In-situ biogas upgrading during anaerobic digestion of food waste amended with walnut shell biochar at bench-scale

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Abstract

A modified version of an *in-situ* CO_2 removal process was applied during AD of food waste with two types of walnut shell biochar (WSB) at bench-scale under batch operating mode. Compared to the coarse WSB, the fine WSB has a higher ash content (43 vs. 36 wt%) and higher concentrations of calcium (31 vs. 19 wt% of ash), magnesium (8.4 vs. 5.6 wt% of ash) and sodium (23.4 vs. 0.3 wt% of ash), but a lower potassium concentration (0.2 vs. 40% wt% of ash). The 0.96 - 3.83 g biochar (g VS_{added})$^{-1}$ fine WSB amended digesters produced biogas with 77.5-98.1% CH_4 content by removing 40-96% of the CO_2 compared to the control digesters at mesophilic and thermophilic temperature conditions. In a direct comparison at 1.83 g biochar (g VS_{added})$^{-1}$, the fine WSB amended digesters (85.7% CH_4 content and 61% CO_2 removal) outperformed the coarse WSB amended digesters (78.9% CH_4 content and 51% CO_2 removal). Biochar addition also increased alkalinity as $CaCO_3$ from 2,800 mg L^{-1} in the control digesters to 4,800-6,800 mg L^{-1} providing process stability for food waste AD.

Keywords Renewable methane, food waste, anaerobic digestion, CO_2 removal, biochar

Introduction

Approximately 13.9% of all US municipal solid waste (MSW) or 31.5 million metric tonnes (MMT) per year is classified as organic food waste (FW) (USEPA 2012, Linville et al. 2015). Landfilling and incineration of FW are not sustainable because of limited land availability and rising landfill fees, and increased energy consumption from the FW's high moisture content, respectively (Linville et al. 2015, Wang et al. 2013). FW is the largest portion of organic waste sent to landfills (USEPA 2012), highly biodegradable (Levis and Barlaz 2011), and the dominant contributor to considerable uncontrolled release of methane (CH_4), a greenhouse gas (GHG) (USEPA 2015). In 2013, landfills were the third largest source of CH_4 emissions in the US (114.6 MMT of CO_2 equivalent (MMT CO_2e)) (USEPA 2015). Less than 5% of discarded FW is being recovered; utilizing anaerobic digestion (AD) is a positive alternative owing to its energy-rich characteristics and high energy-recovery from produced biogas (Grimberg et al. 2015, Wang et al. 2013, Zhang et al. 2013). Biogas is composed of 50-70% CH_4 and 30-50% carbon dioxide (CO_2), with trace amounts of hydrogen sulfide (H_2S) and other impurities (Lombardi and Carnevale 2013, Shen et al. 2015a). Biogas is upgraded to renewable CH_4 through costly upgrading processes for removal of CO_2 and impurities (Lombardi and Carnevale 2013) increasing production costs by 20-72% (Beil et al. 2013, Murray et al. 2014). Financial issues with food waste anaerobic digestion (FWAD) may be eliminated or minimized with cost competitive biogas upgrading and treatment technology (Linville et al. 2015).

The research objective is to enhance FWAD adapted from *in-situ* CO_2 removal process developed for wastewater treatment plants (WWTPs) sludge AD. The process was modified for the increased biomethane potential of FW (290 - 500 m^3 CH_4 (tonne VS)$^{-1}$) compared to sludge (220 - 310 m^3 CH_4 (tonne VS)$^{-1}$) (Linville et al. 2015, Shen et al. 2015b, Snyder et al. 2014). FW poses challenges for AD due to the high solids content, and highly varying composition and volume (Grimberg et al. 2015, Leiva et al. 2014, Zhang and Jahng 2012), increased biodegradation of carbohydrates and lipids leading to increased volatile fatty acids (VFAs) production rate and low digester pH (Bozym et al. 2015, Wang et al. 2013). Failure to maintain the balance between acidogenic and methanogenic microorganisms causes digester instability and upsets due to the differences in physiology, nutritional needs, growth kinetics, and susceptibility to environmental conditions (Linville et al. 2015).

