

# Performance of Anaerobic Bioreactors under Diurnally Cyclic Air Temperatures: A Spectral Analysis Approach to Biogas Production

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## Abstract

The effects of two diurnally cyclic temperature ranges (20-40°C and 15-25°C) and four levels of hydraulic retention times (25, 20, 15, and 10 d) on the performance of anaerobic reactors operated on screened dairy manure were evaluated. The reactor temperature exhibited a lag relative to the chamber air temperature. For the 20-40°C temperature cycle, the average lags period at the maximum and minimum chamber temperatures 3.75 and 4.37 h, respectively. For the 15-25°C temperature cycle, the average lag periods at maximum and minimum chamber temperatures were 3.61 and 4.34 h, respectively. The effluent solids content were not adversely affected by the reactor diurnally cyclic temperature. The effluent total solids and methane content of the biogas diurnally cyclic patterns were out of phase with the diurnally cyclic pattern of the reactor temperature by about 12 hours under most operating conditions. The reductions in total solids and methane yield were all significantly affected by the diurnal temperature range and hydraulic retention time. Biogas production from a healthy digester operating under a diurnally cyclic temperature environment follow a sinusoidal pattern which can be described by a Fourier equation of the form:  $\gamma_t = \gamma_o + \sum_{n=1}^{\infty} [a_n \cos(2\pi n\phi t) + b_n \sin(2\pi n\phi t)]$ . However, where the operating conditions are not favourable, the production followed a sinusoidal pattern which may be embedded in some harmonic and noise.

**Keywords:** Anaerobic reactors; Solids; Volatile fatty acids; Diurnally cyclic Temperature; Biogas; Fourier function; Spectral analysis

## Introduction

Anaerobic digestion is a biological process in which organic materials are decomposed in the absence of free oxygen to yield methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and small quantities of other gases (H<sub>2</sub>S, N<sub>2</sub> and H<sub>2</sub>O). The process requires the concerted actions of a symbiotic population of three groups of facultative and obligate anaerobic bacteria. The first group degrade carbohydrates, lipids and proteins into alcohols and long chain fatty acids. The second group produce acetate, carbon dioxide and hydrogen from the fermentation of degraded organic materials. The third group produce methane and carbon dioxide from the fermentation of acetate.

Compared to other biological treatment processes, anaerobic digestion has several advantages including: (a) production of usable biogas that is about 60-80% methane with a fuel value of 17-23.9 MJ/m<sup>3</sup>, (b) the digested residue is almost odourless with partially stabilized solids content, (c) the inorganic nutrients are conserved resulting in an enhanced fertilizer value of the digested sludge and (d) pathogenic microorganisms such as Salmonella sp. and Brucella sp. as well as weed seeds are destroyed [1-5]. The latter implies that livestock can be grazed on pastures which have been spread with sludge from the anaerobic digester sooner than would be acceptable if raw manure was used [6]. However, despite these advantages, anaerobic digestion has not enjoyed widespread acceptance among many farmers due to the relative instability of the system resulting from the sensitivity of anaerobic bacteria to changes in environmental conditions [7]. Temperature is considered to be one of the most important environmental parameters affecting the growth and survival of anaerobic microorganisms [1,4,6,8-13]. Diurnally cyclic variation in slurry temperature is a common phenomenon in reactors operated under ambient conditions. Echiegu [14], Ghaly et al. [15] Ghaly and Hattab [6] showed that gas production as well as some other operating indices followed a diurnally cyclic pattern with some lag relative to the diurnally cyclic environmental temperature.

The aim of this study was to investigate the performance of a continuous mix anaerobic reactor operating under diurnally cyclic temperatures by evaluating reductions in the effluents solids and the profile of VFA and biogas. Also, the gas production data was subjected to spectral analysis to determine whether gas production under the above operating condition can be described by a perfectly sinusoidal periodic function.

## Modeling

Deterministic data is one which can be described by an explicit mathematical relationship; a non-deterministic data cannot. Deterministic data can be classified as periodic or non-periodic. Periodic data and for which the following relationship holds:

$$Y_t = f(t + T_p) \quad (1)$$

Where:

$Y_t$  is the value of the variable at time t in seconds

$T_p$  is the period in seconds.

Many periodic data can be described in the form of a sine or cosine wave. Such a data is said to be sinusoidal. A sinusoidal data can be represented by a time dependent function of the following form:

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$$Y_t = A(\cos\omega_0 t + \phi) \quad (2)$$

Where:

A is the amplitude

$\omega_0$  is the angular frequency (rad/s)

$\phi$  is the phase angle (rad)

The angular frequency can be expressed as follows:

$$\omega_0 = 2\pi f \quad (3)$$

Where:

f is the frequency in Hz =  $1/T_p$

By invoking trigonometric relationships, equation (2) can be expressed as follows:

$$Y_t = a_1 \cos(\omega_0 t) + b_1 \sin(\omega_0 t) \quad (4)$$

Where:

$$a_1 = A \cos \phi \quad (5)$$

$$b_1 = A \sin \phi \quad (6)$$

Many real life periodic data are not sinusoidal. They are rather complex in nature and can be described as follows:

$$Y_t = f(t + nT_p) \quad (7)$$

Where:

n is harmonic number

More generally, arbitrary periodic functions can be represented by an infinite series of sinusoids of harmonically related frequencies such as Fourier series. A Fourier series representation of a complex periodic data can be written as follows:

$$Y_t = Y_0 + \sum_{n=1}^{\infty} [a_n \cos(n\omega_0 t) + b_n \sin(n\omega_0 t)] \quad (8)$$

Where:

$Y_0$  is the mean value of the periodic function

The terms  $Y_0$ ,  $a_n$  and  $b_n$  are defined as follows:

$$Y_0 = \frac{1}{T_p} \int_0^{T_p} Y_t dt \quad (9)$$

$$a_n = \frac{2}{T_p} \int_0^{T_p} Y_t \cos(n\omega_0 t) dt \quad n = 0, 1, 2, \dots \quad (10)$$

$$b_n = \frac{2}{T_p} \int_0^{T_p} Y_t \sin(n\omega_0 t) dt \quad n = 0, 1, 2, \dots \quad (11)$$

For equally spaced data with N data points, equation (9) to (11) can be written respectively as follows:

$$Y_0 = \sum_{i=1}^N \frac{Y_i(t)}{N} \quad (12)$$

$$a_n = \frac{2}{N} \sum_{i=1}^N [Y_i \cos(n\omega_0 t)] \quad n = 0, 1, 2, \dots \quad (13)$$

$$b_n = \frac{2}{N} \sum_{i=1}^N [Y_i \sin(n\omega_0 t)] \quad n = 0, 1, 2, \dots \quad (14)$$

Equation (8) indicates that a periodic data can be represented by a static component  $Y_0$  and an infinite number of sinusoidal components with a fundamental frequency  $\omega_0$  and harmonic whose frequencies are constant multiples of the fundamental frequency. In periodic data, the

sequence repeats, although the repetition may not be exact. To measure the degree of similarity between two successive positions, the sequence would have to be compared with itself at two successive positions. This is achieved by calculating autocorrelation function. Autocorrelation is defined as the linear correlation between a time series at time (t) and the same series at a later time (t +  $\tau$ ) [16].

Mathematically:

$$R_{xx}(t, t + \tau) = \lim_{n \rightarrow \infty} \left\{ \frac{1}{n} \sum_{i=1}^n [Y_i(t)Y_i(t + \tau)] \right\} \quad n = 0, 1, 2, \dots \quad (15)$$

Where:

$R_{xx}(t, t + \tau)$  is the autocorrelation coefficient

$\tau$  is the time interval in seconds.

Lag is the amount of offset between two successive series being compared. If each observation in a segment of time series is numbered from 1 to i, the autocorrelation has zero lag when element 1, 2, 3, ...i is compared to elements 1, 2, 3, ...i of another segment of the series. When the correlation coefficient of elements 1, 2, 3 ...i-1 of one segment of the series are compared to elements 2, 3, 4, ...I of another segment of the same series, the autocorrelation has a lag of 1. For a perfectly periodic data such as sine wave, the autocorrelation at zero lag equals 1. A typical autocorrelation will start from +1 at zero lag, decrease to a negative value less than or equal to -1 and then rise again. For a perfectly periodic data, the autocorrelation coefficient will vary from +1 to -1.

## Materials and Methods

### Experimental apparatus

The experimental apparatus (Figure 1) consisted of four bioreactors, a feeding system, a temperature control system, a gas collection system, a data acquisition and control system and computer.

