**Enhanced mesophilic anaerobic digestion of food waste by thermal pretreatment:
substrate versus digestate heating**

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1. Introduction

Food waste (FW) is a mixture of organic materials derived from the processing, sorting, preparation, cooking and handling of food. On a global scale, the most common FW stabilization technology at present is still landfilling followed by biological, thermal and thermochemical conversion technologies. Landfills are strongly discouraged by legislations such as the EU Directive on Landfills (1999/31/EC) and the Waste Framework Directive (2008/98/EC), as they contribute to environmental impacts including soil and groundwater pollution, greenhouse gas (GHG) emissions and utilization of huge land areas (Grosso et al., 2010, Holm-Nielsen et al., 2009). Due to the high moisture content and easily biodegradable characteristics of FW, biological treatments (anaerobic or aerobic) are preferred over thermal or thermochemical conversion technologies. Although aerobic treatment like composting provides a promising alternative to landfill disposal, anaerobic digestion (AD) is more favourable due to the following advantages: i) production of renewable energy; ii) less land and space required; iii) more controlled emissions of GHG and toxic gases such as ketones and aldehydes; iv) digestate can be used as soil conditioner or fertilizer; and v) pathogen proliferation is prevented (Ariunbaatar et al., 2014a; Guo et al., 2014; Kastner et al., 2012; Mata-Alvarez et al., 2000; Zhang et al., 2009).

The AD process is mainly operated at mesophilic (30 – 40 °C) or thermophilic (45 – 60 °C) conditions. Theoretically, thermophilic AD (TherAD) is preferred over mesophilic AD (MesAD), as recent studies have shown that: i) TherAD is kinetically favoured over MesAD, thus resulting in a shorter retention time and poses a higher possibility to increase the organic loading rate (El Mashad et al., 1994; Angelidaki et al., 2006; Esposito et al., 2011), ii) TherAD has a higher rate of organic matter degradation with a higher biomethane production (Gavala et al., 2003; Labatut et al., 2014; Suhartini et al., 2014), and iii) TherAD holds a better potential to inactivate pathogens, thus complying with the EU policy for elimination of pathogens as well as obtaining Class A biosolids according to the USEPA guidelines (Labatut et al., 2014; Suhartini et al., 2014; Wagner et al., 2008; Ziemba and Peccia 2011).

Despite the mentioned advantages, TherAD also poses some operational disadvantages including: (i) a relatively higher operating cost, (ii) more sophisticated structural facilities, (iii) a lower process stability, and (iv) a higher susceptibility to inhibition due to sudden environmental changes (De la Rubie et al., 2002; Labatut et al., 2014; Kim et al., 2002). Such

disadvantages are mostly due to the acceleration of the biochemical reaction rates of the hydrolysis and acidogenesis steps producing higher amounts of ammonia, propionate and long chain fatty acids (LCFA) that are known to cause inhibition of methanogenic activity (Kastner et al. 2012; Labatut et al., 2014; Scaglia et al., 2014). Thus, in practice MesAD is preferred over TherAD for prolonged operation of AD of FW.

Coupling the advantages of TherAD with those of MesAD in the same digester could result in an enhanced process; although such a combination has been poorly studied. Therefore, this research aims at investigating the effect of applying thermophilic/hyperthermophilic digestion for a short time prior to mesophilic digestion to potentially accelerate the AD process. This was referred to as thermophilic pretreatment (TPP) in this research. The results from TPP (a combination of biological and thermal pretreatment) were compared with conventional thermal pretreatment (CTP), which heats only the FW without inoculum to 50 – 80 °C prior to MesAD. CTP is one of the easiest and most studied pretreatment methods with proven results even at full-scale. CTP of FW with an aim to improve hydrolysis, increase the biomethane production, and to achieve pasteurization has been studied. Ariunbaatar et al. (2014a) obtained a 52% higher biomethane production with CTP at 80 °C for 1.5 hours, whereas Ma et al. (2011) obtained a 24% increase of biomethane production at 120 °C due to enhanced biodegradation of FW. However, CTP at high temperatures (>140 °C) or longer pretreatment times (> 4 hours) results in the loss of easily fermentable sugars and therefore potential biomethane production (Liu et al., 2012; and Ariunbaatar et al. 2014a).