Parameters and inhibitory substances have also been reported with FWAD including C/N ratio, and VFAs, lipids, H_2S and NH_3, respectively (Wang et al. 2013) which cause lower CH_4 yields and longer digestion times (Zhang et al. 2013). However, biochar amendment can potentially stabilize AD (Shen et al. 2015b, Snyder et al. 2014). In this study, the applicability of the *in-situ* CO_2 removal process for FWAD was determined by optimizing biochar doses to increase CO_2 removal without causing digester toxicity. The process captures and sequesters the CO_2 produced during AD by utilizing biochar from gasification or pyrolysis of lignocellulosic biomass under oxygen-starved conditions (Brown 2011). Biochar has high cation concentrations including calcium, magnesium, sodium, and potassium to sequester CO_2 (Cheah et al. 2014, Gul et al. 2015, Shen et al. 2015b, Tan et al. 2015) which may have stimulatory benefits for FWAD (Bozym et al. 2015, Chen et al. 2008). This study uses walnut shell biochar (WSB) (fine or coarse) instead of previously reported corn stover biochar because there is no economic competition as with the corn stover feedstock for cellulosic biofuels production (Shen et al. 2015b). Walnut shells are a carbonaceous waste product of orchards (Daoyuan et al. 2014) and presents a future scenario where local biomass residues are used for bioenergy production with the biochar available for utilization (Mukome et al. 2013). WSB has been used as a sorbent to remove contaminants (Tan et al. 2015), as soil amendment to increase water holding capacity (Daoyuan et al. 2014) and to reduce GHG emissions (Mukome et al. 2013, Suddick and Six 2013). To date no study has attempted to investigate the effect of WSB on FWAD performance.

The aim of this study is to investigate: 1) the effect of WSB doses on FWAD at mesophilic and thermophilic temperatures, and 2) the effect of particle size and ash content of WSB on digester performance. This process could enhance the economics of FWAD to supply renewable CH_4 in a quality that allows injection into the natural gas pipeline or use as vehicle fuel.. Overall, the process could provide an economically viable waste-to-energy process, reduce GHG emissions, reduce demand for fossil fuels, and reduce environmental impacts associated with a major US waste source.

Materials and methods

Characteristics of food waste, sewage sludge and biochar

77 The AD experiments were conducted using FW as the substrate and AD sludge from a municipal
78 WWTP as the inoculum source. The FW was prepared by mixing various fruits, vegetables, bread,
79 grease, and coffee grounds and filters in a blender, and stored at 4^{o}C (Table S1) (Kovacs et al. 2015).
80 The inoculum was obtained from the outlet of the methane-phase digester at Woodridge Greene
81 Valley Wastewater Facility located at Woodridge, Illinois. The facility operates a two-stage AD
82 system as reported previously (Shen et al. 2015b). Fine WSB (FWSB) and coarse WSB (CWSB)
83 samples were provided by Dixon Ridge Farms located in California which utilizes a 50 kW BioMax
84 50 (Community Power Corporation, Littleton, Colorado, USA) downdraft gasifier at 900 ^{o}C (Suddick
85 and Six 2013).

86 *Anaerobic digestion experiment design*

87 The AD experiments were conducted in 650 mL Wheaton serum bottles at either mesophilic (37 °C ±
88 1 °C) or thermophilic (55 °C ± 1 °C) temperature with a working volume of 550 mL. The first
89 experiment was conducted with a single replicate and tested the FWSB at two dosages at mesophilic
90 temperature; 1.91 and 3.83 g biochar (g VS_{added})-1 and three dosages at thermophilic temperature;
91 0.96, 1.91 and 3.83 g biochar (g VS_{added})$^{-1}$ (5, 10, and 20 g of FWSB per digester), against a positive
92 control (PC) digester without FWSB at both temperatures (Table 1). The dose was selected based on
93 averaged values of other biochar samples tested in the lab (data not shown). The second experiment
94 was conducted at thermophilic temperature in duplicate for the PC and in triplicate for the FWSB and
95 CWSB each at a dosage of 1.83 g biochar (g VS_{added})$^{-1}$ (10 g WSB per digester) (Table 1). Each
96 digester contained inoculum (4.7 g dry matter, 3 g VS), food waste (2.3 g dry matter, 2.2 g VS),
97 varying concentrations of WSB (depends on the experimental condition) and deionized water as the
98 makeup water, and was sparged with helium gas (99.999% purity) (Airgas, Minooka, Illinois) for 2.5
99 min before AD experiments began. Each digester was then either placed in an MPA-200 Biomethane
100 Potential Analyzer system (Challenge Technology, Springdale, Arizona) or in a New Brunswick's
101 model I24 benchtop incubating shaker (Eppendorf, Hauppauge, New York) as described previously
102 (Shen et al. 2015b), otherwise identical continuously stirred digesters. In brief, the MPA-200 system
103 consists of a respirometry-based unit for gas measurement and automated data recording. Each

digester in the incubating shaker was attached to a multi-layer foil gas sampling bag (Restek, Bellefonte, Pennsylvania) for gas volume measurement daily. The gas production volume was adjusted to dry ambient temperature and pressure (20 ^{o}C and 101,325 Pa) to account for temperature and pressure fluctuations in the lab (Walker et al. 2009). All the experiments were operated in batch mode and at 50 rpm agitation. The batch experiments were terminated when the daily biogas production volume reached less than 1% of the total biogas volume.

