Comparison of performance of three different seeding sludge under three different hyper-thermophilic temperatures

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Abstract: Performance of three seeding sludge under three different hyper-thermophilic temperatures, 60°C, 65°C and 70°C was investigated and compared with mesophilic (37°C) and thermophilic (55°C) temperatures using the same seeding sludge and substrate in batch tests. Based on German guidelines for batch tests, the seeding sludge were selected for the batch tests using eudiometer tubes. Daily volumes of methane production for the three sludge were measured. Methane yields and degrees of COD degradation of the black water were also calculated and compared. Cow manure at 65°C recorded the highest net normalised cumulative volume of methane content (387.2 mlNCH_4-%), methane yield (231.7 mlNCH_4/gVS) and degree of COD degradation (79.1%) while BTU at 70°C recorded the least performance of methane yield (0.0 $mlNCH_4/gVS$) and content (0.0 $mlNCH_4$-%) and degree of COD degradation (0.0%). Cow manure at 65°C should be considered when setting-up large-scale hyper-thermophilic digesters.

Keywords: methane yield; net cumulative methane content; degree of COD degradation; hyper-thermophilic temperatures; seeding sludge; biogas.

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1 Background

Anaerobic digestion (AD) is a microbiological process by which organic matter either in the form of liquid or solid is degraded in the absence of oxygen. Technologically, AD process is employed in many sectors fundamentally as waste treatment approach to produce energy in the form of biogas and nutrient-rich digestate even though other chemical parameters like nutrients and heavy metals should also be considered (Tervahauta et al., 2014; Bryant, 2012; van Lier, 2008; de Mes et al., 2003). AD process is preferred to aerobic process (which requires oxygen for organic breakdown) because of high efficiency of COD removal, production of reduced quantities of excess sludge (Dereli et al., 2012), reduction of dependency on fossil fuels, creation of jobs and closing the loop for nutrient cycle (Vögeli et al., 2014). Other advantages include smaller reactor volume, rapid reactor start-up, little or no use of chemicals (van Lier, 2008), reduction of greenhouse gas like methane (CH_4) being released into the atmosphere and generation of renewable energy in the form of biogas which is a mixture of CH_4, CO_2, H_2S, O_2, H_2 and N_2 (Bagge, 2009; Vögeli et al., 2014). The biogas produced serves not only as a renewable energy source but also contributes to the protection of natural resources like forests by reducing the use of firewood and consequently deforestation, especially in developing countries (Vögeli et al., 2014).

The type of microbial biomass present in the seeding sludge for the AD process determines the methane yield as well the stabilisation of the effluent for agricultural purposes (Toumi et al., 2015). Apart from the type of seeding sludge, the temperature regime within which the digester operates also influences the methane volume and yield (Arikan et al., 2015; Navickas et al., 2013; Chae et al., 2008). There exist single, double and multiple stages of anaerobic treatments of wastewater. In the double and multiple stages or phases of anaerobic treatment using thermophilic or hyper-thermophilic conditions, acidogenesis usually occurs in the first phase at a much higher temperature of 70°C, while methanogenesis occurs on either a normal mesophilic temperature of 37°C or optimal thermophilic temperature of 55°C (Lee et al., 2009). Mamimin et al. (2015) reported that double-stage processes employed in the treatment of palm oil effluent produce about 34% better yield compared with single-stage AD processes. Lv et al. (2013) compared thermophilic and mesophilic digesters operating on dairy manure and concluded that thermophilic temperature of 50°C had better volatile solids (VS) removal of 31% and methane yield of 0.22 LCH_4/gVS.

Most of the research on hyper-thermophilic digestion employs double stages based on the advantage above-mentioned. The use of a single-stage hyper-thermophilic anaerobic treatment for black water (BW) has not been investigated. However, single-stage systems are considered to be simple, easy to design and less expensive to be constructed and operated making them common in the anaerobic treatment technology applications (Foley et al., 2015; Rapport et al., 2008). In addition, producing a hygienised digestate fit for usage for agricultural purposes is worth mentioning if hyper-thermophilic temperature is used. Consequently, the type of seeding sludge to be used when employing single-stage hyper-thermophilic anaerobic wastewater treatment is very paramount, since absence of microbial flora in the sludge at hyper-thermophilic temperatures implies no degradation, resulting in no removal of organic matter nor production of biogas (methane).

It is, therefore, important to know the type of seeding sludge suitable for use when considering the use of hyper-thermophilic temperature for anaerobic treatment of wastewater for biogas production and the use of hygienised digestate for agricultural purposes. Consequently, the objectives for this study were to assess the performance of three seeding sludge under three hyper-thermophilic conditions under a single-stage condition through batch tests and assess the effectiveness of the hyper-thermophilic temperatures in hygienising the seeding sludge.