A series of batch experiments to determine the biomethane potential (BMP) and to compare the effects of CTP and TPP were conducted using a synthetic FW as the substrate. As both the improved hydrolysis and the pathogen inactivation are temperature and treatment time dependent (Boušková et al. 2005), a first set of batch tests was carried out to identify the most favourable temperature range and treatment time of the TPP. The second series of BMP tests was conducted with the aim to compare the effects of TPP and CTP when the operating conditions (temperature and time) were set at the same range. The results were compared with the CTP at 80 °C for 1.5 hours, as previous research reported that 52% of biomethane production enhancement was achieved (Ariunbaatar et al. 2014a). Based on these lab-scale experimental data, an energy requirement estimate for the scenarios with the highest biomethane production enhancement by TPP and CTP was done to suggest the most preferable pretreatment method to produce biomethane from FW.

2. Materials and Methods

2.1 Pretreatment procedures

To perform the TPP, FW and inoculum were mixed in the BMP bottles, and then incubated at varying temperatures (50, 60, 70 and 80 °C) for a varying time (12, 24, 36 and 48 hours). After each TPP, the temperature of the incubator was reduced to mesophilic (35 ± 2 °C) conditions. To perform CTP, only FW was put inside the BMP bottles and directly placed in the incubator at the selected temperatures for the desired time, which was identified during the first set of experiments. The inoculum was added in the bottles after the CTP and incubated at mesophilic conditions. Each test was carried out in duplicate and prior to incubation the BMP bottles were flushed with nitrogen to provide anaerobic conditions. The daily biomethane production was measured with the liquid displacement method using a sodium hydroxide (120 gNaOH/L) solution to capture carbon dioxide (Esposito et al., 2012). During the initial 48 hours, the biomethane production was measured every 12 hours, and it was measured once a day afterwards until the plateau was achieved. Cumulative biomethane production (CBP) was normalized to standard temperature and pressure.

2.2 Batch BMP experiments of FW

BMP tests were conducted in 1L glass bottles at mesophilic (35 ± 2 °C) conditions with a substrate to inoculum (S/I) ratio of 0.5 gVS/gVS, following the BMP protocol described by Esposito et al. (2012). Synthetic FW mimicking a typical European FW was prepared as described by Ariunbaatar et al. (2014a) and used as the substrate. Various foods (fruits, vegetables, meat, rice, paste, and dairy products) were bought from the local supermarkets and blended together for homogenization. The synthetic FW slurry was prepared fresh for each set of experiments. Digestate from a full-scale anaerobic digester in Capaccio-Salerno (Italy) treating buffalo manure and dairy waste at mesophilic conditions was used as inoculum.

2.3 Analytical methods

Soluble chemical oxygen demand (CODs) was analyzed with HACH test kits following the manufacturer's instructions (HACH, Loveland, Colorado, USA). Total lipids were extracted with a mixture of chloroform and methanol (50% v/v). The extracted solution was put in

aluminium caps and dried at room temperature in the laminar flow hood until constant weight. The leftover weight was used to calculate the lipids content (Phillips et al., 1997). Total carbohydrates were determined with the phenol-sulfuric method and measured with a spectrophotometer (TUV SR03210002) using glucose as standard solution (Codex Guidelines 1993). Total solids (TS), volatile solids (VS) and total Kjeldahl nitrogen (TKN) were analyzed according to standard methods (APHA, 2005). Total protein content was calculated based on TKN using a correction coefficient of 6.25, as suggested by CODEX Guidelines 1993.