Analyses

Biochar characterization

Particle size distribution of biochar samples was determined using the method as described previously (Shen et al. 2015b). Brunauer-Emmet-Teller (BET) surface area, total pore volume and pore size were determined utilizing argon or nitrogen gas adsorption analysis at 77.35 K (Brewer et al. 2009). Surface morphology was characterized by using scanning electron microscope (SEM) (Shen et al. 2015b). Proximate, ultimate and ash elemental analyses were conducted in triplicate using the methods as reported in detail in our previous publication (Shen et al. 2015b).

Feedstock and digestate characterization

Total solids (TS) and volatile solids (VS) contents were determined per Standard Methods (APHA et al. 2012). Total chemical oxygen demand (COD), total organic carbon (TOC), total alkalinity (TA), total phosphorus (TP), total nitrogen (TN) and ammonia nitrogen (NH_3-N) were determined using Hach test kits (Hach, Loveland, CO). Total metal (Al, Ca, Fe, Mg, Mn, K, Si, and Na) concentrations were analyzed using USEPA Method 200.7 (USEPA 2007a) and 200.8 (USEPA 2007b).

Gas sampling and analysis

Gas samples were taken from the digester headspace periodically as described in (Shen et al. 2015b). Biogas was analyzed for CH_4 and CO_2 by using a Shimadzu GC-2014 gas chromatograph equipped with a thermal conductivity detector (TCD) and a Supelco 80/100 Porapak Q packed column (5m x 3.175mm x 2.1 mm) (Sigma-Aldrich, St. Louis, MO). Helium (99.999% purity) (Airgas, Minooka,

IL) was used as the carrier gas. The column temperature was set at 100 °C isothermally and the TCD temperature was set at 170 °C.

Statistical analysis

All statistical comparisons were conducted using the student's t-test with a 95% confidence interval. Time course data was analyzed with a paired t-test and the initial and final averaged digester values were analyzed with an unpaired t-test. Comparisons were considered statistically different in the p-value was less than 0.05.

RSM model development for methane production and content

This statistical technique is a useful tool for AD optimization when the response (CH_4 production volume and CH_4 content) may be influenced by several variables (Ahmad et al. 2014a, Gonzalez-Fernandez et al. 2011, Jimenez et al. 2014, Linville et al. 2016, Montgomery 2005). The full model methodology was described by Linville et al. (2016). In brief, the software program Design Expert 9 (Jin et al. 2013) was used to determine the model equation for CH_4 production volume and content with variables as X_1 (digestion time, days) and X_2 (biochar dosage, g biochar (g VS_{added})$^{-1}$) (Table S2). Insignificant terms (p-value > 0.05) were removed from the model except when required to support the hierarchy (Causton et al. 2001, Jin et al. 2013).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{122} X_1 X_2^2$$

Multi-objective optimization (MO) analysis was performed using the proposed model equations to maximize the two response variables (CH_4 production volume and CH_4 content) that are dependent on the same inputs (FWS dosage and digestion time), using Matlab® version 7.4.0 (R2015b) Optimization Toolbox (Brule et al. 2014).

Results and discussion

Biochar characteristics

The two WSB were produced by pyrolysis at different temperatures and treated with different activation techniques resulting in varied physical and chemical properties (Tan et al. 2015). The

FWSB has a particle size distribution with approximately 76.9 wt% of the particles below a particle size 500 µm and the largest fraction (31.8 wt%) of particles between 125-177 µm. The CWSB has 23.9 wt% of particles below a particle size of 500 µm and most particles (68.6 wt%) above 707 µm (Figure S1). SEM images of FWSB and CWSB at 200x Magnification (Figure 1A and 1B, respectively) and 1000x Magnification (Figure 1C and 1D, respectively) show the morphologies of tested WSB and differences in their hierarchical structures. Surface area of biochar is dependent on particle size of feedstock pellet, reaction temperature and reactor operational conditions (Manya 2012). Increasing pyrolytic temperature increases the escape of volatile substances and the formation of channel structures thus improves the specific surface area and pore structure (Tan et al. 2015).