2 Methods

2.1 Acclimatisation of the three seeding sludge in a hunger stage

The purpose for carrying out the fermentation batch test was to assess the performance of three seeding sludge with respect to their net normalised cumulative volume of methane content, methane yield and degree of COD degradation of the substrate, BW. This is done to help ascertain which seeding sludge is best suited for a single-stage hyper-thermophilic treatment of BW in a continuous fermentation test both on laboratory and pilot scales. The three seeding sludge were subjected to a hunger phase test at an optimal thermophilic temperature of 55°C to ensure that the methane that would be produced in the batch fermentation tests was from the substrate with minimal contribution from the seeding sludge. In order for the seeding sludge to adjust well to the hyper-thermophilic temperature in this research, the three seeding sludge were acclimatised to optimal thermophilic temperature of 55°C and increased gradually; 1°C per day, until the desired hyper-thermophilic temperatures of 60°C, 65°C and 70°C for this study were reached as was also proposed by Kjerstadius et al. (2013).

2.2 Collection and selection of seeding sludge (inoculum)

About 30 litres of wastewater was collected from the effluent buffer tank of the Lausitzer Wasser GmbH & Co. KG (LWG) wastewater treatment plant in Cottbus and was called *LWG*. The average VS present in it were 0.679 gVS/kg and a pH of 7.27. In addition, ten litres of sieved cow manure (CM) (1.0 mm diameter sieve) which had been stored for six months in a fridge at 4°C in the laboratory of the Department of Waste Management in Brandenburg University of Technology (BTU), Cottbus-Senftenberg was also collected and named *CM*. The CM had average VS of 1.899 gVS/kg and a pH of 7.34. Finally, ten litres of effluent from a thermophilic reactor treating maize silage in the laboratory of the Department of Waste Management in BTU, Cottbus-Senftenberg, operating on a thermophilic temperature of 55°C was collected and was called *BTU*. It had VS of 0.612 gVS/kg and a pH of 7.77. These three seeding sludge were selected based on their easy availability for the purpose of this research. In addition, literature suggests there exists a consortium of methanogens in both sludge of wastewater treatment plant and also in CM, thus making them suitable for investigation in this research (Christy et al., 2014). The seeding sludge from the thermophilic reactor in BTU had a stable daily methane content of at least 65 CH_4-%. Thus comparing these three inocula is not out of place.

2.3 Collection of BW

BW was collected from household BW treatment plants in Cottbus and Konptendorf, Hornower Weg 2, Cottbus, a neighbouring village of Cottbus. One of the sources had 1.5 litres per flush while the other source had four litres per flush. The two streams were put together and homogenised. The homogenised mixture of BW was then stored in the laboratory of Chair of Waste Management at an average temperature of 4°C (to prevent microbial activity) before they were used for the experiments (batch fermentation tests).

2.4 Ratio of substrate and seeding sludge for batch fermentation tests

The Association of German Engineers (VDI, 2006), guidelines recommend that the quantity of substrate and seeding sludge to be used for the batch fermentation tests should comply with the equation below:

$$\frac{VS_{substrate}}{VS_{seeding\ sludge}} \leq 0.5 \tag{1}$$

where

$VS_{substrate}$ is the VS present in the substrate

$VS_{seeding}$ sludge is the VS present in the seeding sludge (inoculum)

0.5 is the desired substrate/seeding sludge ratio for effective performance of the batch fermentation test.

Consequently, based on the values obtained for the VS of the substrate and the different inocula, a $\frac{VS_{substrate}}{VS_{seeding\ sludge}} = 0.49$ was calculated and chosen for all the three different inocula under the same temperature and pressure conditions (see details in subsequent sub-sections).

2.5 Determination of COD, total solids and VS for the substrate

COD for the BW was measured using the New Spectroquant® tests kits (made in Germany, 2017) for COD. The sample was dispensed in the test kit containing potassium dichromate reagent and was mixed thoroughly on the shaking mixing equipment. The cuvette containing the mixed sample was placed in a thermoreactor (TR300 by MERCK, Germany) at 148°C and digested for two hours. After two hours, it was allowed to cool to room temperature and measured in the COD Spectroquant NOVA 60 by MERCK (made in Germany). Total solids (TS) representing the dry matter (DM) and VS were measured following the APHA 1998 standard methods.

2.6 Batch fermentation tests for three different inocula at three hyper-thermophilic temperatures

The three different seeding sludge were subjected to three different hyper-thermophilic temperatures of 60°C, 65°C and 70°C and were compared to assess which of them ensured higher degree of COD degradation of the substrate (BW), produced higher net normalised cumulative methane volume and cumulative methane yield. This was done by subjecting the three seeding sludge to the same treatments (three different hyper-thermophilic temperatures of 60°C, 65°C and 70°C and giving them the same ratio of substrate to seeding sludge; 0.49). The temperature regulator pump in a covered water bath was set depending on which temperature was being investigated.

