dedicated to my husband Wesley. Thank you for picking up my slack (and then some) and waiting patiently for me to emerge from writing.

Acknowledgments

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Figure 5: Running average of methane concentration. The temperature of the digesters is graphed on the secondary axis (right side). The vertically lines mark sharp changes in the slope of pH and the VFA/alkalinity ratio, occurring at the days indicated at the top of the graph. Methane concentration decreased with temperature. T1 and T2 remained sour, with CH_4 concentrations around 20%. T3 recovered, but concentrations varied between 43and 63%.13

Introduction

There are numerous small-scale, variable temperature anaerobic digester systems throughout the world, particularly in China, where they have been present at household scales since the 1960s (Jingjing, Xing, DeLaquil, & Larson, 2001). In tropical locations, unheated biodigesters work well (Lansing, Botero, & Martin, 2008) because the average ambient temperature is within the mesophilic temperature range. However, in temperate climates, winter temperatures fall well below the mesophilic range. Existing large-scale digester systems in these climates maximize gas production by maintaining internal temperature through heating and active mixing processes to increase metabolic reaction rates.

These biodigesters have been installed successfully at wastewater treatment plants and large dairy farms, but the capital investment needed is typically \$1 million, or more (Agstar-US EPA, 2010a). To make this investment worthwhile, a steady supply of organic matter is needed, for example, manure from a herd of 1000 cows. However, over 90% of US dairy farms have less than 200 cows (Macdonald et al., 2007), indicating a large market for smaller, more affordable systems. While many small-scale, variable-temperature systems exist in China, these systems can still benefit from a greater understanding of variable-temperature anaerobic digestion and the development of better management plans to improve energy production.

Developing a viable small-scale digester system could result in significant renewable energy production. According to USEPA estimates, the installation of large-scale dairy digesters on 5,600 suitable US farms would produce 6.8 billion kW-hr/year (Agstar-US EPA, 2010b). Since cows on small farms represent a significant portion of the dairy cow national inventory

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(38% in 2006 (Macdonald et al., 2007), it is reasonable to use this data about large-scale systems as an estimate of the potential of small-scale digesters as well.

Filling the biodigester technology gap on small farms and bringing small-scale digesters alongside these large-scale systems would represent a significant gain in renewable energy production. In addition to developing the new technology for the U.S., the millions of existing small-scale, variable temperature digesters in China, India and elsewhere stand to benefit from this research. Better management strategies will allow the millions of families who utilize digesters to have a more dependable source of heat with less maintenance and less chance of digester failure.

The technologic problems associated with variable temperature digesters are commonly associated with microbiologic issues. The microorganisms responsible for anaerobic digestion can be divided into two groups: acidogenic bacteria and methane-producing archaea (methanogens). The acidogenic bacteria produce acids by the metabolic breakdown of the organic substrates. Methanogens then convert these acids into methane and carbon dioxide. These two groups have different pH and temperature tolerances; the acidogens are tolerant of lower temperatures and pH than are the methanogens (Anderson, Kasapgil, & Ince, 1994). As the temperature decreases, the acidogens continue producing acids at similar rates, but the performance of the methanogens is impaired. The unused acids accumulate, lowering the pH and further inhibiting the metabolic activity and growth of the methanogens. This 'souring' of the digester is characterized by low, acidic pH and, accordingly, low gas production. Souring of digesters can be caused by providing organic matter at a faster rate than it can be fully metabolized (i.e. overloading). Because the metabolic activity of the methanogens changes with

temperature, a loading rate appropriate during warm temperatures may quickly lead to 'souring' as temperatures decrease. Due to these occurrences, and their impact on digester performance, it is essential to identify appropriate organic loading rates for digesters undergoing temperature changes.