Physical and chemical analyses results are shown in Table 2. The BET surface area of the FWSB was measured as 86.5 $m^2 g^{-1}$ and the CWSB as 793 $m^2 g^{-1}$ which are remarkably different than previously reported WSB (227 $m^2 g^{-1}$) (Daoyuan et al. 2014, Mukome et al. 2013). It should be noted that direct comparison of the pore volume and area is difficult due to FWSB sample interaction with Ar/N_2 gas. The FWSB and CWSB both have a high ash content like other WSB (ash content 40.4 - 46.6 wt%) (Daoyuan et al. 2014, Mukome et al. 2013, Suddick and Six 2013). Furthermore, the FWSB and CWSB have a carbon content like other WSB (55.3%) (Mukome et al. 2013, Suddick and Six 2013), but less than woody biomass such as oak (65.3%-84.4%) (Brewer et al. 2009, Cheah et al. 2014). FWSB and CWSB have similar hydrogen content; however, the FWSB has higher oxygen content compared to the CWSB. The H:C molar ratio represents the degree of carbonization as hydrogen is primarily associated with the organic matter in the biomass (Tan et al. 2015). The FWSB and CWSB both have a low molar H:C ratio like other WSB (0.22) (Mukome et al. 2013). The surface hydrophilicity of biochar, described by molar O:C ratio, is indicative of polar-group content (Tan et al. 2015). The O:C ratio of the FWSB and CWSB is again low (Ahmed et al. 2016, Keiluweit et al. 2010). The FWSB ash contains 31.0% CaO, 8.4% MgO, 23.4% Na_2O, and 0.2% K_2O while the CWSB ash contains 19.2% CaO, 5.6% MgO, 0.3% Na_2O, and 40.3% K_2O. Based on the ash percent and cation concentrations, the CWSB requires roughly 1.35 times as much biochar as the FWSB to remove the same amount of CO_2.

In summary, the FWSB has greater ability to promote *in-situ* CO_2 removal due to the smaller particle size and higher ash content leading to a higher cation concentration compared to the CWSB as discussed below.

Anaerobic digestion experiments

The WSB's ability to promote *in-situ* CO_2 removal during FWAD was tested at typical design ranges: mesophilic (35-40 ^oC) and thermophilic (50-55 ^oC) temperatures (Linville et al. 2015). Mesophilic AD is commonly used for organic wastes due to the low capital costs and ease of operation (Linville et al. 2015). However, thermophilic AD has many inherent advantages over mesophilic AD including faster reaction rates, higher biogas production rate and volume, less foaming and enhanced pathogen reduction (De la Rubia et al. 2013, Suryawanshi et al. 2010). The elevated temperature also enhances the leaching and dissolution of the cations (Ca, Mg, Na, and K) from biochar (Pan et al. 2012, Sanna et al. 2014) and the endothermic adsorption capacity of the biochar (Tan et al. 2015). The performance of FWAD is also improved by alkaline condition at thermophilic temperature (Vlyssides and Karlis 2004).

Comparison of fine walnut biochar at mesophilic and thermophilic temperature

The FWSB test lasted 54 days at mesophilic temperature and 26 days at thermophilic temperature. Figure 2 shows the time course of CH_4 content (% v/v), CH_4 production volume (mL), and CO_2 production volume (mL) for the digesters at mesophilic and thermophilic temperature, respectively.

The CH_4 content on day 1 was 100% for the 37FWS10 and 37FWS20 digesters whereas the 37PC digester had a 45% CH_4 content (Figure 2A). The CH_4 content in the 37FWS10 digester and 37FWS20 digester remained statistically higher (p-value < 0.001) than the 37PC digester after day 26. The 37FWS10 and 37FWS20 digesters have a statistically lower CH_4 volume (p-value <0.0095 for both, Figure 2B) due to lower biogas production volume (Figure S2). Interestingly, the CH_4 yields were similar between the 37PC (484 mL CH_4 (g $VS_{degraded})^{-1}$) and the 37FWS10 (492 mL CH_4 (g $VS_{degraded})^{-1}$) digesters; however, the 37FWS20 CH_4 yield was much lower (131 mL CH_4 (g $VS_{degraded})^{-1}$) indicating that the FWSB dose was inhibitory at higher concentrations. Furthermore, the CO_2

208 production volume (mL) was also much lower in the 37FWS10 and 37FW20 digester compared to the
209 37PC due to a combination of lower biogas production volume and higher CH_4 content (Figure 2C).