2.4 Energy balance calculations

The energy balance of both CTP and TPP was calculated only for the pretreatment step. The energy considerations related to the MesAD operation and the capital cost for the pretreatments were neglected in this study, because the main purpose of the study is to compare the efficiency of the pretreatment methods in terms of enhancing the biomethane production from FW. The energy balance was estimated based on the differences of the total energy requirements for the pretreatment of 1 ton FW, and the extra energy produced (E_{EXTRA}) due to the enhanced biomethane production. E_{EXTRA} and the energy requirement for thermal pretreatment (E_{CTP}) were calculated as described in details by Ariunbaatar et al. (2014a). The implicit ambient temperature and the initial temperature of the FW were considered as 10 °C. The insulation material for both the digester and the pretreatment chamber for CTP were assumed as polyurethane, as its thermal conductivity is low ($0.022 \text{ W}\cdot\text{m}^{-1}\text{K}^{-1}$). The energy requirement for TPP (E_{TPP}) was estimated for the whole digester. Considering the substrate to inoculum ratio of 0.5 gVS/gVS the digester volume was calculated as 31 m³, which contains 1 ton of FW. Since TPP is conducted in the same digester, the initial temperature was assumed to be equal to the digester operating temperature ($35 \pm 2 \text{ °C}$).

3. Results

3.1 Characterization of substrate and inoculum

The physical and chemical properties of both the synthetic FW and the inoculum are shown in Table 1. Synthetic FW contains mostly carbohydrates ($71.5 \pm 0.5 \text{ \% VS}$) and a considerable amount of lipids ($10.6 \pm 0.3 \text{ \% VS}$) and proteins ($17.2 \pm 0.8 \text{ \% VS}$), whereas the

inoculum contains a higher amount of proteins (56.5 ± 2.5 % VS) and lipids (40.7 ± 3.7 % VS) than carbohydrates (3.1 ± 0.5 % VS). These characterization results suggest that the FW is a suitable substrate for AD (Angelidaki et al., 2006, Kastner et al., 2012) and the VS in the inoculum mainly contained microbial biomass (Ariunbaatar et al., 2014a).

Table 1. Characterization of FW and inoculum

3.2 Effect of TPP on temperature and treatment time on MesAD

The CBP curve plateau was obtained after 20 – 25 days of incubation, although the methanogenic activity was completely ceased reaching zero production after 134 days (Fig. 1). TPP at 50 °C resulted in the highest biomethane production during both the TPP and mesophilic biomethanation stage, yielding $44.6 (\pm 0.6)$, $36.1 (\pm 4.5)$, $31.1 (\pm 0.8)$, $15.7 (\pm 0.8)$ % higher CBP than the control (i.e. MesAD of untreated FW) with 12, 24, 36, and 48 hours pretreatment time, respectively (Fig. 1A). FW pretreated at 60 °C produced a higher amount of biomethane as compared to the control during the TPP, but the biomethane production decreased instantly as soon as the temperature was changed to mesophilic conditions (Fig. 1B). At the end of the BMP test, the CBP of FW pretreated at 60 °C for 12, 24, 36 hours was higher by 28.0 ± 1.6 , 16.2 ± 0.9 , 1.9 ± 0.6 % respectively, while pretreatment for 48 hours resulted in a 16.9 ± 2.8 % lower CBP as compared to the control. Pretreatment at 70 °C yielded a lower biomethane production than the control for any length of the pretreatment time investigated (Fig. 1C), while 80 °C pretreatment resulted in no biomethane production at all.

The highest biomethane production rates of the FW pretreated with TPP were achieved with 12 hours pretreatment at all temperatures tested. Fig. 1 also shows that most of the organics (85 – 95%) are converted into biomethane during the initial 20 days of biomethanation, which is also considered as the typical hydraulic retention time (HRT) for AD (Ariunbaatar et al. 2014a). Hence, the net specific biomethane production (SBP) of the control as well as all the 12 hours TPP scenarios were calculated using the data obtained during the initial 20 days (Fig. 2).