Goals and Objectives

One goal of this study was to determine how different organic loading rates (OLRs) affect the performance of digesters during simulated annual temperature changes in temperate climates. A second goal was to identify a constant loading rate that would prevent souring and allow the digester to recover after a period of cold temperature. To accomplish these goals, the following specific aims were pursued during an experiment in which temperature was varied to simulate an annual cycle of a temperate climate. Three loading rates were applied, as well as, a fourth treatment consisting only of inoculum.

- Determine the impact of temperature change and different loading rates on biogas production, methane production, pH, alkalinity, and volatile fatty acid (VFA) concentrations.
- Determine how different loading rates impacted the recovery of the digesters as temperature increased following low temperatures.

• Make management recommendations for loading rates of variable temperature digesters. It was anticipated that the digesters that soured would do so at different points in the experiment; specifically, that digesters with the highest loading rate would fail first. The temperatures associated with the failure point of each loading rate can be used to design a loading schedule for small-scale digesters to maximize summertime use, but prevent souring during the winter.

Experimental Design

The experiment was composed of eight lab-scale digesters submerged in a water bath to control temperature. Each 4-liter HDPE digester included a port for the addition and extraction of liquid samples, and a valve for biogas measurement and sampling. These eight digesters were divided into four treatments of two replicates each. Treatments 1-3 (T1-T3) had high, medium, and low organic loading rates. Treatment 4 (T4) was loaded with inoculum only (Table 1).

Treatment	Organic Loading Rate (kg VS/m ³ day)	Average Volumetric Loading Rate (ml/day)	Hydraulic Retention Time (days)			
1	1.3	69.22	43.34			
2	0.8	42.5	70.59			
3	0.3	15.94	188.26			
4	0.19	14.5	171.43			

Table 1: Organic and volumetric loading rates, as well as hydraulic retentions times associated with each treatment.

At start-up, the digesters were filled with 3 liters of inoculum from the Columbus, OH Quasar anaerobic digester. (pH 7.90, VS 3.3%, wet basis). Dairy manure from Waterman Agricultural Laboratory, collected weekly, was used as the substrate (pH 6.72 \pm 0.49, VS 5.76% \pm 0.83%, wet basis). Using the VS content of the manure and the OLR set for each digester, a sampling and loading volume was calculated for each week. Every other day, the same volume was sampled and loaded with slight variations, as needed, in order to maintain a liquid volume of three liters in each digester.

The digesters were first held at a stable temperature (27°C for 14 days) then the temperature was decreased by 0.5°C each sampling/loading day until a temperature of 10 °C was

reached. After holding this temperature for 19 days, the temperature was increased by 0.5°C each sampling day until a temperature of 27 °C was once again reached and maintained for 13 days (Figure 1). These temperature changes were based on annual temperature data from a pilot scale variable temperature digester located in Columbus, OH but were accelerated in order to shorten the duration of the experiment (216 days).



Figure 1: Temperature changes of digesters during the experiment. The three temperature periods used in Results and Discussion are also defined: Decreasing, Stable, and Increasing temperature periods.

Analysis

Biogas production and quality were measured as well as the following indicators of digester quality: pH, alkalinity, and VFA concentration. Biogas production was measured with wet tip gas meters (one per digester), and was recorded each sampling day. Gas samples were taken every sampling day for the first half of the experiment. After analyzing these samples, it was determined that collecting gas samples twice a week during the second half of the

experiment was sufficient. The biogas samples were analyzed on a Shimadzu GC-14A, using the TCD detector and Helium carrier gas. The pH was measured using a Fisher Scientific Education pH/Ion 510 bench pH meter. VFA concentrations and alkalinity were measured using a titration method (Lossie & Putz, 2008), and standard methods (APHA, 2005) were used to determine total (TS) and volatile (VS) solids.

Results

To determine if the performance of the replicates within each treatment were similar, ttests (two-tailed, unequal variance) were performed. As seen from the results (Table 2), the replicates are statistically similar in every parameter measured except for Biogas production in T2 and T3. When the data were separated into temperature periods (Figure 1) the t-tests performed indicated that the digesters in T2 were similar in their biogas production except during the final increasing temperature phase. Similar analyses revealed that digesters in T3 had similar biogas production while the temperature was decreasing, but dissimilar biogas production while the temperature was stable and increasing. Following these results, the average of the two digesters in each treatment was used in this paper for the following analyses unless individual digesters are specified.