210 The CH_4 content on day 1 was above 93% for the 55FWS5 digester and at 100% for the 55FWS10
211 and 55FWS20 digester whereas the 55PC had a 54% CH_4 content (Figure 2D). The CH_4 content
212 stabilized by day 7. Compared to the 55PC, the averaged CH_4 content is statistically higher in the
213 FWSB supplemented digesters(p-value <0.0001 for all). There is no inhibition in the 55FWS5
214 digester based on the CH_4 production volume compared to the 55PC (Figure 2E) (p-value 0.7615).
215 There is also no statistical difference in the CH_4 production volume between the 55FWS10 and
216 55FWS20 digesters (p-value 0.7843); however, there is a 20.5% reduction in CH_4 volume between the
217 55PC/55FWS5 and 55FWS10/55FWS20 digesters (Figure 2E). Likewise, the CH_4 yields were similar
218 between the 55PC and 55FWS5 digesters (633 and 606 mL CH_4 (g $VS_{degraded}$)$^{-1}$, respectively), and
219 between the 55FWS10 and 55FWS20 digesters (478 and 466 mL CH_4 (g $VS_{degraded}$)$^{-1}$, respectively)
220 indicating an inhibition at the higher FWSB dosages. Most remarkably, the CO_2 production volume
221 (mL) is reduced by 39.2% in the 55FWS5 digester compared to the 55PC without any loss in CH_4
222 volume. Furthermore, the 55FWS10 digester had a 73.1% reduction and the 55FWS20 digester had a
223 95.8% reduction in CO_2 volume compared to the 55PC (Figure 2F) due to lower biogas production
224 volume (Figure S2) and higher CH_4 content due to addition of higher FWSB concentrations.

225 The FWAD benefited from the FWSB addition regarding the CO_2 removal from the biogas for both
226 temperatures. CO_2 adsorption increased with increasing FWSB addition attributed to increased
227 available adsorption surface and more reactive sites (Tan et al. 2015). Adsorption, electrostatic
228 interactions and/or polarity attraction are some of the possible mechanisms for CO_2 removal due to
229 biochar in the digester (Ahmad et al. 2014b). H:C and O:C are indicators of biochar's stability and
230 hydrophobicity, and their contribution to the absorption capacity is not quite clear. However, the small
231 particle size provides a large surface area for CO_2 adsorption, as discussed before. The high ash
232 content and high concentration of base cations (Ca, Mg, Na, and K) in FWSB contributes to the
233 accelerated carbonation reaction (Shen et al. 2015b). Total concentrations of base cations (Ca, Mg, Na
234 and K) are positively correlated to biochar's alkalinity (Fidel et al. 2017), and therefore can be used as

indicator of CO_2 absorption capacity. Shen et al., reported CO_2 sorption processes based on a pseudo-second-order kinetic model for corn stover biochar which indicates physical adsorption, and a weak bonding between adsorbate and adsorbent (Shen et al. 2015b). Other studies have shown a decrease in CO_2 emissions from biochar amended soil (Daoyuan et al. 2014; Mukome et al. 2013).

The FWAD was more efficient at thermophilic temperature with the 55PC having a digestion period 28 days shorter than the 37PC while producing 23% more biogas. AD was inhibited from higher concentrations of FWSB at both temperatures. The 55FWS10 digester and 37FW10 digester saw a 37% decrease in biogas production volume compared to the PC digester at the respective temperature (Figure S2). The biogas production volume was less inhibited for the 55FWS20 compared to the 37FWS20 digester. The decrease in inhibition may be due to the faster reaction rate and higher biogas production volume at thermophilic temperature (De la Rubia et al. 2013, Suryawanshi et al. 2010).

Response surface methodology (RSM) modeling

The dosage of adsorbent has significant influence on the adsorption efficiency; therefore, applying the optimum dosage of WSB for CO_2 removal is crucial for its cost-effective application (Tan et al. 2015). The studied parameters (digester operating time and FWSB dosage) was optimized by response surface methodology (RSM) at each temperature (Figure S3 and Table S2). The interactive effects of the independent variables on CH_4 production volume and CH_4 content were illustrated by three-dimensional plots (Figure 3). The results of the MO determined that near pipeline-quality methane content (>90% CH_4) (Shen et al. 2015b) can be achieved with only a slight reduction in CH_4 production volume. The maximum volume of pipeline-quality methane that can be achieved at mesophilic temperature is 670 mL with a digestion time of 47 days and 1.94 g biochar (g VS_{added})$^{-1}$ FWSB addition, and at thermophilic temperature is 1186 mL with a digestion time of 20 days and 2.46 g biochar (g VS_{added})$^{-1}$ FWSB addition.