**Bioreactors:** Each bioreactor (Figure 2) was constructed of a cylindrical PVC vessel, (458 mm length, 300 mm diameter and a 1.0 mm wall thickness). It provided a liquid volume of 25 L and a gas head space of 7.4 L. The cover of the bioreactor was made of a transparent Plexiglas plate (370 mm diameter and 1.0 mm thickness). It was secured to the bioreactor, through PVC lugs glued onto the top outer periphery of the vessel by means of six bolts. A Vaseline-coated rubber gasket was fitted between the cover and the vessel to provide a gas-tight seal. The

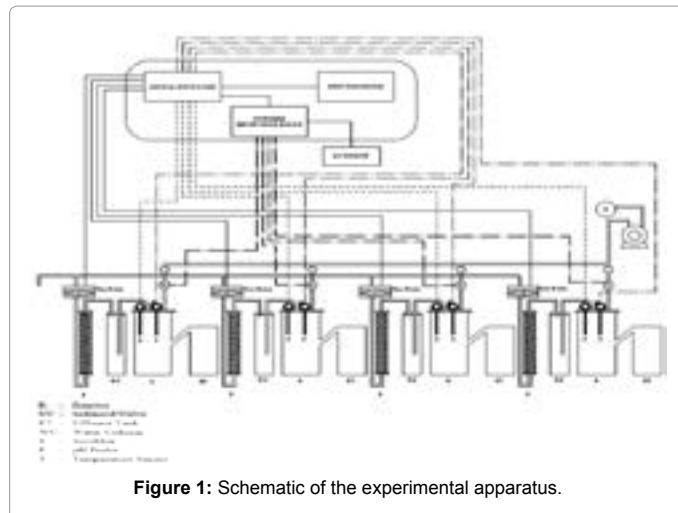


Figure 1: Schematic of the experimental apparatus.

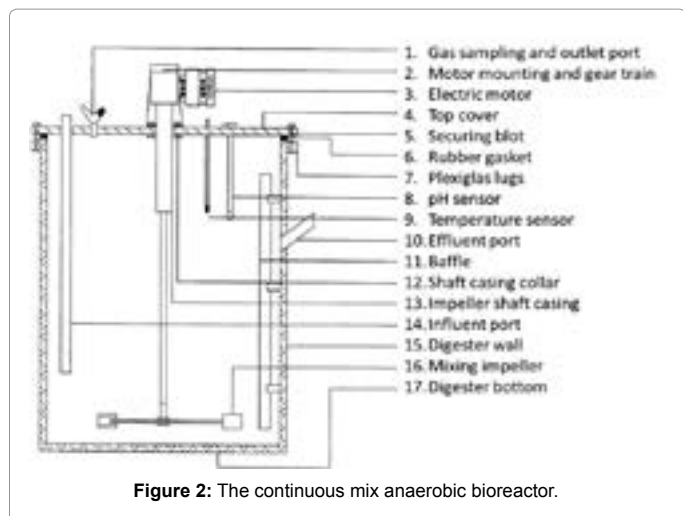


Figure 2: The continuous mix anaerobic bioreactor.

temperature and pH sensors were mounted on special holders threaded through the reactor cover and immersed to a depth of 100 mm below the liquid surface. The contents of the bioreactor were stirred by means of a flat-bladed impeller driven by a 1/12 HP electric motor (Model No6105061401, Franklin, Bluffton, Indiana, USA) at a speed of 125 rpm. The manure feed inlet consisted of a 25 mm diameter PVC tube inserted through the bioreactor cover to a depth of 246 mm below the liquid surface. The effluent port was located opposite to the feed inlet port at a distance of 300 mm above the bottom of the bioreactor.

**Feeding system:** The manure feeding system consisted of a manure storage tank, a feed pump, a set of tubing, a distribution manifold and a set of solenoid valves. A 60 L plastic container was used to store the manure and was fitted with a stirring paddle driven by a 1/12 HP electric motor (Model 55CP1OFG17AX, General Electric, Mississauga, Ontario, Canada) mounted on the tank cover. A feeding and ventilation ports were provided on the tank cover. The outlet port of the tank was located at the lower portion of the tank side. A peristaltic pump (Model 110-030, TAT Engineering Co., Logan, Ohio, USA) was used to feed the manure. At a rotational speed of 2 rpm and an output of 0.138 L of manure per revolution, the pump delivered the manure at a capacity of 16.56 L/h from the storage tank through the valve, tygon tubing, the distribution manifold and a set of automatically controlled solenoid valves to the individual reactors. A digital timer controlled and synchronized the operation of the feed tank stirrer (Model 5935932, Type NSI-10R93, Bodine Electric Co, North York, Ontario, Canada), the feed pump and the solenoid valves to ensure the delivery of a well mixed manure to the bioreactors at a predetermined sequence and loading rate. Eight feeding cycles were carried out daily at an interval of 3 hours.

**Temperature control:** The objective of the temperature control system was to maintain the profiles of the two temperature cycles (Figure 3) in the enclosed chamber in which the digesters were located. It consisted of an insulated chamber, a chiller, two heaters, two cross flow heat exchangers, two fans, temperature transducers and ducts for directing the circulation of air (Figure 4). The chamber was made of a 20 mm thick wooden box (2440 mm long, 710 mm wide and 525 mm deep) and divided into two compartments each was padded with 40 mm of Styrofoam insulation (R-value of 0.88 m<sup>2</sup> KI/W). Each compartment was maintained at one of the two temperature profiles and contained two bioreactors. On the floor of each compartment was a U-shaped galvanized steel duct of 100 mm inside diameter. A fan, a heat exchanger and a heater were located

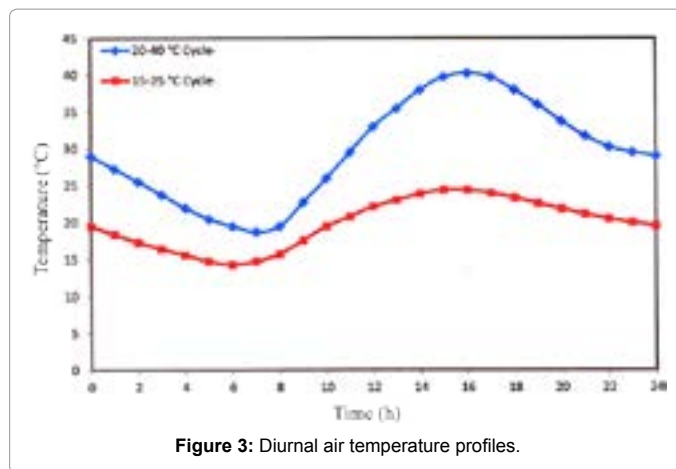


Figure 3: Diurnal air temperature profiles.

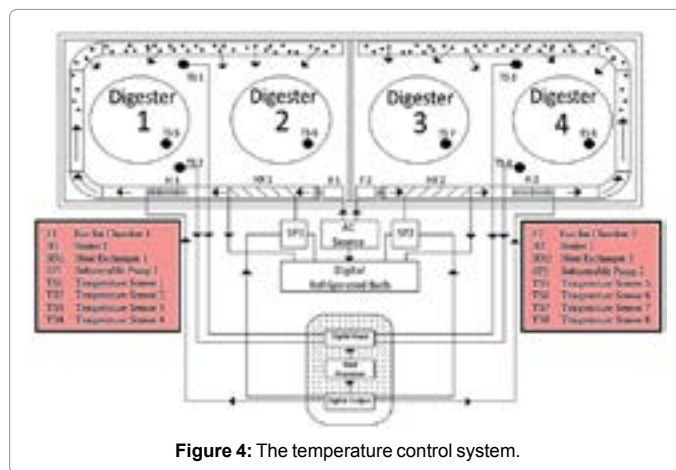


Figure 4: The temperature control system.