Digesters	Biogas	%CH ₄	CH ₄ prod	pH	VFAs	Alkalinity	VFA/Alkalinity Ratio
1,2 (T1)	0.20	0.47	0.27	0.99	0.94	0.63	0.92
3,4 (T2)	0.01	0.98	0.36	0.71	0.61	0.69	0.62
5,6 (T3)	0.03	0.51	0.95	0.50	0.14	0.41	0.08
7,8 (T4)	0.30	0.82	0.47	0.12	0.45	0.30	0.28

Table 2: T-test results between replicates. Two-tailed, unequal variance t-tests were performed between each replicate pair. Grey highlight denotes similarity between the data being compared (p-value above 0.05). No highlight denotes statistically significant differences. The pair of digesters that compose each treatment are similar to each other in almost every parameter.

For each temperature period (Figure 1), the data from each parameter was averaged for each digester (Table 3). The overall performance of the digesters was clearly impacted during the simulated annual temperature cycle.

The performance of the four treatments was similar for most of the decreasing period. T1 (digesters 1 and 2) had the lowest pH of the four treatments (Table 3), but had the highest biogas production (Table 3 and Figure 4). Treatment 2 had the highest biogas production when normalized for the OLR (Table 3). The average VFA/alkalinity ratios for all treatments were between 0.18 and 0.29 during the decreasing temperature period, below the recommended threshold of 0.3-0.4 ((Liu et al., 2012; Lossie & Putz, 2008), but the ratio for T1 increased rapidly at the end of the decreasing temperature period (day 64 of the experiment), when the temperature approached 15°C (Figure 3). The pH of T1, though trending downward, also remained fairly stable until 15°C. At 15°C, the downward slope increased, and was maintained until the end of the stable temperature period (Figure 2).

Digester	OLR (kg VS/ m ³ ·day)	Biog	as Produ (ml/day)	iction)		% CH ₄	l	CH (ml C	4 produc H ₄ / kg VS	tion S·day)	рН		рН		рН		рН		VFAs (g Hac/L)		Alkalinity (mg CaCO ₃)			VFA/Alkalinity Ratio ((mg Hac/L)/(mg CaCO3/L))		
		D	S	Ι	D	S	Ι	D	S	Ι	D	S	Ι	D	S	Ι	D	S	Ι	D	S	Ι				
1	1.3 (T1)	764.3	60.5	69.7	51.0	22.1	17.8	322.3	14.18	12.38	7.46	6.51	6.01	2.7	6.5	8.4	10.4	5.7	2.8	0.29	1.23	3.12				
2	1.3 (T1)	907.7	84.3	107.2	53.2	25.1	18.4	384.2	23.9	15.48	7.46	6.62	5.96	2.6	5.9	8.6	10.7	6.4	2.9	0.26	0.97	3.35				
3	0.8 (T2)	558.2	109.3	68.5	52.6	40.2	21.0	379.3	89.94	19.92	7.57	6.98	6.15	2.6	4.3	8.4	12.1	8.9	3.7	0.22	0.53	2.45				
4	0.8 (T2)	587.7	101.2	282.2	52.9	37.3	21.4	396.3	86.49	25.95	7.58	7.00	6.21	2.4	3.7	8.2	12.3	9.3	4.0	0.20	0.42	2.26				
5	0.3 (T3)	225.8	57.4	299.0	54.1	41.9	55.7	412.5	104.6	1425	7.69	7.54	7.30	2.6	3.0	3.5	13.8	12.3	9.3	0.19	0.25	0.39				
6	0.3 (T3)	228.7	80.0	439.5	54.9	46.0	58.0	416.1	108.6	1442	7.69	7.51	7.36	2.5	2.9	3.0	13.8	12.8	10.1	0.18	0.23	0.29				
7	.19 (T4)	43.8	0.2	8.1	54.4	18.8	29.5	199.0	3.27	63.85	7.85	7.91	7.87	2.9	3.4	2.8	14.4	14.7	13.1	0.21	0.23	0.21				
8	.19 (T4)	52.0	1.9	12.2	53.0	26.0	27.5	172.2	19.53	29.21	7.85	7.88	7.86	2.9	3.9	2.9	13.9	14.4	13.1	0.21	0.27	0.22				