Comparison of fine and coarse walnut biochar performance at thermophilic temperature

The impacts of different biochar addition (FWSB versus CWSB) on AD performance were investigated at thermophilic temperature for 25 days. Figure 4 shows the time course of CH_4 content

(% v/v), CH_4 production volume (mL), and CO_2 production volume (mL) for the digesters with FWSB and CWSB. The initial CH_4 content for the 55FWS10 digester was higher than the 55CWS10 digester and both were greater than the 55PC digester (Figure 4A). The CH_4 content stabilized in the 55PC digester after day 4. The CH_4 content was statistically higher in the 55CWS10 digester and the 55FWS10 digester compared to the 55PC digester(p-value <0.0001 for both). The CH_4 content of the 55FWS10 digester is statistically higher than the 55CWS10 digester (p-value 0.0093). The CH_4 production volume (Figure 4B) was statistically different (p-value <0.005 for all conditions) showing slight inhibition from the WSB concentration in the digester. Furthermore, the CH_4 yield for the 55PC digesters (600±29 mL CH_4 (g $VS_{degraded}$)$^{-1}$) is higher than the 55CWS10 digesters (492±30 mL CH_4 (g $VS_{degraded}$)$^{-1}$, p-value 0.0275) and the 55FWS10 digesters (510.0±31 mL CH_4 (g $VS_{degraded}$)$^{-1}$, p-value 0.0461). The limitation of the CWSB due to its larger particle size, and lower ash content thus lower cation concentration can be seen in the CO_2 removal efficiency. The 55CWS10 digesters only had a 51.0% CO_2 removal and the 55FWS10 digesters had a 61.0% CO_2 removal compared to the 55PC (Figure 4C).

To validate the RSM models at thermophilic temperature, the results for the 55FWS10 digesters were compared. The estimated CH_4 production volume was 3.3% above the actual value and the estimated CH_4 content was the same at 81.1%. This result shows that the RSM models provide an acceptable model accuracy and high degree of fit to experimental data.

Impact of biochar addition on digester performance

The characteristics of the digester environment were compared before and after AD. Both the TS and VS increased with increasing FWSB addition (Figure S4), which was expected. The thermophilic temperature digesters had higher solids destruction compared to the mesophilic temperature digesters. The pH increased with increasing concentration of FWSB, with the initial pH ranging from 8.6-9.2 (Figure S4). The 55CW10 digester had a higher pH than the 55FW10 digester (Figure S4). FWAD can be characterized by low pH (Leiva et al. 2014); therefore, FWAD can be facilitated by the alkaline pH treatment from WSB addition. It was also demonstrated that thermophilic alkaline pretreatment (pH >8) could maximize VFAs yields during sludge AD because of faster reaction

kinetics and enrichment of VFA producing bacteria (Zhang et al. 2010). Furthermore, the final pH values of all WSB digesters were still in a slightly alkaline range (pH > 7.4) regardless of digester temperature or biochar dosage.

The cations in the WSB increased the buffering capacity of the biochar-amended digesters which enhanced the process stability as compared to the PC digester. All WSB digesters had a final TA concentration 1.9 – 2.7 times higher than the PC digester at both temperatures (Figure 5A and 5C). The final TA concentrations were not statistically different in the 55FWS10 and 55CWS10 digesters (Figure 5C). The final TA concentrations in digesters amended with 10g WSB dose or greater were above the desirable range (2000-5000 mg L^{-1}) (Chen et al. 2008). The increase in alkalinity during AD was the result of metabolic alkalinity from organic nitrogen degradation, hence ammonia formation (Figure 5) or cation release from WSB (Speece 1996).

The NH_3-N concentration increased by approximately 30% in the PC digesters after AD at both temperatures compared to the FWSB amended digesters which ranged from 29% increase in the 55FWS5 digester to 2.0% increase in the 37FWS20 digester (Figure 5A and 5B). The degradation of organic nitrogen-compounds caused the increase in ammonia (Speece 1996). There was no significant difference in the initial or final NH_3-N concentrations for the 55CWS10 or 55FWS10 compared to the PC digesters which averaged 21.5% increase during the experiment (Figure 5C). An increase in pH typically increases free ammonia (NH_3) inhibition (Chen et al. 2008) by shifting the NH_3-NH_4^+ equilibrium towards NH_3 formations in the digesters. However, the experimental results showed that the change in ammonia concentration decreased with increasing WSB dose. The biochar's large surface area promotes NH_3 adsorption thereby mitigating ammonia inhibition (Taghizadeh-Toosi et al. 2011).