Based on the VS present in both the substrate (BW) and the different seeding sludge, specific mass of the substrate and seeding sludge was weighed in accordance with the VDI (2006) 4630 specification as was proposed in equation (1). NB: in this report, *i* = inoculum, *s* = sample. Thus, *Mi* is represents mass of the inoculum (g), whereas *Ms* is the mass of the substrate (g). *T* (*s* + *i*) is the total mass (g) of the inoculum and substrate in the batch fermentation bottle (500 ml). *Cmi* represents inoculum for CM, *Cmi + s* represents both the CM inoculum and substrate. *BTUi* represents inoculum for *BTU*, *BTUi + s* represents both the BTU inoculum and substrate. Finally, *LWGi* represents inoculum for *LWG*, *LWGi + s* represents both the LWG inoculum and substrate. Tables 1–3 give details of mass of substrate and inoculum used in this research for the various hyper-thermophilic temperatures based on VS ratio of 0.49 of the substrate and the inoculum as stated above. The batch fermentation tests ran for at least 21 days [VDI (2006) 4630, guidelines suggest at least 20 days] when biological degradation is expected to have been completed. The results in terms of degree of COD degradation, methane yield and net normalised cumulative volume of methane content were assessed at the end of the tests.

Table 1 Quantities of substrate and inoculum used for batch test at 60°C

Name of sample (ID)	*Mass of the inoculum, Mi (g)*	*Mass of sample, Ms (g)*	*Total of inoculum and sample, T (s + i), (g)*
Cmi	400	0.0	400.0
Cmi + s	205.8	194.3	400.1
BTUi	400	0.0	400.0
BTUi + s	309.4	96.2	405.6
LWGi	400	0.0	400.0
LWGi + s	304.9	95.1	400.0

Table 2 Quantities of substrate and inoculum used for batch test at 65°C

Name of sample (ID)	*Mass of the inoculum, Mi (g)*	*Mass of sample, Ms (g)*	*Total of inoculum and sample, T (s + i), (g)*
Cmi	400	0.0	400.0
Cmi + s	205.8	194.3	400.1
BTUi	400	0.0	400.0
BTUi + s	309.4	96.2	405.6
LWGi	400	0.0	400.0
LWGi + s	304.9	95.3	400.2

Table 3 Quantities of substrate and inoculum used for batch test at 70°C

Name of sample (ID)	*Mass of the inoculum, Mi (g)*	*Mass of sample, Ms (g)*	*Total of inoculum and sample, T (s + i), (g)*
Cmi	400.1	0.0	400.1
Cmi + s	204.3	196.0	400.3
BTUi	400	0.0	400.0
BTUi + s	350.3	50.3	400.6
LWGi	400.1	0.0	400.1
LWGi + s	311.5	89	400.5

In order to compare the performance of the selected optimal hyper-thermophilic temperature and preferred seeding sludge in this research with optimal mesophilic and thermophilic temperatures of 37°C and 55°C, respectively, in terms of cumulative methane yield, net normalised cumulative volume of methane content and degree of COD degradation, batch fermentation tests were also performed for temperatures 37°C and 55°C using the same substrate and inoculum ratio of 0.49. Tables 4 and 5 give details of quantities of substrate and inocula used.

Table 4 Quantities of substrate and inoculum used for batch test at 37°C

Name of sample (ID)	*Mass of the inoculum, Mi (g)*	*Mass of sample, Ms (g)*	*Total of inoculum and sample, T (s + i), (g)*
Cmi	400	0.0	400
Cmi + s	219.8	180.2	400.0
BTUi	400.3	0.0	400.3
BTUi + s	350.2	50	400.2
LWGi	400.3	0.0	400.3
LWGi + s	368.9	31.2	400.1

Table 5 Quantities of substrate and inoculum used for batch test at 55°C

Name of sample (ID)	*Mass of the inoculum, Mi (g)*	*Mass of sample, Ms (g)*	*Total of inoculum and sample, T (s + i), (g)*
Cmi	400.1	0.0	400.1
Cmi + s	204.4	195.6	400.0
BTUi	401.1	0.0	401.1
BTUi + s	350.4	49.8	400.2
LWGi	400.2	0.0	400.2
LWGi + s	311.5	89.5	401.0

Methane yield and degree of COD degradation were calculated using equations (2) and (3) respectively.