Table 3: Summary of Results. D: average value for data in the decreasing temperature range (see Figure 1), S: average value for data in the stable temperature range, I: average value for data in the increasing temperature range.

The performance (as measured by biogas production, pH, VFA/alkalinity ratio, and methane concentration) of all the digesters decreased with declining temperature (Table 3) and remained low during the period of stable, cold temperature. The percentage decrease in biogas production from the decreasing temperature (D) period to the stable temperature (S) was between 65% and 99.5%. T1 experienced a decrease of roughly 90%, T2 80%, T3 70% and T4 97%. T1-T3 had significant drops in pH levels from D to S (Table 3, Figure 2). T1 experienced a drop of nearly 1unit to an average of 6.45, T2 decreased by 0.59 pH to 7.0. T3 only decreased in pH by 0.17, from 7.7 to 7.3. Unlike the other treatments, T4 increased in pH, from 7.85 to 7.91 (Table 3).



Figure 2: Running average of pH. The temperature of the digesters is graphed on the secondary axis (right side). The vertically lines mark sharp changes in the slope of pH and the VFA/alkalinity ratio, occurring at the days indicated at the top of the graph. The final pHs were T1-5.54, T2-5.85, T3-7.46, and T4-7.9. The pH of T3 and T4 stayed above 7.0, but that of T1 and T2 dropped below 6.0 at 21.5 and 24.5 °C, respectively, both in the increasing temperature period.

Similar to the abrupt rise in VFA/alkalinity ratio for T1 at 15°C, T2 also showed a point of distinct change. At the beginning of the S temperature period (day 84, 10°C), the VFA/alkalinity ratio rises abruptly (Figure 3). As would be expected, a correlating steeper slope of pH decline appears at the same point in the experiment (Figure 2).



Figure 3: Truncated graph of the running average of VFA/alkalinity ratio. The temperature of the digesters is graphed on the secondary axis (right side). The vertically lines mark sharp changes in the slope of pH and the VFA/alkalinity ratio, occurring at the days indicated at the top of the graph. Final values were T1 4.91, T2 3.47, T3 0.214, and T4 0.269. The maximum desired ratio of 0.04 is shown.

As the temperature increased towards 27°C, T1 and T2 continued to sour (Figure 2,

Figure 3, Figure 4, Figure 5). The little gas that was produced was low in methane, the pH continued to decline, and the VFA/alkalinity ratio increased dramatically to final values of 4.91 and 3.47 for T1 and T2, respectively. The average biogas production for T1 during the I period was only 10.5% of the D average production. Gas production for Digester 3 decreased by 88% from the decreasing temperature period to the increasing (I) temperature period, and Digester 4 decreased by 52%. The t-test results recorded in Table 2 indicated that the biogas production for Digesters 3 and 4 was statistically different, and further t-tests showed that they diverged during the I period. Though T1 and T2 continued to decrease in performance, T3 made a recovery. Gas production started to increase first, when the temperature reached 16°C (day 150), then pH increased at 24.5°C (day 180). While the I average pH (7.33) did not rebound to the D average pH (7.69), the gas production of Digesters 5 and 6 were 32% and 92.2% higher during the I period than the D period (Table 3). While T1 soured without recovery before the conclusion of this experiment, it still had the highest total biogas production, 40.1 liters. T2 and T3 produced 34.2 and 28.4 liters, respectively.