Fig 1. Cumulative biomethane production curves of FW pretreated with: A) TPP at 50 °C; B) TPP at 60 °C; and C) TPP at 70 °C

The SBP of the control was $407.4 (\pm 7.8)$ mlCH₄/gVS_{added}, and was enhanced by $41.1 (\pm 4.7)\%$ with a TPP at 50 °C, while a TPP at higher temperatures (60 and 70 °C) resulted in a decrease of the SBP (Fig. 2). As the decrease of SBP at 60 °C pretreatment is negligible (2%), the preferable experimental condition for TPP was chosen as 50 – 60 °C for 12 hours. Since the main purpose of TPP is not only to enhance the hydrolysis through enzymatic and thermal processes (Ferrer et al., 2008), but also to eliminate the pathogens, a minimum of 12 hours of TPP was desirable. A TPP time below 12 hours could initially inactivate the pathogens but may not permanently eliminate them (Lund et al., 1996, Popat et al., 2010). However, the possibility to enhance MesAD while saving energy by reducing the pretreatment time until 6 hours at the preferred temperature range was also investigated during the second series of experiments.

Figure 2 Temperature effect of TPP for 12 hours on the SBP during the initial 20 days

3.3 Comparison of the TPP and CTP efficiencies

Fig. 3 shows the CBP curves from AD of FW pretreated with TPP and CTP at 50, 55, and 60 °C for 6 and 12 hours. All the tested scenarios except for TPP at 60 °C resulted in a higher biomethane production than the control. Similar to the first set of experiments, the CBP curves obtained a plateau after 20 – 25 days of biomethanation. Hence, the experiments were stopped and the SBP was calculated for the initial 20 days as well (Fig. 4). The SBP of the control was $413.90 (\pm 4.87)$ mlCH₄/gVS_{added}, which is consistent with the previous set of experiments.

Figure 3 CBP curves of FW pretreated with TPP and CTP at: A) 50 °C; B) 55 °C; and C) 60 °C

The highest enhancement ($46.39 \pm 8.70\%$) of the SBP was obtained with a CTP at 80 °C for 1.5 hours. It agrees with the results obtained by Ariunbaatar et al. (2014a). Fig. 4 also illustrates that the highest SBP enhancement of 44 – 46% was obtained with a TPP at 50 °C for 6 and 12 hours; but the same condition with CTP of FW resulted in a lower (12 – 39%) enhancement of the SBP. Both TPP and CTP at 55 °C yielded a higher SBP than the control. When the effects of pretreatment times are compared, a negligible difference can be seen between 6 and 12 hours. It can also be observed that the enhancement of SBP with TPP is

higher than those with CTP, suggesting that TPP performs better than CTP at 50 – 55 °C temperatures. However, at a temperature higher than 60 °C TPP had a negative effect on the SBP, while CTP had a positive effect resulting in a higher SBP at those temperatures.

Figure 4 Effect of TPP and CTP: A) Specific biomethane potential of the initial 20 days; and
B) Increased percentage of biomethane production

To investigate the effects of TPP and CTP on organic material removal by AD, the CODs of each bottle was analysed after the pretreatment and BMP tests. Fig. 5A shows that the TPP temperature and digestion time have a direct correlation with COD solubilisation, whereas such an intensive CTP resulted in a reduction of CODs. Fig. 5B shows that most of the digestates had a similar concentration of CODs as the blank (1934 ± 34 mg/L) and the control (2284 ± 4 mg/L) after the BMP tests.

In detail, both TPP and CTP at 50 and 55 °C resulted in a 20 – 59% higher COD solubilisation than the control, and after 25 days of digestion all digestates except for the one produced by TPP at 55 °C for 12 hours showed a similar level of CODs concentration. Even though higher temperatures (55 and 60 °C) and a longer TPP treatment time (>12 hours) caused a higher COD solubilization as shown in Fig. 5A, the methanogenic activity was inhibited, and thus the CODs concentration at the end of the BMP test was 16 – 60% higher than the control. On the contrary, the CTP at 60 °C resulted in a loss of organics yielding a 1 – 9 % lower CODs. It is also interesting to note that a CTP at 80 °C for 1.5 hours yielded the highest COD solubilization that at the end of the BMP test was almost completely consumed, as the final CODs concentration was only 144 (± 72) mg/L higher than the control.