Methane yield (Y) is given by:

$$\text{Methane yield} = \frac{\text{Net cumulative normalised volume of methane after the batch test (mlNCH4)}}{Mass\ of\ volatile\ solids\ (gVS)} \quad (2)$$

Degree of COD degradation (η) is given by:

$$\frac{\text{Volume of gas} * \text{amount of methane} * 100\%}{320 * \text{mass of subtrate} * \text{COD in substrate}} \quad (3)$$

NB: 320 represents the approximate degradation value of methane (approx. 320 $mlNCH_4$) for 1 gCOD under normalised conditions.

Net normalised cumulative volume of methane content is given by:

$$\begin{aligned} &\text{Net normalised cumulative volume of methane content (mlNCH4} - \%) \\ &= \text{Total of normalised cumulative volume of methane content in sample} \\ &-\text{Total of normalised cumulative volume of methane conetent in seeding sludge} \end{aligned} \tag{4}$$

2.7 Hygienisation tests for the three seeding sludge

Concentrations of pathogens like *Escherichia coli* and *Salmonella spp* in the seeding sludge were assessed before the batch tests were performed to assess the effectiveness of the different hyper-thermophilic temperature regimes in hygienising the digestate for possible agricultural applications. In addition, concentrations of *Escherichia coli* and *Salmonella spp* were assessed in the BW which was used as the substrate. The concentrations of pathogens were compared with both positive and negative controls for two selective media; Endo agar (EA) and brilliant green agar (BGA). Endo selective agar (SIFIN, made in Germany) was prepared by following manufacturer's instruction. Similarly, BGA (modified) (OXOID, made in Germany) was prepared by following manufacturer's instruction. *Escherichia coli* were cultured on EA while *Salmonella spp* were cultured on BGA. One ml each of BTU, CM and LWG seeding sludge as well as the substrate, BW was pipetted and inoculated on either the EA or BGA and incubated at 37°C for at least 24 hours before plate counting (APHA/AWWA/WEF, 2012).

3 Results

Figure 1 compares the net normalised cumulative volume of methane content for the three seeding sludge at three different hyper-thermophilic temperatures. CM at 65°C recorded the highest net normalised cumulative volume of methane content (387.2 $mlNCH_4$-%), followed by LWG at 60°C (197.8 $mlNCH_4$-%) and BTU at 65°C (164.5 $mlNCH_4$-%). At 70°C, BTU was the most inhibited with a net normalised cumulative volume of methane content of 0.0 $mlNCH_4$-% followed by LWG (13.3 $mlNCH_4$-%) and CM (18.9 $mlNCH_4$-%).

Comparison was made on the methane yield for the three inocula for the five temperature regimes of 37°C, 55°C, 60°C, 65°C, 70°C. CM at 65°C recorded the highest net normalised cumulative methane yield of 231.7 $mlNCH_4$/gVS, followed by LWG at 60°C and 55°C which had 216.5 $mlNCH_4$/gVS and 180.3 $mlNCH_4$/gVS, respectively. The least net normalised cumulative methane yield for the three inocula was recorded by BTU at 70°C (0.0 $mlNCH_4$/gVS) followed by CM at 37°C which recorded 0.9 $mlNCH_4$/gVS (Figure 2).

Comparing net normalised cumulative volume of methane content of the optimal hyper-thermophilic temperature and inoculum (CM, 65°C), to optimal temperatures for mesophilic (37°C) and thermophilic conditions (55°C) for the three seeding sludge (in this research), CM at 65°C recorded the highest net normalised cumulative volume of methane content of 387.2 $mlNCH_4$-%. For CM at 65°C, net normalised cumulative volume of methane content increased sharply within the first eight days during the fermentation test, thereafter, increased gradually till the end of the batch fermentation test. The net normalised cumulative volume of methane content for LWG at optimal thermophilic temperature of 55°C was the second highest (208.9 $mlNCH_4$-%). LWG at

55°C had a curve that increased rapidly within the first 14 days of the batch fermentation test but slowed down and increased slowly till the end of the batch fermentation test. Inoculum called BTU at 55°C was the third highest with respect to net normalised cumulative volume of methane content (54.5 $mlNCH_4$-%). It had a curve that increased very slowly even within the first two weeks and more steady increase till the end of the test (Figure 3).

Figure 4 gives a comparison of degree of COD degradation for the three inocula at different hyper-thermophilic temperatures with optimal mesophilic temperature of 37°C and thermophilic temperature of 55°C. CM at optimal hyper-thermophilic temperature of 65°C had the highest degree of COD degradation of 79.1%. LWG at hyper-thermophilic temperature of 60°C recorded the second highest degree of COD degradation of 73.9%. This was followed by BTU at 65°C which recorded degree of COD degradation of 57.3% and LWG at 55°C (55.2%). At hyper-thermophilic temperature of 70°C, BTU was the most inhibited as it recorded degree of COD degradation of 0.0%, while CM and LWG recorded degree of COD degradation of 4.3% and 5.9% respectively.