(in that order) inside one arm of each duct which circulated air in a closed loop. The fan drew air from the chamber into the duct and the air circulated over the heat exchanger and the heater and then left the duct through perforations on the other two sections of the U-shaped duct to bathe the bioreactor. The air was circulated using a thermally protected electric fan (Model 4C02A, Dayton Electric Manufacturing Co., Nues, Illinois, USA). The capacities of the heaters were 750 and 500 W for the compartments operated at the higher and lower cyclic temperature ranges, respectively. A digital rapid cool refrigerated bath (Series 900, Polyscieńce, Nues, Illinois, USA) with an operating temperature range of -35 to 150 °C and an accuracy of  $\pm 0.02^\circ\text{C}$  was used. The chiller had a maximum cooling capacity of 425 W at 20°C (or 225 W at -10°C), a bath capacity of 13 L and can deliver a maximum flow of 15 L/min at zero head. Two submersible pumps (Model 1 -MA, Little Giant, Bluffton, Indiana, USA), each delivered chilled water to one of the two heat exchangers, were immersed inside the chiller bath. With a power consumption of 3.73 W, each pump had a rated capacity of 10.72 L/min at 0.3 m and a maximum pumping height of 1.85 m. A Styrofoam cover of the same thickness as the wall insulation was provided for each of the chamber compartments in such a way that visual examination of the content of the reactors was possible without removing the covers. Four temperature sensors (TS 1 & TS2 and TS3 & TS4) were mounted on the chamber covers (two for each compartment). During operation, the temperature transducers continuously sampled the air temperature in each of the chamber compartments and transmitted the signals

through the digital input node of the data acquisition system to the host processor. Every 30 seconds, the host processor computed the average temperature of each compartment and compared these with set point values. Depending on the error signal, the host processor generated a correction signal which either turned the heaters and the chilled water submersible pumps on and off (or vice versa), respectively.

**Gas collection:** Gas collection was effected through a Y-shaped nipple fitted on the cover. One end of the nipple carried a rubber septum through which gas samples were drawn with a syringe. The other arm of the nipples was fitted with flexible tubing which conveyed the evolved gases through a water column, gas scrubber and gas meter to the gas collection and storage system. The water column was used to provide back pressure in the reactor to maintain a constant liquid level. The scrubber was made of a gas-tight Plexiglas column (80 mm diameter and 450 mm long) filled with steel wool to strip the biogas of hydrogen sulfide. The gas meter was a tipping balance meter which indicated when  $50 \pm 2$  mL of gas had been collected. The collected gas was subsequently pumped into a gas cylinder by means of a compressor.

**Manure collection, preparation and storage:** Dairy manure was collected from a commercial dairy farm located in Stewiacke, Nova Scotia, Canada. The manure was scrapped off the floor of the dairy barn and screened using a modified fish de-boner (Model SDX16, Bibun, Japan). During the sieving process, tap water was added to dilute the manure to 6.5% solid content. The manure was placed in buckets of 30 L and the buckets were sealed and transported to a commercial freezing plant (Associated Freezers, Dartmouth, Nova Scotia, Canada) where they were stored at  $-25^\circ\text{C}$  until needed. When needed, buckets of manure were removed from the freezer and kept at room temperature in the Waste Management Laboratory to thaw for 48 hours and the manure was then loaded into the feeding tank. Some characteristics of raw manure are shown in Table 1.

### Start-up procedure

The bioreactors were started by adding 18.0 L (to each reactor) of actively digesting sewage sludge obtained from Mill Cove Municipal Wastewater Treatment Plant (Bedford, Nova Scotia, Canada). This

was followed by the addition of 7.0 L of screened dairy manure. The temperature control and the data acquisition systems were activated and the digesters were operated in batch mode for 48 hours. The feeding system was used to feed the reactors. Reactors R1 and R2, were operated at the diurnal temperature cycle of  $20\text{--}40^\circ\text{C}$  and received feed at rates of 0.33 and 0.42 L/d, respectively. Reactors R3 and R1 were operated at the diurnal temperature cycle of  $15\text{--}25^\circ\text{C}$  and received feed at rates of 0.33 and 0.42 L/d, respectively. Feeding rates were equivalent to hydraulic retention times (HRTs) of 75 days for the feeding rate of 0.33 L/d and 60 days for the feeding rate of 0.42 L/d. The feeding rates were then adjusted to 0.5 L/d for reactors R1 and R3 and 0.63 L/d for R2 and R1 and were held constant for 72 hours. These rates corresponded to HRTs of 50 and 40 days, respectively. These rates were maintained for another 48 hours.

### Operating procedure

After 7 days from start-up, R1 and R3 were operated at an HRT of 25 days while R2 and R4 were operated at an HRT of 20 days. The start-up period was concluded after a period of 32 days. Following the initial start-up period, monitoring of the biogas production was started on day 33 (from the start). Steady state was construed to have been achieved when a uniform gas production and/or uniform effluent quality were achieved. Once the steady-state was achieved at a given retention time, the system parameters were measured monitored for a period of at least five days. The feeding rates of the reactors were adjusted for the next set of retention times (15 days for reactors R1 and R3 and 10 days for reactors R2 and R4). When the steady state was attained, sampling monitoring was continued for five days.

**Analyses:** The liquid samples were analyzed for total solids (TS), total volatile solids (TVS), total fixed solids (TFS) and volatile fatty acids (VFA) whereas the biogas samples were analyzed for gas composition.

**Solids:** The total solids analyses were performed according to procedures described in the Standard Method for the Examination of Water and Wastewater [17].

**Volatile fatty acids:** The individual volatile acids (C2-C7) contained in a sample were determined using a Hewlett-Packard gas chromatograph (Model 5890 series II, Mississauga, Ontario, Canada) equipped with an HP 76734A automatic injector. Extraction of the VFA was carried out by acidifying 3.0 mL of each of the manure samples using 0.1 mL 30% sulphuric acid. The acidified samples were well mixed and centrifuged at 7000 rpm for 20 minutes. 2.0 mL of the supernatants were decanted and an equal amount of diethyl ether was added. The mixtures were well shaken and then centrifuged at 5000 rpm for 5 minutes to break down the emulsion layer. The upper layer which consisted of di-ethyl ether was removed for analysis. Volatile acids were, also, extracted from a volatile acid standard mixture (No4-6975, SupelCo, Oakville, Ontario, Canada) using di-ethyl ether. The chromatograph was calibrated by injecting 1.0 mL of the extracted standard VFA mixture into the 25 mm x 0.2 mm capillary column of the liquid chromatography whose film thickness is 0.33 mm. 1.0 mL of the extracted samples were injected into the column. A split ratio of 1:5 was applied. The column temperature was first maintained at  $60^\circ\text{C}$  for 3 minutes and then increased at a rate of  $10^\circ\text{C}$  per minute until a temperature of  $150^\circ\text{C}$  was attained. The column temperature was maintained at  $150^\circ\text{C}$  for 2 minutes. The injector was set to  $180^\circ\text{C}$  while the flame ionization detector was set at  $250^\circ\text{C}$ . The carrier gas was

Parameter	Mean Value*
√ Total Solids (g/L)	64.25
• Total Volatiles Solids (g/L)	50.26
• (% of Total Solids)	78.23
• Total Fixed Solids	13.99
√ Total Suspended Solids (g/L)	42.25
• Volatile Suspended Solids (g/L)	31.00
• Fixed Suspended Solids (g/L)	11.25
√ Total COD (g/L)	98.80
√ Soluble COD (g/L)	27.90
√ Total Kjeldahl Nitrogen (g/L)	3.80
• Ammonium Nitrogen (g/L)	2.45
• NH <sub>4</sub> -N/TKN Ratio	0.64
√ Volatile Fatty Acid (VFA)	
• Acetic (g/L)	1.55
• Propionic (g/L)	0.28
• i-Butyric (g/L)	0.04
• Butyric (g/L)	0.06
• i-Valeric (g/L)	0.04
• Valeric (g/L)	0.02
• i-Caprioc (g/L)	0.01
• Caprioc (g/L)	0.01
• Heptanoic (g/L)	0.04
Total VFA as acetic acid (g/L)	1.91

\*Each value represents the average of 5 samples

**Table 1:** Characteristics of raw manure.

helium at a flow rate of 1.2 mL/mm.

**Biogas composition:** The composition of biogas was determined using a gas chromatograph (Model HP 5980A, Hewlett Packard, Mississauga, Ontario, Canada). Samples of 0.1 mL were taken from the gas collected in the sampling tube using a gas tight locked syringe. The samples were injected into 152.4 mm x 3.2 mm (6 in x 1/8 in) OD porapak Q stainless steel column of the gas chromatograph which is connected in a series bypass arrangement with a 152.4 mm x 3.2 mm (6 in X 1/8 in) OD molecular sieve 5A 60180 stainless steel column. The switch valve of the gas chromatograph was adjusted to permit the molecular sieve column to store nitrogen, methane and carbon monoxide until the elution of the CO<sub>2</sub>, C<sub>2</sub>H<sub>2</sub> and C<sub>6</sub>H<sub>6</sub> through the porapak Q stainless steel column. The column was maintained at 45°C with helium as the carrier gas at 30 ml/min. The injector was set at 150°C while the thermal conductivity detector was set at 250°C.