Figure 5 Effect of TPP and CTP temperature and treatment time on the CODs concentrations after A) Pretreatment; and B) The BMP tests

4. Discussion

4.1 Effects of pretreatment methods

This study showed that the advantages of a faster hydrolysis of TherAD and a stable operation of MesAD can be achieved in the same digester (Fig. 2 and Fig. 4). Compared to the sophisticated two-stage AD system, the temperature-phased anaerobic digestion (TPAD),

TPP followed by MesAD prevents the possible reduction of the symbiotic activities between anaerobic microbes (hydrolytic/acidogenic and methanogenic) and thus this system is preferred over TPAD (Ariunbaatar et al., 2014b; Bolzonela et al., 2012; Carrère et al., 2010).

The net SBP of the control (e.g. untreated FW) was $407.4 (\pm 7.8)$ mlCH₄/gVS_{added} and $413.90 (\pm 4.87)$ mlCH₄/gVS_{added} during the first and second set of experiments, respectively. It is in a good agreement with previous research (Ariunbaatar et al. 2014a,b; Esposito et al. 2013; Guo et al. 2014; Mata-Alvarez et al. 2000; Kastner et al. 2012). The temperature and treatment time of TPP play an important role for the enhancement/inhibition of MesAD. TPP at 50 – 55 °C had a positive effect on the MesAD (Fig. 1A, 3A and 3B) both during the pretreatment and the MesAD stage. This can be attributed to the increased solubilisation of organic solids (e.g. the COD) at thermophilic temperatures as compared to the control test, operated at mesophilic (35 ± 2 °C) temperature only (Fig. 5A). Increased solubilisation of organic solids and/or the increased hydrolysis made the substrates more available for the anaerobic microorganisms, thus the biomethane production was enhanced (Ariunbaatar et al. 2014b; Carrère et al. 2010; Esposito et al. 2012).

The TPP at 50 – 55 °C is also likely to have altered the microbial community. The temperature shock could have induced the sporulation of some hydrolytic microbial community while the major part of the microbial community was deactivated. The microbial community thus became more specialised able to provide an increased efficiency of digestion. Such an improved microbial community was also obtained with a focused pulse shock pretreatment by Zhang et al. (2009), and with a repeated pulse feeding at mesophilic conditions by Vrieze et al. (2013).

Chen et al. (1983) suggested that 9% of the microbial population in a mesophilic inoculum consists of facultative thermophiles. During TPP at 60 °C, the methanogenic activities were higher than the control, but reduced as soon as the temperature was decreased, which agrees with the results obtained by Chachkhiani et al. (2004), who showed that thermophiles adapt to MesAD more hardly than mesophiles to TherAD. The biomethane production at 70 °C during both the pretreatment and mesophilic biomethanation stages indicates the mesophilic anaerobes could tolerate such temperatures but the activities are decreased. Applying TPP at 80 °C resulted in a complete cease of anaerobic activities (Fig. 2), suggesting that no

indigenous obligate thermophilic methanogens that can survive such hyperthermophilic conditions were present in the inoculum (Gavala et al., 2003).

The effects of CTP have been well-documented CTP results in the disintegration of macromolecules and disruption of chemical bonds as well as cell walls. Thus, the solubilisation of organic solids is easier (Fig.5), and became more available for the anaerobes. However, CTP is only effective to certain temperatures and pretreatment times (Ariunbaatar et al. 2014a,b; Ma et al. 2011; Montgomery and Bochmann 2014). This research showed that the highest enhancement ($> 40\%$) of biomethane production due to CTP was obtained at 80°C for 1.5 hours. It agrees with Ariunbaatar et al. (2014) and can be compared with the results obtained with TPP at 50°C for 6 – 12 hours. Although CTP at 50 and 55°C for 6 – 12 hours resulted in a similar increase of CODs as the TPP, the enhancement of biomethane production was lower (Fig. 4) than of TPP. It is another indication of the increased microbial activities due to TPP at $50 - 55^{\circ}\text{C}$. CTP at 60°C for 6 – 12 hours yielded lower CODs as compared to both TPP as well as the control tests (Fig. 5A). The lower CODs can be explained by the loss of easily fermentable sugars as suggested by Ariunbaatar et al. (2014a,b) and Carrère et al. (2010).