The initial concentrations of *Salmonella spp* in the three seeding sludge: BTU, CM and LWG were 2×10^2 CFU/ml, 1×10^2 CFU/ml and 2×10^3 CFU/ml, respectively. The concentrations of *Escherichia coli* in the BTU, CM and LWG before the batch tests were 7×10^4 CFU/ml, 1×10^3 CFU/ml and 1×10^4 CFU/ml, respectively. In BW, the concentrations of *Salmonella spp* and *Escherichia coli* were 2×10^5 CFU/ml and 1×10^7 CFU/ml, respectively, before the batch tests. After the batch tests, no growth was found on any of the seeding sludge for all the three hyper-thermophilic temperatures of 60°C, 65°C and 70°C, indicating that all the pathogens that were present before the batch tests had been destroyed by the hyper-thermophilic temperatures.

Figure 1 Net normalised cumulative volume of CH_4 content for three inocula at three hyper-thermophilic temperatures of 60°C, 65°C and 70°C (see online version for colours)

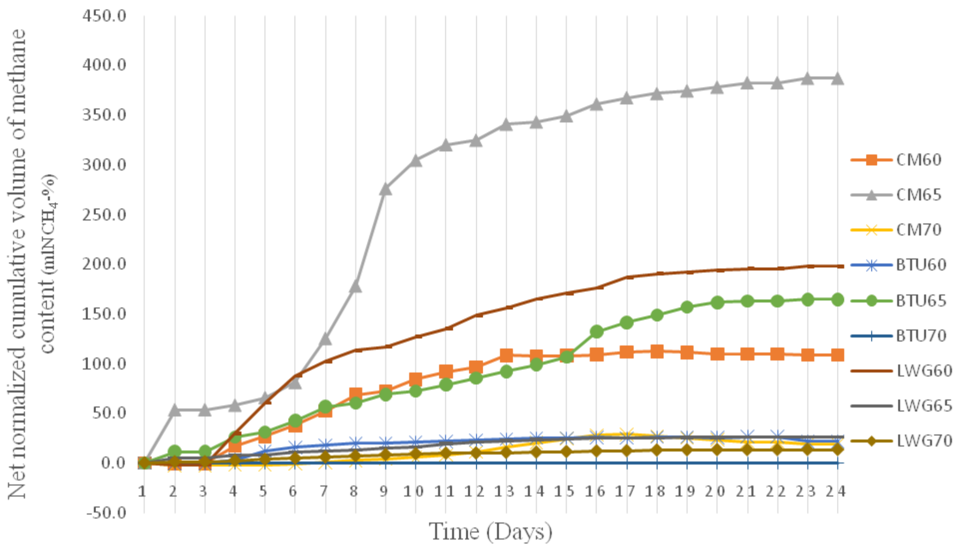


Figure 2 Comparison of net normalised cumulative methane yield for three inocula at hyper-thermophilic temperatures and optimal mesophilic temperature of 37°C and thermophilic temperature of 55°C (see online version for colours)

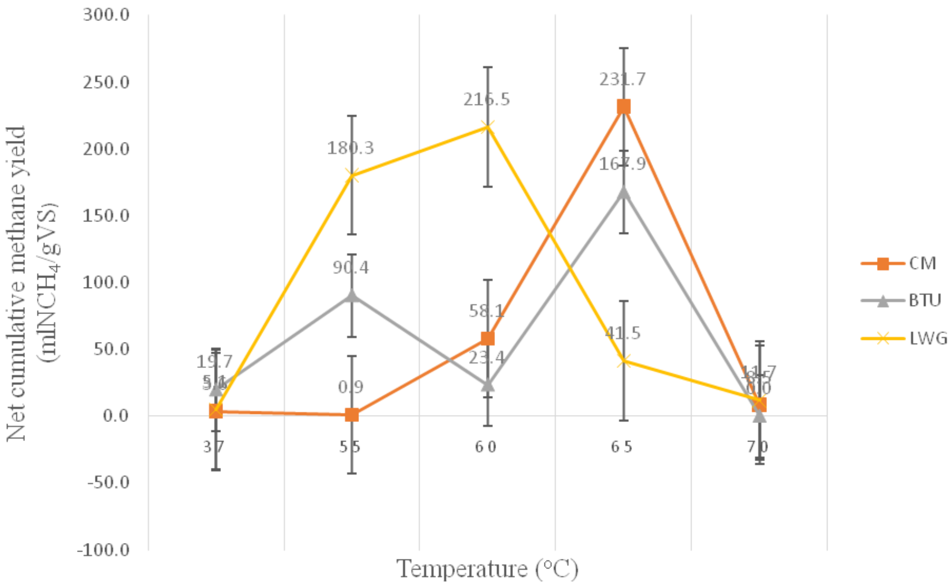


Figure 3 Comparison of net normalised cumulative volume of methane content for three inocula at optimal temperatures of 37°C, 55°C and 65°C (see online version for colours)