## Results and Discussion

### Temperature

Typical variations of reactor temperatures with those of the chambers for the 20-40°C and 15-25°C diurnal temperature cycles are shown in Figure 5. A summary of the reactor temperature profiles at the various operating conditions is presented in Table 2. The mean minimum and maximum temperatures for the reactors operating under the 20-40°C cycle were 25.5°C and 33.8°C, respectively. The mean amplitude for the 20-40°C cycle was ± 8.3°C. The mean minimum and maximum temperatures for the 15-25°C cycle were 18.6°C and 22.8°C, respectively. The mean amplitude for the 15-25°C cycle was ± 4.2°C.

Relative to air temperature, there was a lag in the reactor temperature at both the maximum and minimum temperatures of both temperature cycles. The mean lag at the maximum and minimum temperatures for the 15-25°C cycle and the 20-40°C cycle were 3.37 h and 3.95 h and 3.38 h and 4.35 h, respectively. This was due to the significant differences between the density of air in the chamber and the liquid medium in the reactor and the thermal properties of the reactors' walls which affected the rate of heat transfer to and from the reactors.

### Solids

The average values of total, fixed and volatile solids concentrations of the effluent samples collected during the steady state and their reductions are shown in Table 3. The diurnal variations in the total

solids of the effluent collected over a 24 h period during the steady state are shown in Figure 6.

### Total solids

Diurnally cyclic variations in the effluent total solids were observed under the 20-40°C cycle. However, the diurnally cyclic variations in total solids were not distinct at the 15 and 10 d HRTs under the 15-25°C cycle. Similar diurnally cyclic variations were observed for the effluent total volatile solids and total fixed solids.

For the 20-40°C cycle, the TS reductions ranged from 30.3 to 36.8% (19.45 to 23.65 g/L), the TVS reductions ranged from 34.9 to 40.3% (17.56 to 20.26 g/L) while the TFS reductions ranged from 13.5 to 24.2% (1.89 to 3.39 g/L), depending on the hydraulic retention time. For the 15-25°C cycle, the TS reductions ranged from 24.4 to 38.3% (15.65 to 24.55 g/L), the TVS reductions ranged from 25.2 to 39.7% (12.66 to 19.96 g/L) while the TFS reductions ranged from 21.4 to 32.8% (2.99 to 4.59 g/L), depending on the hydraulic retention time. Higher TS reductions were achieved with the high diurnal temperature cycle (20-40°C) than those with the lower diurnal temperature cycle (15-25°C) at all HRTs.

Wilkie et al. [18] obtained the highest TS reduction of 46.7% during the treatment of dairy manure at a low temperature range of 11.7-32.4°C. Elango et al. [19] achieved a TS reduction of 87.6 % during the treatment of solid wastes over the temperature range of 26 to 36°C. The TS reduction achieved in the present study (25.2-39.7) is within the values reported in the literature. The hydraulic retention time (HRT) had a significant effect on the TS reduction in the present study. Generally, the shorter the HRT, the lower the solids reductions. Ribas and Barana [20] reported that a decrease in the HRT from 16.6 to 9.7 d increased the TS of anaerobically treated wastewater from 2.3 to 4.8%. Umana et al. [21] reported an increase in the TS removal efficiency from 14.6 to 68.8% when the HRT was increased from 1 to 5.5 d during the treatment of dairy manure. Rico et al. [22] noted that the concentration of TS increased from 39.2 to 45.3 g/L as the HRT was decreased from 20 to 10 d during the treatment of dairy manure.

### Volatile solids

The absolute values of TVS reductions (g/L) were generally lower than those of the TS. This may be explained by the fact that volatile solids are converted to microbial biomass, volatile fatty acids (VFAs)

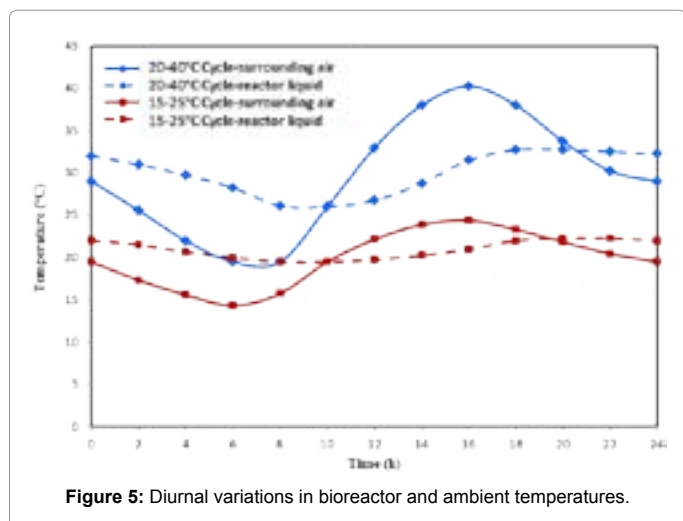


Figure 5: Diurnal variations in bioreactor and ambient temperatures.

Diurnal Temperature Cycle (°C)	HRT (d)	Reactor Temperature (°C)			Lag (h)	
		T <sub>min</sub>	T <sub>max</sub>	A	L <sub>min</sub>	L <sub>max</sub>
20-40	25	25.5	33.7	8.2	3.38	4.35
	20	25.1	33.6	8.4	3.30	4.69
	15	26.0	33.9	7.9	3.37	3.19
	10	25.2	33.8	8.6	3.27	3.49
	Mean	25.5±0.4	33.8±0.1	8.3±0.31	3.33±0.1	3.93±0.7
15-25	25	18.6	22.9	4.3	3.51	4.37
	20	18.3	22.4	4.1	3.47	4.47
	15	18.5	22.9	4.4	3.30	3.37
	10	18.8	22.8	4.0	3.19	3.60
	Mean	18.6±0.2	22.8±0.2	4.2±0.2	3.37±0.2	3.95±0.5

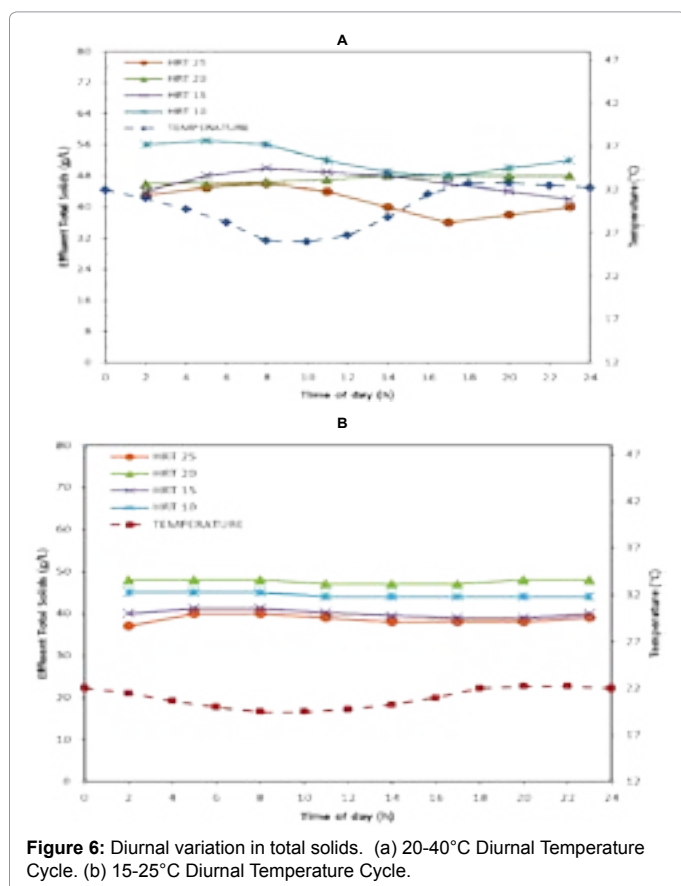
T<sub>max</sub> = Maximum temperature  
 T<sub>min</sub> = Minimum temperature  
 A = Amplitude  
 L<sub>min</sub> = Lag at minimum temperature  
 L<sub>max</sub> = Lag at maximum temperature

Table 2: Summary of reactor temperature profiles.