4.2 Energy efficiency

Table 2 shows that the extra energy produced by applying the pretreatment methods ranges between 219 – 229 kWh/tonFW. The energy requirement for TPP (E_{TPP}) is much higher than CTP (E_{CTP}), and thus a CTP is more favourable than a TPP in terms of energy efficiency. E_{TPP} was calculated for the whole digester (31 m^3) containing 1 ton of FW, whereas E_{CTP} was estimated for a separate pretreatment chamber containing 1 ton of FW. Also the high-energy requirement for TPP is due to the high water content in the digester. The main energy requirement ($> 98\%$) for TPP was the energy to heat up the digester, and a very low amount of heat is lost. Hence, if such a TPP is applied for a MesAD with a higher solids content, the extra energy produced could be enough to make the process self-sufficient, assuming the enhancement of the biomethanation process would be high as well. As there is a limited research on the TPP of FW, the total energy requirement was calculated per ton of wet biowaste. The total energy requirement was 20.4 – 20.6 kWh, which is less than the results (29 – 31 kWh/wet ton of sewage sludge) of Ziemba and Paccia (2011). The relatively lower energy requirement could be due to the different experimental conditions of the pretreatment.

Table 2. Energy requirements for the pretreatment methods

5. Conclusions

The net specific biomethane production of synthetic food waste (FW) was $413.90 (\pm 4.87)$ $\text{mlCH}_4/\text{gVS}_{\text{added}}$, which represents a high potential of renewable energy from this type of waste. The possibility to enhance the AD of FW at mesophilic conditions by applying a thermophilic (heating the full reactor content prior to mesophilic digestion) or conventional thermal pretreatment (heating only the substrate) was studied through a series of batch experiments. The highest enhancement of the biomethane production (higher than 40%) was obtained with a thermophilic pretreatment at 50 °C for 6 – 12 h or thermal pretreatment at 80 °C for 1.5 h. The main effect of these pretreatments was a higher solubilisation of COD due to various physical and microbial processes. Although, thermophilic pretreatment of FW and inoculum simultaneously could result in a higher biomethane production than the control, the extra energy produced by the enhanced process is insufficient to heat up the reactor to the desired temperature. Despite the capital cost of the separate chamber, conventional thermal pretreatment has a higher energy efficiency than thermophilic pretreatment and the extra energy can actually be utilized for short pretreatment.

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Reference

1. Angelidaki I, Chen X., Cui J., Kaparaju J., Ellegaard L. (2006) Thermophilic anaerobic digestion of source sorted organic fraction of household municipal solid waste: Start-up procedure for continuously stirred tank reactor. *Water Research*, 40: 2621-2628
2. APHA, Standard Methods for the examination of water and wastewater 21st edition ISBN: 0875530478
3. Ariunbaatar J., Panico A., Frunzo L., Esposito G., Lens P.N.L., Pirozzi F. (2014a) Enhanced anaerobic digestion of food waste by thermal and ozonation pretreatment methods. *Journal of Environmental Management*, 146: 142-149
4. Ariunbaatar J., Panico A., Esposito G., Pirozzi F., Lens P.N.L. (2014b) Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy*, 15: 143-156
5. Bolzonela D., Cavinato C., Fatone F., Pavan P., Cecchi F. (2012) High rate mesophilic, thermophilic, and temperature phased anaerobic digestion of waste activated sludge: A pilot scale study. *Waste Management*, 32(2): 1196 - 1201
6. Boušková A., Dohányos M., Schmidt J.E., Angelidaki I. (2005), Strategies for changing temperature for mesophilic to thermophilic conditions in anaerobic CSTR reactors treating sewage sludge. *Water Research*, 39: 1481-1488
7. Carrère H., Dumas C., Battimelli A., Batstone D.J., Delgenès J.P., Steyer J.P., Ferre I. (2010) Pretreatment methods to improve sludge anaerobic biodegradability: A review. *Journal of Hazardous Materials*, 183(1-3): 1 – 15
8. Chachkiani M., Dabert P., Abzianidze T., Partskhaladze G., Tsiklauri L., Dudaauri T., Godon J.J. (1993) 16SrDNA characterization of bacterial and archaeal communities during start-up of anaerobic thermophilic digestion of cattle manure. *Bioresource Technology*, 93 (3): 227 – 232
9. Chen M. (1983) Adaptation of mesophilic anaerobic sewage fermenter population to thermophilic temperatures. *Applied Environmental Microbiology*, 45(4): 1271 - 1276
10. Codex Guidelines on Nutrition Labelling CAC/GL 2-1985 (Rev.1 – 1993)
11. De la Rubia, M. A., Perez, M., Romero, L. I., & Sales, D. (2002). Anaerobic mesophilic and thermophilic municipal sludge digestion. *Chemical and biochemical engineering quarterly*, 16(3): 119-124.