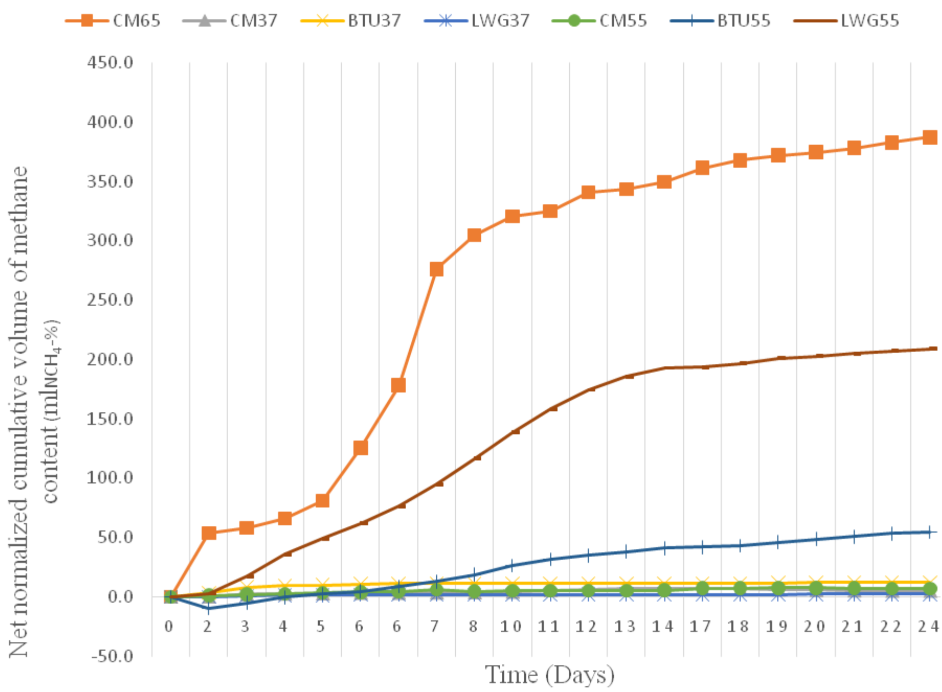
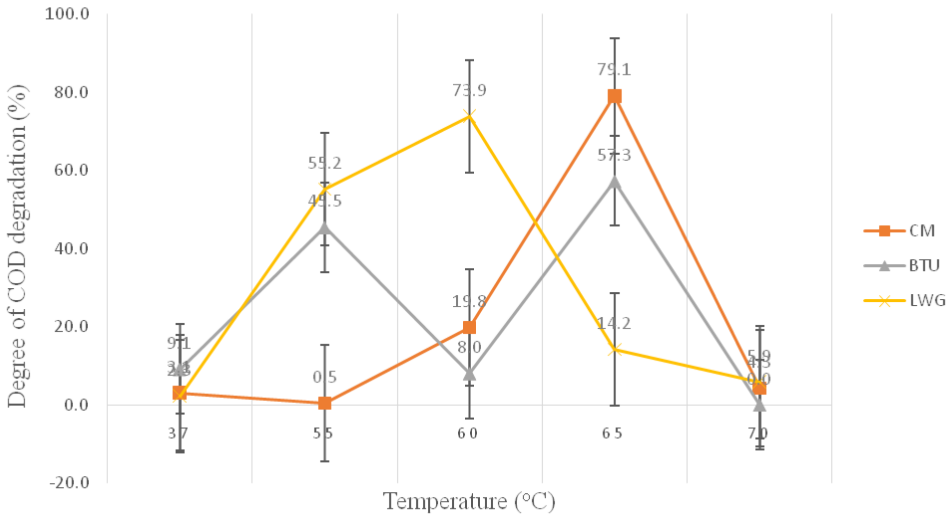


Figure 4 Comparison of degree of COD degradation for three inocula at hyper-thermophilic temperatures and optimal mesophilic temperature of 37°C and thermophilic temperature of 55°C (see online version for colours)