Figure 4: Running average of daily biogas production for each treatment. The temperature of the digesters is graphed on the secondary axis (right side). The vertically lines mark sharp changes in the slope of pH and the VFA/alkalinity ratio, occurring at the days indicated at the top of the graph.

The VFA Alkalinity Ratio of T3, which stayed below 0.35 during the D and S periods,

was above 0.4 for approximately 15 days during the increasing temperature period (Figure 3).

Like the other parameters, methane concentration of the biogas produced in all treatments

declines as the temperature decreases (Figure 5). The concentration of methane in T3 increases

as temperature increases, while T1 and T2 remain sour, with low concentrations of methane

(20%).



Figure 5: Running average of methane concentration. The temperature of the digesters is graphed on the secondary axis (right side). The vertically lines mark sharp changes in the slope of pH and the VFA/alkalinity ratio, occurring at the days indicated at the top of the graph. Methane concentration decreased with temperature. T1 and T2 remained sour, with CH_4 concentrations around 20%. T3 recovered, but concentrations varied between 43and 63%.

T-tests were performed to analyze the similarity of the treatments (Table 4). T1 and T2

were distinct for only two of the seven parameters, pH and Alkalinity. T1 and T3 were different

for all seven parameters.

Treatments	Biogas	$%CH_4$	CH ₄ prod	pН	VFAs	Alkalinity	VFA/Alkalinity Ratio
T1,T2	0.26	0.32	0.40	0.03	0.41	0.02	0.08
T1,T3	1.74E-02	0.001	0.02	9.91E-23	7.28E-08	5.26E-14	1.18E-07
T1,T4	8.97E-14	0.07	1.56E-4	5.71E-34	4.56E-07	1.14E-20	6.39E-08
T2,T3	0.14	0.01	0.05	7.70E-17	3.35E-06	1.31E-07	2.36E-06
T2,T4	1.49E-19	0.30	1.5E-6	1.43E-29	1.96E-05	6.45E-14	1.03E-06
T3,T4	4.37E-21	0.20	4.49E-6	1.66E-36	0.1764	6.04E-06	0.06

Table 4: T-test results between treatments. Two-tailed, unequal variance t-tests were performed between the averages of the treatments. Grey highlight denotes similarity between the data being compared (p-value above 0.05). No highlight denotes statistically significant differences. Treatments 1 and 3 were significantly different from each other in every parameter.

Discussion

Past studies have found declines in digester performance associated with decreasing temperature and recommend low organic loading rates (and long HRTs) for digesters at low temperatures (Alvarez & Lidén, 2008, 2009; Khoiyangbam et al., 2004; Meher, Murthy, & Gollakota, 1994; Safley & Westerman, 1994). The results presented in this analysis confirm performance declines with temperature, as well as the need for low organic loading rates in low temperature systems.

Urmila, Zisengwe, Meriggi, & Buysman (2008) recommend longer HRTs with decreasing temperature. A study of psychrophillic digesters at 20°C recommended an HRT of at least 40 days (Zeeman, Sutter, Vens, Koster, & Wellinger, 1988). The shortest HRT used in this experiment was 43 days (Treatment 1, associated with an OLR of 1.3 kg VS m⁻³day⁻¹, see Table 1), and did not perform well at lower temperatures. While 20°C is contained within the temperature range of this experiment, the difference between 20°C and 10°C is significant. A longer HRT, such as the 188 days used in T3, is needed for colder psychrophillic temperatures such as 10°C. While successful AD can be carried out at 10°C (Singh, Maurya, Ramana, & Alam, 1995) with relatively short HRTs (25-40 days), this was accomplished using an inoculum adapted first to 20°C for three months, then slowly adapted down to 10°C over an additional four months. The defining characteristics of a variable temperature digester prevent an adapted inoculum approach such as this from being utilized year round, but selective inoculation with communities adapted for different temperatures could be a possible management strategy to combine the advantages of these two types of systems.