Diurnal Temperature Cycle (°C)	HRT (d)	TS			TFS			TVS		
		Mean	Reduction		Mean	Reduction		Mean	Reduction	
		(g/L)	(g/L)	(%)	(g/L)	(g/L)	(%)	(g/L)	(g/L)	(%)
20-40	25	40.60	23.65	36.8	10.60	3.39	24.2	30.0	20.26	40.3
	20	41.30	22.95	35.7	10.90	3.09	26.4	30.4	19.86	39.5
	15	44.10	20.45	31.4	11.80	2.19	15.7	32.3	17.96	35.7
	10	44.80	19.45	30.3	12.10	1.89	13.5	32.7	17.56	34.9
15-25	25	39.70	24.55	38.2	9.40	4.59	32.8	30.3	19.96	39.7
	20	44.90	19.35	30.1	10.20	3.79	27.1	34.7	15.56	30.9
	15	47.30	16.95	26.4	10.60	3.39	25.7	36.7	13.56	26.9
	10	48.60	15.65	24.4	11.00	2.99	21.4	37.4	12.66	25.2

TS = Total solids  
 TFS = Total fixed solids  
 TVS = Total volatile solids  
 Initial TS = 64.25 g/L  
 Initial VS = 50.26 g/L  
 Initial FS = 13.99 g/L

Table 3: Total solids.



and biogas. While microbial biomass contributes to the total solids content of the effluent, the VFAs are evaporated at 105 °C and therefore, are not detected in TS measurements [23].

The HRT had a significant effect on the effluent TVS. Generally, the shorter the HRT the lower the TVS reduction. De la Rubia et al. [24] reported an increase in the TVS reduction (from 53 to 73 %) as the HRT increased from 27 to 75 d. Ribas and Barana [20] reported an increase in the TVS from 1.6 to 4.3% in a wastewater treatment system as the HRT was decreased from 16.6 to 9.7 d. Umana et al. [21] reported an increase in the TVS reduction (from 26.3 to 78.8 %) during

the treatment of dairy manure as the HRT was increased from 1 to 5.5 d. Rico et al. [22] noted that the concentration of TVS increased from 23.7 to 27.8 g/L during anaerobic treatment of dairy manure as the HRT decreased from 20 to 10 d. The reduction in the TVS obtained in this study (25.2-39.7 %) was within the range reported in the literature.

The diurnal cycling temperature also affected the TVS reductions, the higher the temperature the higher the TVS reduction. Wilkie et al. [18] achieved the highest TVS reduction of 60.4% at a temperature of 32.4°C during the treatment of dairy manure wastewaters. Elango et al. [19] reported 88.1% reduction in TVS over the temperature range of 26 to 36°C. Trazcinski and Stuckey [25] reported TVS reductions in the range of 40-75% at the temperatures of 21-35°C during anaerobic digestion of solid waste. The TVS reductions achieved in this study (25.2-39.7%) are within the range reported in the literature.

### Fixed solids

Fixed solids represent the inorganic components of the waste and it consists of potassium, sodium, calcium, magnesium, iron, copper and other minerals [23]. Unlike carbon and nitrogen, little or no significant losses in these inorganic compounds are generally expected to occur since they do not play a very significant role in the anaerobic digestion process. In this system, a fixed solid recovery ranging from 64.3 to 112.0 % was recorded.

Converse et al. [26] reported a fixed solid recovery ranging from 90 to 104 %. In this study, the losses in fixed solids may have been due to inadequate mixing of the bioreactor contents which may have resulted in the settling out of the inorganic mineral resulting in the lower concentration of these compounds in the effluent. They may, also, have been due to the fact that changes in the nature of these inorganic compounds may have occurred at the higher temperature (550°C) used in the determination of total ash content.

### Volatile fatty acids

The concentrations of volatile fatty acids (VFAs) in the effluent samples taken during the steady state conditions are shown in Table 4. The VFAs included: acetic, propionic, isobutyric, iso-valeric, valeric, iso-caprioc and heptanoic acids.

The VFAs concentration in the effluent ranged from 45 to 1200 mg/L, depending on the operating conditions. The effluent VFAs concentration generally increased with decreases in the HRT and higher VFAs concentrations were recorded at the lower temperature cycle. At

Temp (°C)	HRT (d)	Volatile Acid Concentration (mg/L)									
		Acetic Acid	Propionic Acid	i-Butyric Acid	n-Butyric Acid	i-Valeric Acid	n-Valeric Acid	i-Caproic Acid	n-Caproic Acid	Heptanoic Acid	Total as Acetic Acid
20-40	25	5.5	26.6	15.6	8.4	9.8	<0.01	11.7	3.9	5.7	60.0
	20	44.0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	44.7
	15	3.0	26.0	10.7	11.3	9.7	1.3	3.3	2.4	2.5	50.0
	10	53.8	56.9	35.7	8.2	11.5	5.	9.0	4.7	16.9	154.8
15-25	25	54.1	73.6	13.7	18.3	3.9	<0.01	2.2	5.4	20.0	151.0
	20	70.8	102.1	19.1	26.0	19.8	4.7	6.1	6.1	58.4	231.8
	15	207.4	92.0	18.5	27.6	19.7	4.1	6.6	4.3	59.4	362.5
	10	645.0	431.4	139.7	38.6	36.0	25.0	22.7	17.8	30.1	1187.0
Raw Manure		1548.4	283.5	44.5	60.5	40.2	21.0	7.0	11.3	37.1	1913.0

Table 4: Volatile fatty acids.

the 20-40°C diurnal temperature cycle, the VFAs (measured as acetic acid) first decreased from 60 to 44.7 mg/L as the HRT was reduced from 25 to 20 d. Further reductions in the HRT resulted in increasingly higher concentrations of VFAs, up to 154.8 mg/L was recorded at the 10 day HRT. Among the individual volatile acids, propionic acid had the highest concentration at all but 20 d HRT. At the 20 d HRT, only acetic acid was detected in measurable levels. At the 15-25°C diurnal temperature cycle, the total volatile acids concentration increased from 151.0 mg/L at the 25 d HRT to 1187.0 mg/L at the 10 d HRT. Propionic acid concentration was higher than that of the rest at the 25 and 20 d HRTs while acetic acid concentration was the highest at the 15 and 10 d HRTs.

The HRT has a significant influence on the production of VFAs, the shorter the HRT the higher the VFAs concentration. Colmenarejo et al. [27] reported that the highest VFAs production was achieved when the HRT was decreased from 5.2 to 1.7 h. El-Mashad et al. [28] reported that the VFA concentration increased when the HRT was decreased from 24 to 3 h during the treatment of domestic wastewater. Elefsiniotis and Oldham [29] reported an increase in VFA production when the HRT was increased from 6 to 12 h which then decreased with further increase in the HRT to 15 h. Maharaj and Elefsiniotis [30] noted highest VFA concentration at an HRT of 30 h as appose to 48 and 60 h while anaerobically treating wastewaters. De la Rubia et al. [24] found that the VFA production increased from 5532 to 6432 mg/L as the HRT decreased from 75 to 20 days.

The results also showed the importance of temperature on the production of VFAs. Higher VFA production was observed with lower diurnal cyclic temperature. Maharaj and Elefsiniotis [30] noted increased VFA concentrations as the temperature was increased from 8 to 25°C, which then decreased upon further increase in temperature (from 25 to 35°C). Choorit and Wisarnwan [31] noted that the VFA concentration slightly increased from 160.71 to 166.64 mg/L as the temperature decreased from 43 to 37°C and from 245.07 to 338.71 mg/L as the temperature decreased from 55 to 49°C. De la Rubia et al. [24] observed an increase in VFA production from 830 to 5688 mg/L during digestion anaerobic municipal sludge as the temperature increased from 35 to 55°C.

However, values of effluent VFAs concentrations reported in the literature vary depending on the type of waste. Converse et al. [27] reported effluent VFAs concentrations of 423-1113 mg/L (as acetic acid) using CSTR operated on dairy manure of 12.8-13.4 % TS content and a temperature of 35°C. Jeyanayagam and Collins [32] reported effluent VFAs concentrations of 754-1515 mg/L using laboratory digesters operated on dairy manure of 4.0-6.5 % TS content at hydraulic

retention times of 15-20 d and a temperature of 35°C. Lo and Liao [33] reported a VFAs concentration of 584 mg/L using a fixed film reactor operated on a screened dairy manure of 3.7 % TS content at an HRT of 20 d and a temperature of 12°C. Peck et al. [34] reported effluent VFAs concentrations of 64-98 mg/L using manure of 3458-4109 mg/L TS at 10-25 d HRTs and a temperature of 35°C. The VFA concentrations observed in this study are within the range of values reported in the literature.