The TP concentration increased with increasing FWSB dose (Figure 5A and 5B) due to the FWSB ash containing 6.0 wt% P_2O_5 (Table 2). The TP was higher in the 55FWS10 digester compared to the 55CWS10 digester (Figure 5C), despite similar P_2O_5 wt% in the ash due to the smaller particle size or increased ash content having higher dissolution for the FWSB.

The initial COD increased with increasing WSB addition (Figure 5A and Figure 5B). The COD did not decrease for the higher concentrations of WSB addition at either temperature due to inhibition. The initial TOC also increased with increasing WSB addition which probably resulted from the volatile matter in the biochar. However, the TOC decreased by similar amounts in all digesters at the same temperature except for the 55FWS5 which decreased less. It should also be noted that TOC and COD measurements include harsh conditions including low pH (pH=2) and high temperature (105-150 ^{o}C). Nevertheless, biochar stores the organic carbon in a recalcitrant form which is represented by the low O:C molar ratio (Table 2); hence, the biochar addition hardly increased bioavailable organic matter concentration in the digester (Shen et al. 2015b). The COD and TOC concentrations were higher in the 55FWS10 digester compared to the 55CWS10 digester (Figure 5C) which may be due to the higher volatile matter and higher ash content in the FWSB (Table 2).

Impact of biochar addition on cation concentrations

Moderate levels of cations (Na, K, Mg, Ca) are essential for microbial growth, affect specific growth rates in microorganisms, and reduce inhibition; however, excessive levels have serious toxic effects inhibiting microbial growth (Bozym et al. 2015, Chen et al. 2008). While high WSB dosage increases CO_2 removal, it results in digester cation toxicity; therefore, WSB dosage optimization is important. The optimum cation concentrations have been reported as 200 mg L^{-1} Ca, 100-200 mg L^{-1} Na and <400 mg L^{-1} K with inhibitory concentrations ranging from 2500-4000 mg L^{-1} Ca, >400 mg L^{-1} Mg, 3500 -5500 mg L^{-1} Na and >5800 mg L^{-1} K (Bozym et al. 2015, Chen et al. 2008).

In all experiments, the PC digester had concentrations below the inhibitory levels (Figure 5). Na was only detected in the PC digesters in the second experiment. However, the detection limit for Na was 50 mg L^{-1} at the dilution factor used; therefore, it is likely that the Na concentration in the other samples is below the detection limit (Figure S5). The decrease in biogas production volume for the 37FWS10, 37FWS20, 55FWS10 and 55FWS20 digesters compared to the PC digester (Figure 2B and 2E) may be due to the inhibitory Ca and Mg concentrations in the FWS10 and FWS20 digesters (Figure 5A and 5B) (Bozym et al. 2015, Chen et al. 2008). However, the Ca and Mg concentration was lower in the final 55CWS10 digesters leading to a smaller reduction in biogas production volume

compared to the 55FWS10 digesters (Figure 5C). Na was below the inhibitory level but detected inconsistently due to the dilution factor (Figure S5). Although still below the inhibitory level, the K concentration was higher in the final 55CWS10 digester than the final 55FWS10 digester (Figure 5C) due to increased K content in the CWSB (Table 2). There is no significant difference in release of cations from the FWSB at mesophilic versus thermophilic temperature. Although inhibitory levels of each cation were compared with the literature data, the synergistic impacts of all cations also need to be considered. However, to the best of authors' knowledge there is no information on this adverse effect in the literature. Also, as expected from the biochar characterization results (Table 2), the concentrations of aluminum, iron, manganese, and silicon increased with increasing concentration of biochar (Figure S5).

Conclusion

This study resolved challenges surrounding FW, as a highly degradable AD substrate, reducing the overall digester performance due to reactor instability. The impacts of two WSB's on digester performance and *in-situ* CO_2 removal during FWAD were compared in this study. The FWSB has better CO_2 absorption capacity compared to the CWSB due to the increased surface area and ash content which leads to a higher cation concentration and alkalinity in the digester. The FWSB could remove 61.0% of the CO_2 produced by volume whereas the CWSB could remove 51.0% of the CO_2 produced by volume compared to the control digester. A decrease in biogas/methane production was observed with higher dosages of biochar due to higher concentrations of mono- and divalent cations released from the biochar into the digester environment. However, impact of high biochar dosages on methane production in thermophilic digesters (665 mL of methane produced at 55FW20) was less than that of mesophilic digesters (220 mL of methane produced at 37FW20). This is due to better hydrolysis efficiency and faster microbial reaction rates, which lead to higher biogas/CH_4 production. The biochar increased process stability by increasing the total alkalinity and pH of the digesters. RSM modeling showed that near-pipeline quality methane (>90% CH_4) can be achieved utilizing a dose of 1.94 g biochar $(g\ VS_{added})^{-1}$ at mesophilic temperature and a dose of 2.46 g biochar $(g\ VS_{added})^{-1}$ at thermophilic temperature. This hypothesis will be tested by conducting AD experiments under