A variable temperature study conducted in Janata, India (Kalia & Kanwar, 1998) found 23-27% decreases in biogas production associated with decreases in temperatures from 23-24°C to 13-14°C. These decreases are much lower than those experienced by the digesters in this experiment, ranging from 70% (T3) to 90% (T1) reduction in biogas production, but the study in India examined a digester under a temperature difference of only 10°C (from 23-24°C to 12-14°C), compared with the 17°C difference used in this study.

Implications for Management and Further Research

A simple management system consisting of a constant low loading rate (and, accordingly, a long HRT), can be successfully used to permit a digester to recover from a cold season and resume performance with increasing temperatures, as was observed in T3. This method of management, while preventing souring during the winter, does not realize the potential gas production enabled by warm summer weather. While the higher loading rages of treatments 1 and 2 did sour with lower temperatures, they still produced a greater amount of biogas during the experiment than treatment 3.

A more sophisticated management regime with a variable loading rate would result in higher biogas production during periods of warm weather (high loading rate during the summer, lower the loading rate as the temperature decreases), but would be recoverable in the spring (slowly increase the loading rate as the digester recovers). This variable approach needs more research, but the failure points of the two sour treatments can provide an initial framework to test in future experiments (Table 5). For example, since T1, the high loading rate, started to sour at 15°C, the loading rate should be reduced before reaching this temperature.

Temperature Range (°C)	OLR (kg VS/m ³ day)	HRT (days)
27-20	1.3	43
20-15	0.8	71
15-10	0.3	188

Table 5: Recommended loading schedule for variable temperature digesters. This schedule is symmetric (e.g. once the temperature rises above 15°C, the loading rate should be increased from 0.3 to 0.8 kg VS/m³day). Loading rates were reduced 5°C before the souring point to allow acclimation. For example, T1 soured at 15°C, but this schedule recommends reducing the loading rate at 20°C.

The loading strategy adopted must be analyzed in light of the goals and purpose of the digester. T1, though sour by the end of the study, still produced 41% more biogas than T3, which did not sour. If yearly re-inoculation of the digester is feasible and the goal is to maximize gas production, then the loading level should be high until souring occurs. Re-inoculation and subsequent continuation of a high loading rate can occur each spring. If inoculation is difficult and/or steady gas production is desired, then a management strategy that allows recovery (a variable loading rate or constant low loading rate) should be used.

The ratio between the concentration of VFAs and carbonate alkalinity is an indicator of digester health, and a ratio less than 0.4 is commonly considered desirable (Liu et al., 2012). This indicator appears to be more sensitive to changes in digester chemistry than pH. As noted in the Results, T1-3 experienced an increase in the rate of change of the pH. These points occurred at day 64, 84, and 106 for T1-T3 respectively. These days, for each treatment, are approximately when the VFA/alkalinity ratio reached 0.3. These days have been marked on the pH, VFA/alkalinity ratio, and biogas production graphs in Figure 2,Figure 3Figure 4, andFigure 5. While the pH and VFA- Alkalinity ratio seem to negatively correlate (decreases in pH coincide with increases in , biogas production did not appear to be a reliable indicator of digester health. Biogas production was already decreasing in each treatment before the change in pH slope was observed.

In addition to investigating variable loading schedules, future research in this field should include more full scale temperature schedules. This study condensed a year of temperature changes to 216 days. Extending such studies to actual time periods will allow the effects of acclimation to be properly observed.

Conclusions

There is a distinct difference in the performance of T1 and T3 (Table 4). At this timescale, the constant loading rate of 0.3 kg VS/m³day allows recovery of the system after experiencing cold temperatures, while the higher loading rates soured, finishing the study with low pH, high VFA/alkalinity ratio, low biogas production, and low methane concentration. A variable loading schedule has been proposed, based on the failure points of each treatment.