Organic wastes are first converted to long chain fatty acids in the acidogenic stage of anaerobic digestion processes. Volatile acids are further converted to acetic acid which together with hydrogen and formate serve as substrates for methane bacteria. Different species of anaerobic bacteria are involved in the conversion of specific long chain fatty acids to acetic acid. The increased proportion of propionic acid relative to acetic acid indicated that the different species of anaerobic bacteria did not respond in a similar manner to the effect of diurnally cyclic temperature. The fact that individual VFAs are removed at different rates under adverse conditions such as a drop in temperature has been observed by other researchers [34] A lower methane yield was recorded under the conditions in which the propionic acid concentration was high indicating that propionic acid was not available for conversion to methane.

## Biogas

Figure 7 shows the daily biogas production under various operating conditions. The diurnal variations in the mean rate of biogas production for the 25 h HRT are shown in Figure 8. A summary of the average composition of the biogas collected under the various treatment combinations is shown in Table 5. Typical results of the autocorrelation and Fourier analysis are shown in Figures 9-12.

## Biogas production

There appears to be a definite relationship between the bioreactor temperature and the rate of biogas production. Biogas production followed a diurnally cyclic pattern similar to that of the bioreactor temperature under most operating conditions. The highest reactor temperature and the maximum rate of biogas production occurred at the 18 hour while the lowest reactor temperature and the minimum biogas production rate occurred at the 9 hour. This trend was observed at all HRTs with 20-40°C cycle and at the 20 d HRT with 15-25°C cycle. The daily amplitude of the gas production cycle was higher under the 20-40°C cycle than that observed under the 15-25°C cycle. Smaller amplitudes in the gas production cycle were recorded in the lower temperature cycle at all HRTs because of the smaller diurnal amplitude of the reactor temperature. With digesters operated under stressful

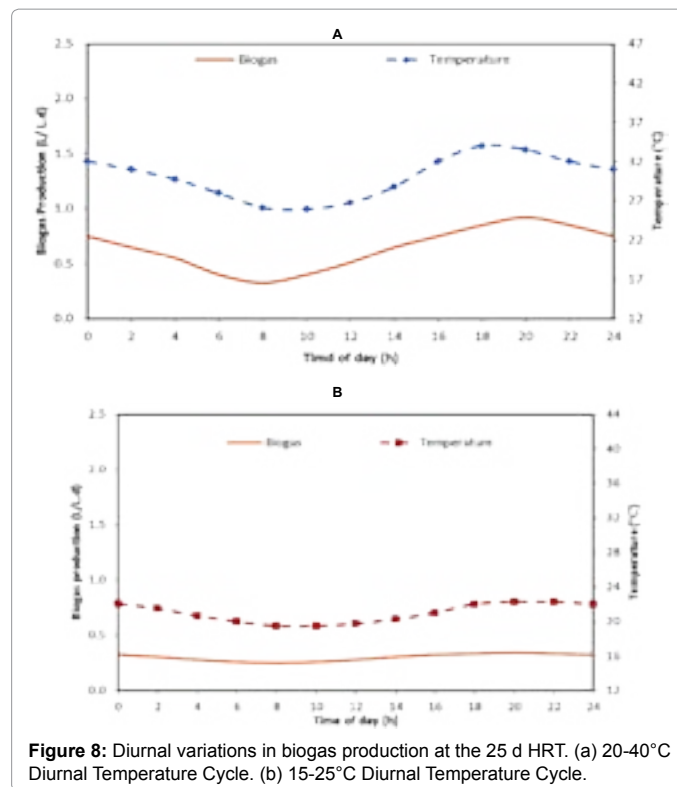
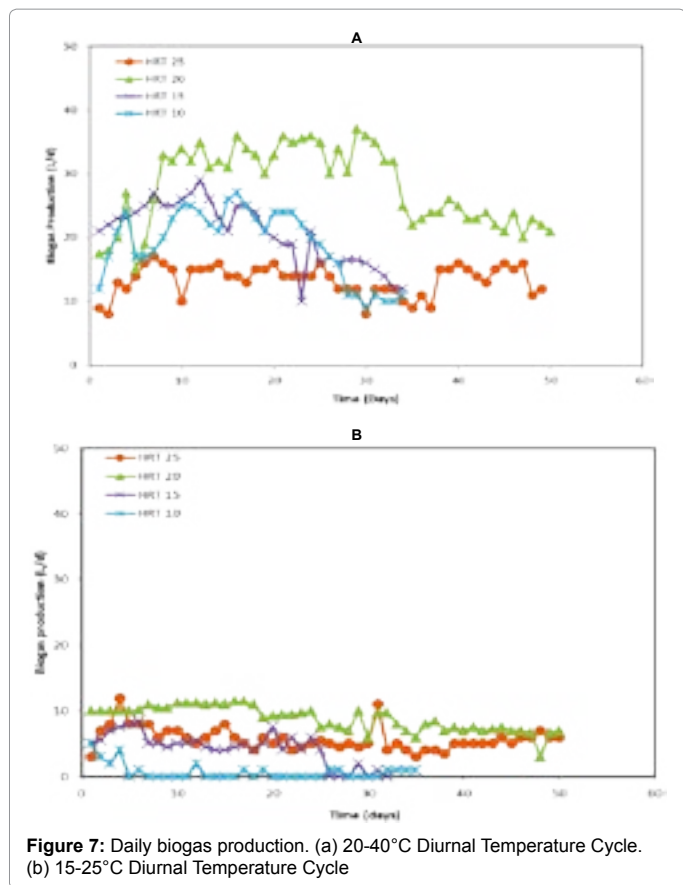


Figure 8: Diurnal variations in biogas production at the 25 d HRT. (a) 20-40°C Diurnal Temperature Cycle. (b) 15-25°C Diurnal Temperature Cycle.

conditions such as a combination of low temperature (15-25°C) and high loading rate (short HRT), the recorded diurnal variations in the rate of biogas production were either less pronounced or non-existent. The direct relationship between the rate of biogas production and the reactor temperature is supported by the known fact that microbial activity increases with increases in the reaction temperature.

Under the operating temperature cycle of 15-25°C, a steady gas production was achieved at the 25 d HRT only after a few days whereas at the 20 d HRT, a steady, but not rapid, decline in biogas production was observed after the 15<sup>th</sup> day, following the initial rise in biogas production. At the 15 d HRT, the gas production declined unsteadily after the first 8 days until it completely stopped at the 28<sup>th</sup> day. At the 10 d HRT, the gas production declined rapidly from the start, ceasing completely after the 5<sup>th</sup> day. These results indicated that the bioreactors did not operate satisfactorily at HRTs shorter than 25 d under the 15-25°C diurnal temperature cycle. The failure of the digesters at 15 and 10 d HRTs is due to the combined effects of the low cyclic diurnal temperature and high loading rate.

The biogas production observed at the 20-40°C diurnal temperature cycle was characterized by rapid rise in production followed by a rapid decline, the shorter the retention time the more pronounced was the decline. This rapid rise in biogas production experienced at the beginning of each retention time can be attributed to the response of anaerobic bacteria to increased food supply as a result of the higher loading rate. Apparently, the acid forming bacteria responded faster to the increased loading rate. However, the accumulation of VFAs resulted in inhibition of the methane producing bacteria and hence reduction in

Diurnal Temperature Cycle (°C)	HRT (d)	Gas Composition		
		CH <sub>4</sub> (%)	CO <sub>2</sub> (%)	Others** (%)
20-40	25	69.0±1.7	25.8±1.5	5.2
	20	67.6±1.3	28.6±2.3	3.8
	15	62.6±1.0	37.3±1.0	0.1
	10	64.9±1.0	34.9±1.0	0.2
15-25	25	71.8±2.1	25.3±1.0	3.0
	20	69.6±1.8	27.3±1.0	3.1
	15	47.8±0.5	44.0±1.2	8.2
	10	44.6±0.5	43.6±1.8	11.8

\*Each mean represents the average of 12 samples  
 \*\*Nitrogen, hydrogen sulphide, carbon monoxide, etc