4 Discussions

The results from this research with respect to optimal hyper-thermophilic temperature confirms what was proposed by Singh (2008). In the work by Singh (2008), extensive studies on extreme environments and extremophiles were investigated and reported that the majority of thermophilic bacteria have optimal growth temperature ranging from 50°C to 70°C even though some thermophilic bacteria could grow slowly at a lower temperature of 40°C. In this research, the inoculum, CM, had optimal performance with respect to net normalised cumulative volume of methane content, degree of COD degradation and methane yield at 65°C, probably because it had methanogens that had optimum activity levels at 65°C. Gupta et al. (2016) reported in their work that CM has a consortium of methanogens which could thrive for biogas production and this could account for why CM had the best performance compared to the other seeding sludge. LWG at 60°C, on the other hand, was the second highest in terms of net normalised cumulative volume of methane content, degree of COD degradation and methane yield because it also had a consortium of bacteria that could survive under varied conditions for varied purposes. Markiewicz et al. (2014) and Fong and Tan (2000) in separate research isolated microbial consortium in activated sludge and confirmed that about nine different species exist in activated sludge that could degrade organics and even ionic liquids (ILs) (Markiewicz et al., 2014). This is probably because the content of activated sludge has more than 50% of VS/TS ratio. Once the content of VS in a seeding sludge is above 50% of the DM, bacterial species in the sludge could have enough biodegradable substrates to convert to biogas and methane. This was also confirmed in the work by Tabatabaei and Ghanavati (2018).

None of the tested seeding sludge performed very well under a hyper-thermophilic temperature of 70°C. BTU at temperature 70°C was the most inhibited as it recorded 0.0 $mlNCH_4$-% net cumulative volume, followed by LWG at 70°C (13.3 $mlNCH_4$-%) and CM at 70°C (18.9 $mlNCH_4$-%). The performance was similar for net normalised cumulative methane yield for the three inocula as well as degree of COD degradation. This could be to the fact that none of the methanogens in the BTU seeding sludge could survive at hyper-thermophilic temperature of 70°C. Most research in AD and biogas production has failed to focus on hyper-thermophilic temperature of 70°C for methanisation. Forster-Carneiro, et al. (2008) reported on thermophilic digestion of organic waste using activated sludge from wastewater treatment plant in a batch test at 55°C and concluded that cumulative volume of 4045.2 $mlCH_4$ could be produced within 90 days with only 32.4% of VS removed and methane yield of 0.18 LCH_4/gVS. This is similar to what was reported by Lv et al. (2013) who compared thermophilic and mesophilic digesters operating on dairy manure and concluded that thermophilic temperature of 50°C had better removal of VS of 31% and methane yield of 0.22 LCH_4/gVS.

In the LWG and CM, even though some methanogens could be present and thus producing some volume of methane at that high temperature of 70°C, there is an indication that a substantial quantity of the methanogens in the LWG and CM could not survive at that high temperature, consequently, leading to a reduced volume of methane. The results from the LWG and CM are in agreement to what was proposed by Singh (2008) that some methanogenic thermophiles can also grow at very high extreme temperatures of 80°C and 110°C, with examples being eubacteria and archaeabacteria. Consequently, some of the methanogens and the other microbes that existed in the LWG and CM seeding sludge used in this research could either be thermophiles or hyper-thermophiles and could be in the groups of eubacteria and archaeabacteria. The methanogens could not be extreme hyper-thermophiles since most extreme hyper-thermophiles are micro-organisms that cannot grow below 90°C, with an example being *Pyrolobus fumarii* (Singh, 2008).

The findings in this research also confirmed what was reviewed by van Lier (2008). In a review by van Lier (2008), it is reported that raising the temperatures to extreme values may disturb the performance of the sludge bed systems and thus affecting the stability of methanogenic granular sludge. This was also confirmed by Ozgun et al. (2013), that sludge biomass of AD is greatly affected by thermophilic temperatures. Thus, at temperature 70°C, the methanogens in the three seeding sludge were inhibited probably because they were disturbed and thus resulting in no methane production as well as no or low COD degree of degradation as was seen in the seeding sludge, BTU. It is reported that when the C/N ratio (organic carbon: organic nitrogen) decreases, it implies more of the carbon is being degraded unlike the nitrogen. This implies a sudden build-up of ammonia in the reactor and consequently leading to an increase in the pH level to a basic medium, inhibiting methanogenic bacteria (Vögeli et al., 2014). This was most probable in the batch fermentation tests at hyper-thermophilic temperature of 70°C, since the measured pH values after the tests period ranged between 8.14–8.38 for all the inocula. Ammonia has been reported to inhibit methanogenic bacteria and decrease methane yield by 66% (Ho and Ho, 2012; Sasaki et al., 2011; Sung and Liu, 2003) while methane production is decreased up to 80%, especially when co-digestion is practiced in a thermophilic anaerobic process (Yenigün and Demirel, 2013).