Bibliography

- APHA. (2005). Standard methods for the examination of water and wastewater. (A. D. Eaton, L. S. Clesceri, & A. E. Greenberg, Eds.) Test (Vol. 2, pp. 2-59,2-60). American Public Health Association.
- Agstar-US EPA. (2010a). Anaerobic Digestion Capital Costs for Dairy Farms (Vol. 117, pp. 1-2).
- Agstar-US EPA. (2010b). US Anaerobic Digester Status Report.
- Alvarez, R., & Lidén, G. (2008). The effect of temperature variation on biomethanation at high altitude. *Bioresource technology*, *99*(15), 7278-84. doi:10.1016/j.biortech.2007.12.055
- Alvarez, R., & Lidén, G. (2009). Low temperature anaerobic digestion of mixtures of llama, cow and sheep manure for improved methane production. *Biomass and Bioenergy*, 33(3), 527-533. Elsevier Ltd. doi:10.1016/j.biombioe.2008.08.012
- Anderson, G. K., Kasapgil, B., & Ince, O. (1994). Microbiological Study of Two-Stage Digestion During Start-up. Water Research, 28(11), 2383-2392.
- Jingjing, L., Xing, Z., DeLaquil, P., & Larson, E. D. (2001). Biomass energy in China and its potential. *Energy for Sustainable Development*, 5(4), 66-80. International Energy Initiative, Inc. doi:10.1016/S0973-0826(08)60286-0
- Kalia, A. K., & Kanwar, S. S. (1998). Long-term evaluation of a fixed dome Janata biogas plant in hilly conditions. *Bioresource technology*, *65*, 61-63.
- Khoiyangbam, R. ., Kumar, S., Jain, M. ., Gupta, N., Kumar, A., & Kumar, V. (2004). Methane emission from fixed dome biogas plants in hilly and plain regions of northern India. *Bioresource Technology*, 95(1), 35-39. doi:10.1016/j.biortech.2004.02.009
- Lansing, S., Botero, R. B., & Martin, J. F. (2008). Waste treatment and biogas quality in smallscale agricultural digesters. *Bioresource technology*, 99(13), 5881-90. doi:10.1016/j.biortech.2007.09.090
- Liu, X., Wang, W., Shi, Y., Zheng, L., Gao, X., Qiao, W., & Zhou, Y. (2012). Pilot-scale anaerobic co-digestion of municipal biomass waste and waste activated sludge in China: Effect of organic loading rate. *Waste management (New York, N.Y.)*. Elsevier Ltd. doi:10.1016/j.wasman.2012.03.003
- Lossie, U., & Putz, P. (2008). Targeted control of biogas plants with the help of FOS / TAC. Hach Lange.

- Macdonald, J. M., Donoghue, E. J. O., Mcbride, W. D., Nehring, R. F., Sandretto, C. L., & Mosheim, R. (2007). *Profits*, *Costs*, and the Changing Structure of Dairy Farming Cataloging Record :
- Meher, K. K., Murthy, M. V. S., & Gollakota, K. G. (1994). Psychrophilic anaerobic digestion of human waste. *Bioresource technology*, 50(1994), 103-106.
- Safley, L. M., & Westerman, P. W. (1994). Low-temperature digestion of dairy and swine manure. *Bioresource technology*, 47, 165-171.
- Singh, L., Maurya, M. S., Ramana, K. V., & Alam, S. I. (1995). Productin of biogas from night soil at psychrophilic temperature. *Bioresource Technology*, 53(2), 147-149. doi:http://dx.doi.org/10.1016/0960-8524(95)00075-P
- Urmila, B., Zisengwe, L. S., Meriggi, N., & Buysman, E. (2008). *Biogas production in climates* with long cold winters.
- Zeeman, G., Sutter, K., Vens, T., Koster, M., & Wellinger, a. (1988). Psychrophilic digestion of dairy cattle and pig manure: Start-up procedures of batch, fed-batch and CSTR-type digesters. *Biological Wastes*, *26*(1), 15-31. doi:10.1016/0269-7483(88)90146-2