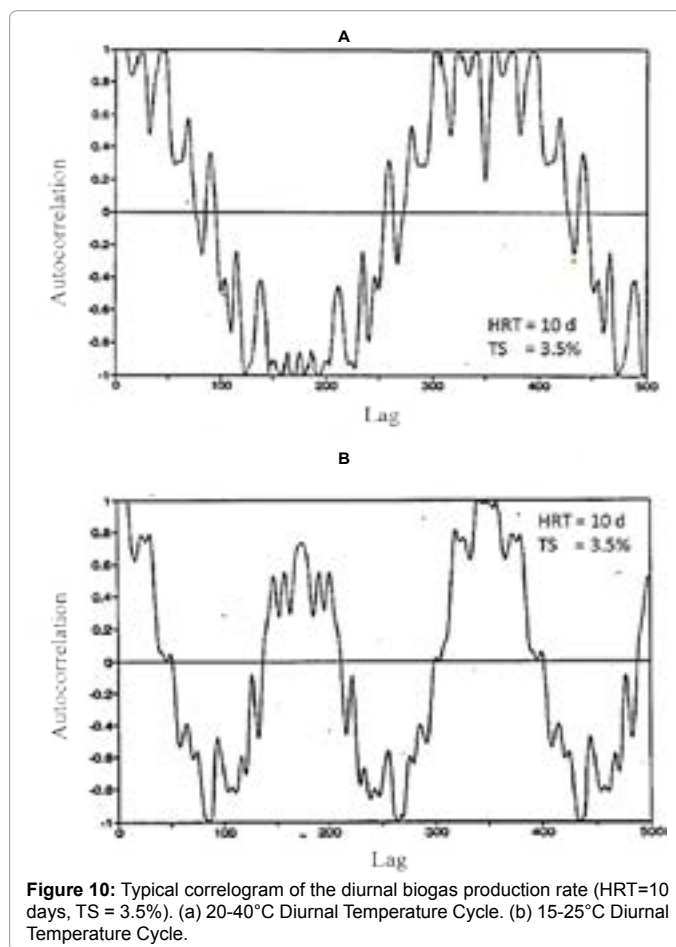
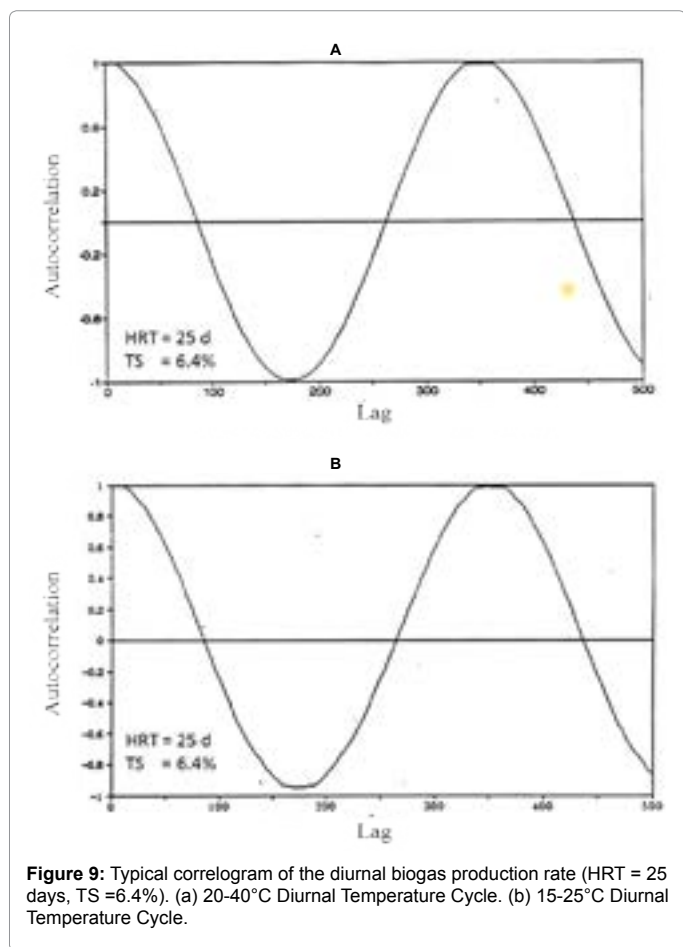
Table 5: Biogas composition\*.

biogas production. The higher rate of biogas production attained at a 20 d retention time indicated that at HRT longer than 20 d, the reactors may have been underfed whereas at shorter retention times the reactors may have been overloaded.

In this study, higher biogas production was obtained with the 20 h HRT. However, literature reports on effect of HRT on biogas production vary depending on the type of waste and system used. Hossain et al. [35] noted that the biogas production increased in wastewaters as the HRT increased from 2 to 8 h, but a further increase in the HRT from 8 to 14 h lead to a decrease in biogas production. Huang et al. [36] reported that the higher biogas yields were achieved at lower HRTs (8-12 h) during wastewater treatment. Rico et al. [22] noted that as the HRT decreased from 20 to 12.5 d, the biogas production increased from 0.66 to 1.21 m<sup>3</sup> during the anaerobic digestion of dairy manure.

In this study, higher biogas production was observed with higher temperatures (within the temperature range studied). However the





reports on the effect of temperature on biogas production vary. Hossain et al. [35] reported that biogas production increased as the temperature increased from 30 to 40°C during wastewater treatment, but further increase in temperature from 40 to 45°C resulted in a decrease in biogas production. Wu et al. [37] noted that the biogas production increased as the temperature increased from 20 to 40°C during anaerobic digestion of solid wastes and decreased with further increase in temperature to 45°C while a further increase to 55°C lead to a rapid increase in biogas production. Yu et al. [38] reported that more biogas production was achieved at temperatures of 55°C during anaerobic digestion of wastewaters than those obtained at 37°C. Banerjee and Biswas [28] illustrated that during anaerobic digestion of wastes over the temperature range of 35 to 55°C maximum biogas production was achieved at 50°C.

### Gas composition

Diurnal variations in gas composition were observed at the all HRTs under the 20-40°C diurnal temperature cycle. Similar variations were observed at the 20 d HRT under the 15-25°C diurnal temperature cycle. A better gas quality (higher methane content) was obtained at all HRTs under the 20-40°C diurnal temperature cycle. Higher methane content was also obtained at the 25 and 20 HRTs under 15-25°C diurnal temperature cycle. Rises in the methane content of biogas as a result of a decrease in the bioreactor temperature have been reported by other researchers [34]. The increase in the quality of biogas is attributed to the raised solubility of carbon dioxide at the lower temperature cycle. Exceptions were observed with failed digesters. In stressed

methanogenic systems, hydrogen production and volatile fatty acids (more reduced than acetic acid such as propionate, butyrate etc.) have been reported to be produced in greater proportions [39].

The mean percentage of methane in the biogas ranged from 44.6 to 71.8 %, the carbon dioxide content ranged from 25.3 to 44.0 % while the composition of the other gases (nitrogen, hydrogen sulphide etc.) ranged from 0.1 to 11.8 %. Methane values reported in the literature ranged from 40 to 75 % [40,26,32-34]. The observed values are, thus, within the range reported in the literature.

### Autocorrelation analysis

A typical correlogram of diurnal biogas production is shown in Figure 9. Figure 9a was obtained at 25 days HRT using manure of 6.4% TS content and the temperature cycle of 20-40°C, while Figure 9b was obtained using similar HRT and manure TS content but a temperature cycle of 15-25°C. From the correlogram, it is seen that the diurnal biogas production rate predominantly followed a pattern similar to a sine wave. This pattern was observed with the operating temperature cycle of 20-40°C for all HRTs using the manure of 6.4% TS content and for the 25, 20 and 15 days HRTs using the manure of 3.5% TS content. However, at the 15-25°C temperature cycle, the sinusoidal pattern was only observed at the 25 day HRT using the manure of 6.4% TS content.

The autocorrelation analysis shown in Figure 10 indicated that under low HRT (10 days) and low influent TS Concentration (3.5%), the biogas production rate follows a sine wave sandwiched in some

Temperature Cycle (°C)	Solids Content (%)	HRT (days)	Sinusoidally Periodic
20 – 40	6.4	25	Yes
		20	Yes
		15	Yes
		10	Yes
	3.5	25	Yes
		20	Yes
15 – 25	6.4	15	No
		10	No
		25	No
		20	No
	3.5	15	No
		10	No

\*Each mean represents the average of 12 samples  
 \*\*Nitrogen, hydrogen sulphide, carbon monoxide, etc

**Table 6:** Summary of Autocorrelation Analysis.

harmonics and noise. Various variants of Figure 10 were observed under the operating temperature cycle of 15–25°C for all retention times using the manure of 3.5% TS content and under the operating temperature cycle of 20–40°C with retention times of 10 to 20 days using the manure of 6.4% TS content. Furthermore, a similar correlogram was obtained under the retention time of 10 days using the manure of 3.5% TS concentration at the operating temperature cycle 20–40°C. These results are summarized in Table 6.