Dereli et al. (2012) further confirmed that thermophilic temperatures even though produce similar amounts of methane as mesophilic temperatures at double organic loading rates, it produces mobile anaerobic biomass since the sludge is dispersed and has poor settling characteristics. Gao et al., (2012), also affirmed that extracellular polymeric substances (EPS) in the bulk sludge, soluble microbial products (SMP) and content of colloidal particles in the seeding sludge and the substrate further increase with increase in temperature of the reactor from mesophilic to thermophilic conditions, thus under hyper-thermophilic conditions more mobile anaerobic biomass is expected.

This may affect the overall conversion of organics to methane even though the digestate will be safe for application on agricultural lands. Contrary to what El-mashad (2003) reported in his work that increase in temperature from 40°C to 60°C corresponds with decrease in methane content in biogas, in this research, the content of methane did not depend on the increase in temperature, rather, on the type of seeding sludge. This is because inoculum called CM at a hyper-thermophilic temperature of 65°C recorded the highest methane volume of 387.2 mlNCH_4-%, higher than the LWG and BTU at both thermophilic temperature of 55°C and hyper-thermophilic temperature of 60°C.

Mamimin et al. (2015) reported that double-stage processes employed in the treatment of palm oil effluent produce about 34% better yield compared to single-stage AD processes.

According to Bartkowska (2015), the least expensive method of sludge handling after treatment of wastewater is to return it to the environment as soil conditioner. However, the presence of pathogens like *Salmonella spp* and eggs of intestinal parasites like *Ascaris*, *Trichuris* and *Toxocara* raises safety concerns making it mandatory to hygienise the digestate before it can be reused on agricultural lands. One system that has been identified and applied in Olecko, Poland is the Autothermal Thermophilic Aerobic Digestion (ATAD) process which operated on a temperature of 59°C to 65°C. This was used after a pre-treatment of wastewater in a sequential biologic reactor (SBR) operating on a temperature of 40°C to 55°C for stabilisation of sludge from a wastewater treatment plant (Bartkowska, 2015). The ATAD had a COD removal of 52.4%, however, no information was provided concerning the bacterial growth and survival vis-à-vis their methanogenic activities (Bartkowska, 2015). Sheth (2009) reported that pathogenic microbes are totally destroyed at thermophilic temperature greater than 55°C with a hygienisation retention time of 24 hours. This is also confirmed in this study since pathogenic microbes could not survive under any of the hyper-thermophilic temperatures used in this study after 24 hours. It is very important to consider at which optimal temperature both higher methane and pathogen-free digestate can be produced. Failure to identify this optimal temperature implies trading-off either large volume of methane for pathogen free digestate or vice versa. Apart from ensuring that the sludge is hygienised in terms of pathogens, Tervahauta et al. (2014), proposed that heavy metals in the sludge should also be assessed before its application on agricultural land. Consequently, the use of seeding sludge with less contribution of heavy metals to the effluent and sludge to be applied on agricultural land is worth considering.

5 Conclusions

The performance of three seeding sludge under three different hyper-thermophilic temperatures were investigated in batch tests. Based on the results from the tests, it can be concluded that CM at 65°C can be considered as the preferred seeding sludge and optimal hyper-thermophilic temperature when a bigger setup is being considered. This is because CM produced the highest net normalised cumulative volume of methane content of 387.2 mlNCH_4-%. In locations where CM is not available to be used as a seeding sludge, a probable option that can be considered for hyper-thermophilic treatment of BW is sewage sludge from wastewater treatment plant, however, its optimal operational temperature is 60°C. It can also be concluded that treating BW at hyper-thermophilic temperature of 70°C is not really feasible irrespective of the type of seeding sludge used. This is because at 70°C, BTU was the most inhibited in terms of methane content as it recorded 0.0 mlNCH_4-% net cumulative volume, followed by LWG (13.3 mlNCH_4-%) and CM (18.9 mlNCH_4-%). CM at 65°C recorded the highest net normalised cumulative methane yield of 231.7 mlNCH_4/gVS, followed by LWG at 60°C and 55°C which had 216.5 mlNCH_4/gVS and 180.3 mlNCH_4/gVS, respectively. The least net normalised cumulative methane yield for the three inocula was recorded by BTU at 70°C (0.0 mlNCH_4/gVS) followed by CM at 37°C which recorded 0.9 mlNCH_4/gVS. At least, about 70% of the COD present in the black was degraded at both 60°C and 65°C for LWG and CM, respectively. CM at optimal hyper-thermophilic temperature of 65°C had the highest degree of COD degradation of 79.1% followed by LWG at hyper-thermophilic temperature of 60°C which recorded 73.9%. At hyper-thermophilic temperature of 70°C, BTU was the most inhibited as it had degree of COD degradation of 0.0%, while CM and LWG recorded degree of COD degradation of 4.3% and 5.9%, respectively.

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