12. El Mashad H.M, Zeeman G., van Loon W.K.P., Bot G.P.A., Lettinga G. (1994) Effect of temperature and temperature fluctuation on thermophilic anaerobic digestion of cattle manure. *Bioresource Technology*, 95: 191-201
13. Esposito G., Frunzo L., Panico A., Pirozzi F. (2011) Model calibration and validation for OFMSW and sewage sludge co-digestion reactors. *Waste Management*, 31: 2527-2535
14. Esposito G., Frunzo L., Panico A., Pirozzi F. (2012) Enhanced bio-methane production by co-digestion of different organic wastes. *Environmental Technology*, 33: 2733-2740
15. Ferrer I., Ponsá S., Vázquez F., Font X. (2008) Increasing biogas production by thermal (70 °C) sludge pretreatment prior to thermophilic anaerobic digestion. *Biochemical Engineering Journal*, 42(2): 186 – 192
16. Gavala, H. N., Yenal, U., Skiadas, I. V., Westermann, P., & Ahring, B. K. (2003). "Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature." *Water Research* 37.19 (2003): 4561-4572.
17. Grosso M., Motta A., Rigamonti L. (2010) Efficiency of energy recovery from waste incineration, in the light of the new Waste Framework Directive. *Waste Management*, 30(7): 1238 – 1243
18. Guo X., Wang C., Sun F., Zhu W., Wu W. (2014) A comparison of microbial characteristics between the thermophilic and mesophilic anaerobic digesters exposed to elevated food waste loadings. *Bioresource Technology*, 152: 420-428
19. Holm-Nielsen J.B., Al Seadi T., Oleskowicz-Popiel P. (2009) The future of anaerobic digestion and biogas utilization. *Bioresource Technology* 100(22): 5478 – 5484
20. Kastner V, Somitsch W., Schnitzhoger W. (2012) The anaerobic fermentation of food waste: a comparison of two bioreactor systems. *Journal of Cleaner Production*, 34: 82-90
21. Kim M., Ahn Y., Speece A.E. (2002) Comparative process stability and efficiency of anaerobic digestion; mesophilic VS. thermophilic. *Water Research*, 36: 4369-4385
22. Labatut R.A., Angenent L.T., Scott N.R. (2014) Conventional mesophilic vs. thermophilic anaerobic digestion: A trade-off between performance and stability? *Water Research*, 53: 249-258