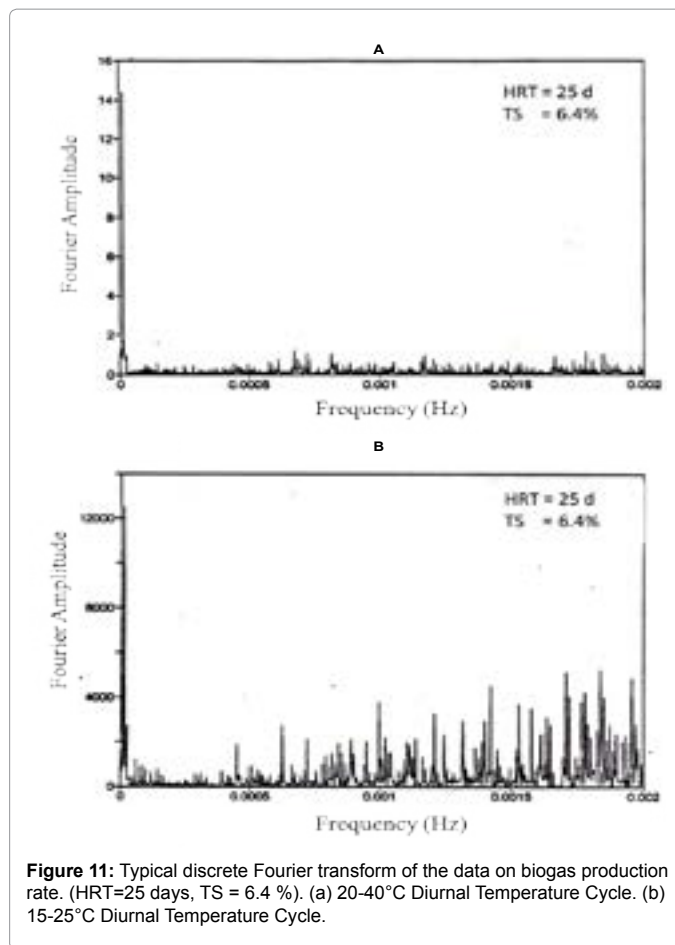
The sinusoidal periodic variations in biogas production rates were obtained at almost all loading rates under the 20–40°C temperature cycle because the temperature of the reactor used in the study was designed to vary in a diurnally cyclic (sinusoidal) manner and microbial activity and gas production increases with increase in temperature. However, sinusoidal variations were not obtained under most of the loading rates at the temperature cycle of 15–25°C due to fact that the amplitude of diurnal fluctuation in reactor temperature was relatively so small and it was impossible to detect a true sine wave. At the higher loading rate, pure sinusoidal variation was not obtained probably because of the higher frequency of the feeding cycle (i.e. shorter feeding interval) as most of the evolved gases are scavenged from the headspace of the reactors and measured during the feeding process.

The result of the fast Fourier analysis on the diurnal biogas production data shown in Figures 11 and 12 showed that, where the diurnal biogas production cycle followed true sinusoidal relationships, the highest Fourier amplitude appeared to occur at a frequency of about  $1.22 \times 10^{-5}$  Hz. Thus, the dominant frequency of such gas production cycle lies at about  $1.22 \times 10^{-5}$  Hz. This frequency roughly corresponds to a period of 24 hours. However, when the biogas production cycle did not follow a true sinusoidal cycle, the dominant frequency (indicated by the highest Fourier amplitude) appeared to lie between 0.0013 and 0.0014.

For the dominant frequency, the following regression equation, based on Fourier series, can be written to describe the diurnal variation in biogas production under diurnally cyclic temperature environment:

$$\gamma_t = \gamma_o + \sum_{n=1}^{\infty} [a_n \cos(2\pi n\phi t) + b_n \sin(2\pi n\phi t)] \quad (16)$$

Where:



**Figure 11:** Typical discrete Fourier transform of the data on biogas production rate. (HRT=25 days, TS = 6.4 %). (a) 20-40°C Diurnal Temperature Cycle. (b) 15-25°C Diurnal Temperature Cycle.

$\gamma_t$  is the biogas production at a time  $t$  (L/L.d)

$\gamma_o$  is the average biogas production per unit volume of reactor (L/L.d)

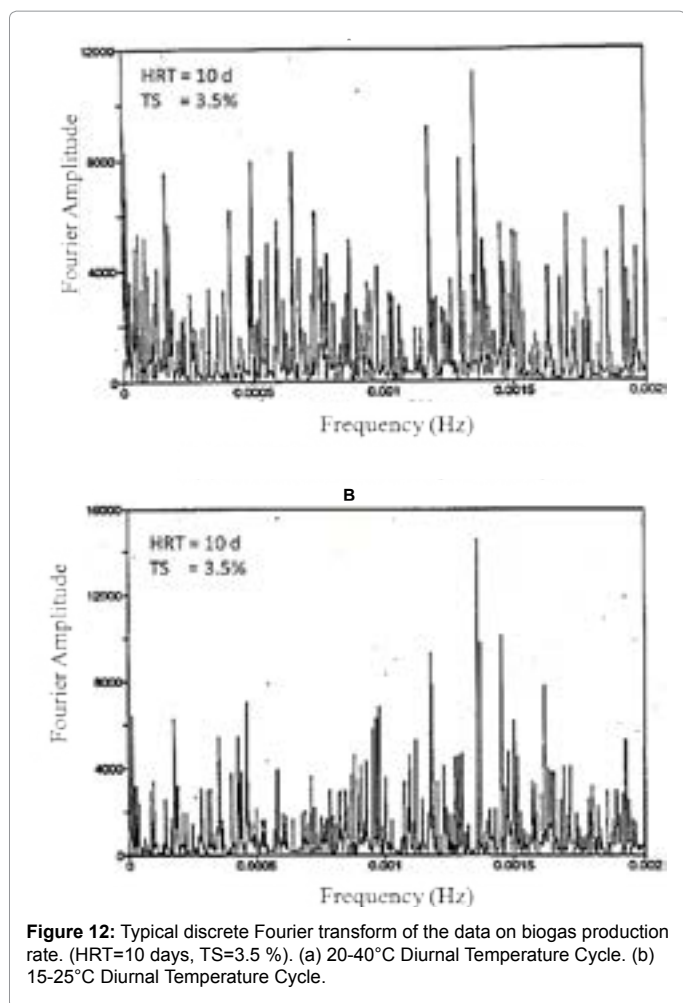
$\phi$  is the dominant frequency of the cycle =  $1.22 \times 10^{-5}$  (Hz)

$a_n, b_n$  are Fourier coefficients.

The Fourier coefficients and mean daily biogas production rate can be obtained by regression analysis. These values will vary with operating conditions (cyclic temperature ranges and loading rates).

## Conclusion

The effects of two diurnally cyclic temperature ranges (20-40°C and 15-25°C) and four levels of hydraulic retention times (25, 20, 15, and 10 d) on the performance of anaerobic reactors operated on screened dairy manure were evaluated. The reactor temperature exhibited a lag relative to the chamber air temperature. For the 20-40°C temperature cycle, the average lag period at the maximum and minimum chamber temperatures were 3.75 and 4.37 h, respectively. For the 15-25°C temperature cycle, the average lag periods at maximum and minimum chamber temperatures were 3.61 and 4.34 h, respectively. The effluent solids content were not adversely affected by the reactor diurnally cyclic temperature. The effluent total solids and methane content of the biogas diurnally cyclic patterns were out of phase with the diurnally cyclic pattern of the reactor temperature by about 12 hours under most operating conditions. The reductions in total solids and methane yield



**Figure 12:** Typical discrete Fourier transform of the data on biogas production rate. (HRT=10 days, TS=3.5 %). (a) 20-40°C Diurnal Temperature Cycle. (b) 15-25°C Diurnal Temperature Cycle.

were all significantly affected by the diurnal temperature range and hydraulic retention time. Biogas production from a healthy digester operating under a diurnally cyclic temperature environment follow a sinusoidal pattern which can be described by a Fourier equation of the form:  $\gamma_t = \gamma_o + \sum_{n=1}^{\infty} [a_n \cos(2\pi n\phi t) + b_n \sin(2\pi n\phi t)]$ . However, where the operating conditions are not favourable, the production followed a sinusoidal pattern which may be embedded in some harmonic and noise.

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