23. Liu X., Wang W., Gao X., Zhou Y., Shen R. (2012), Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste, *Waste Management* 32: 249-255
24. Lund, B., Jensen, V. F., Have, P., & Ahring, B. (1996). Inactivation of virus during anaerobic digestion of manure in laboratory scale biogas reactors. *Antonie van Leeuwenhoek*, 69(1): 25-31.
25. Ma J., Duong T H., Smits M., Vestraete W., Carballa M. (2011), Enhanced biomethanation of kitchen waste by different pretreatments, *Bioresource Technology* 102: 592-599
26. Mata-Alvarez J., Macé S., Llabrés P. (2000) Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology*, 74(1): 3 – 16
27. Montgomery, L. F., & Bochmann, G. (2014). Pretreatment of feedstock for enhanced biogas production. IEA Bioenergy. Ireland.
28. Phillips K.M., Tarrago-Trani M.T., Grove T.M., Grun I., Lugogo R., Harris R.F., Stewart K.K. (1997) Simplified gravimetric determination of total fat in food composites after chloroform-methanol extraction, *Journal of American Oil Chemists' Society*, 74(2): 137-142
29. Popat, S. C., Yates, M. V., & Deshusses, M. A. (2010). Kinetics of inactivation of indicator pathogens during thermophilic anaerobic digestion. *Water research*, 44(20): 5965-5972
30. Scaglia B., D'Imporzano G., Garuti G., Negri M., Adani F. (2014) Sanitation ability of anaerobic digestion performed at different temperature on sewage sludge, *Science of the Total Environment*, 466-467: 888-897
31. Suhartinin S., Heaven S., Banks C.J. (2014) Comparison of mesophilic and thermophilic anaerobic digestion of sugar beet pulp: Performance, dewaterability and foam control. *Bioresource Technology*, 152: 202-211
32. Vrieze J.D., Verstaete W., Boon N. (2013) Repeated pulse feeding induces functional stability in anaerobic digestion, *Microbial biotechnology*, 6(4): 414-424
33. Wagner A.O., Gstraunthaler G., Illmer P. (2008) Survival of bacterial pathogens during the thermophilic anaerobic digestion of biowaste: Laboratory experiments and in situ validation. *Anaerobe*, 14: 181-183

- 432 34. Ziemba C. and Peccia J. (2011) Net energy production associated with pathogen
433 inactivation during mesophilic and thermophilic anaerobic digestion of sewage
434 sludge. *Water Research*, 45: 4758-4768
- 435 35. Zhang H., Banaszak J.E., Prathap P., Alder J., Krajmalnik-Brown, R., Rittman B.E.
436 (2009) Focused-pulsed sludge pretreatment increases the bacterial diversity and
437 relative abundance of acetoclastic methanogens in a full-scale anaerobic digester.
438 *Water Research*, 43: 4517-4526

A)

B)

C)

Figure 1. Cumulative biomethane production curves of FW pretreated with: A) TPP at 50 °C; B) TPP at 60 °C; and C) TPP at 70 °C

Figure 2 Temperature effect of TPP for 12 hours on the SBP during the initial 20 days

A)

B)

C)

Figure 3 CBP curves of FW pretreated with TPP and CTP at: A) 50 °C; B) 55 °C; and C) 60 °C

A)

B)

Figure 4 Effect of TPP and CTP: A) Specific biomethane potential of the initial 20 days; and
B) Increased percentage of biomethane production

A)

B)

Figure 5 Effect of TPP and CTP temperature and treatment time on the CODs concentrations after: A) Pretreatment; and B) BMP tests

Table 1. Characterization of FW and inoculum

	FW	Inoculum
TS, %	23.7 ± 0.8	2.24 ± 0.1
VS, %	21.6 ± 0.3	1.43 ± 0.3
Lipid, % VS	17.2 ± 0.8	40.7 ± 3.7
Carbohydrates, % VS	71.5 ± 0.5	3.1 ± 0.5
Protein, % VS	10.6 ± 0.3	56.5 ± 2.5
TKN	4.0 ± 0.1 g/kg	1.3 ± 0.1 g/L

Table 2. Energy requirements of the pretreatment methods

	Unit	TTP 50 °C 6 h	TTP 50 °C 12 h	CTP 80 °C 1.5 h
Extra biomethane	m ³ /tonFW	41.3 ± 0.9	39.7 ± 0.9	41.5 ± 5.8
Extra energy produced	kWh/tonFW	227.9 ± 5.1	219.1 ± 5.1	229.4 ± 32.3
Total energy requirement	kWh/tonFW	633.1	639.5	71
Net energy	kWh/tonFW	- 405.1 ± 5.1	- 420.4 ± 5.1	158.4 ± 32.3