continuous operation in the future. This process can provide a new path towards efficient and economical renewable CH_4 production from FWAD, with respect to enhanced CO_2 removal, improved process stability at thermophilic temperatures and elimination of energy/cost intensive biogas cleanup and upgrading process as well as reduction in the amount of FW being sent to landfills.

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386 **List of Figures**

402

403 **Table 1: Experimental Conditions**

Condition	Temperature	Ingredients
37PC[a]	37 ºC	Inoculum + substrate
37FWS10	37 ºC	Inoculum + substrate + biochar (1.91 g (g VS_{added})$^{-1}$)
37FWS20	37 ºC	Inoculum + substrate + biochar (3.83 g (g VS_{added})$^{-1}$)
55PC[b]	55 ºC	Inoculum + substrate
55FWS5	55 ºC	Inoculum + substrate + biochar (0.96 g (g VS_{added})$^{-1}$)
55FWS10	55 ºC	Inoculum + substrate + biochar (1.91 g (g VS_{added})$^{-1}$)
55FWS20	55 ºC	Inoculum + substrate + biochar (3.83 g (g VS_{added})$^{-1}$)
55FWS10	55 ºC	Inoculum + substrate + biochar (1.83 g (g VS_{added})$^{-1}$)
55CWS10	55 ºC	Inoculum + substrate + biochar (1.83 g (g VS_{added})$^{-1}$)

404 [a] Positive Control, [b] Condition used in both thermophilic experiments

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Table 2. Physical and Chemical properties of FWSB and CWSB

		FWSB	CWSB
Physical Properties	BET surface area ($m^2 g^{-1}$)	86.5	792.7
	Total volume of mesopores ($cm^3 g^{-1}$)	0.16	0.11
	Average diameter of mesopores (nm)	7.06	3.67
	Total area of micropores ($m^2 g^{-1}$)	109.98	899.04
	Total volume of micropores ($cm^3 g^{-1}$)	0.15	0.40
Proximate Analysis (wt %)	Moisture	2.7 ± 0.1	2.0 ± 0.1
	Ash	43.2 ± 0.2	36.3 ± 0.1
	Volatile Matter	21.2 ± 0.3	12.9 ± 1.1
	Fixed Carbon	32.8 ± 0.4	48.8 ± 1.1
Ultimate Analysis (wt %)	Moisture	2.7 ± 0.1	2.0 ± 0.1
	Ash	43.2 ± 0.2	36.3 ± 0.1
	S	1.5 ± 0.03	0.03 ± 0.02
	C	47.0 ± 0.3	61.0 ± 0.4
	H	0.8 ± 0.04	0.5 ± 0.1
	N	0.8 ± 0.1	0.6 ± 0.04
	O	3.9 ± 0.6	0.03 ± 0.03
Atomic Ratio	H:C molar	0.20 ± 0.01	0.09 ± 0.02
	O:C molar	0.06 ± 0.01	0.0003 ± 0.0004
	C:N molar	68.6 ± 4.9	117.3 ± 6.6
Elemental Analysis of Ash (wt % of ash)	SiO_2	1.7 ± 0.1	1.4 ± 0.6
	Al_2O_3	0.7 ± 0.2	1.1 ± 0.3
	TiO_2	0.02 ± 0.00	0.1 ± 0.02
	Fe_2O_3	0.4 ± 0.01	0.3 ± 0.01
	CaO	31.0 ± 0.7	19.2 ± 0.7
	MgO	8.4 ± 0.1	5.6 ± 0.03
	Na_2O	23.4 ± 0.4	0.3 ± 0.01
	K_2O	0.2 ± 0.01	40.3 ± 1.5
	P_2O_5	6.0 ± 0.03	6.2 ± 0.00
	SO_3	8.0 ± 0.3	0.1 ± 0.02
	Cl	3.2 ± 0.03	1.9 ± 0.06
	CO_2	14.8 ± 0.5	25.2 ± 0.3

Data are shown in average values based on triplicate measurements ± standard deviations.

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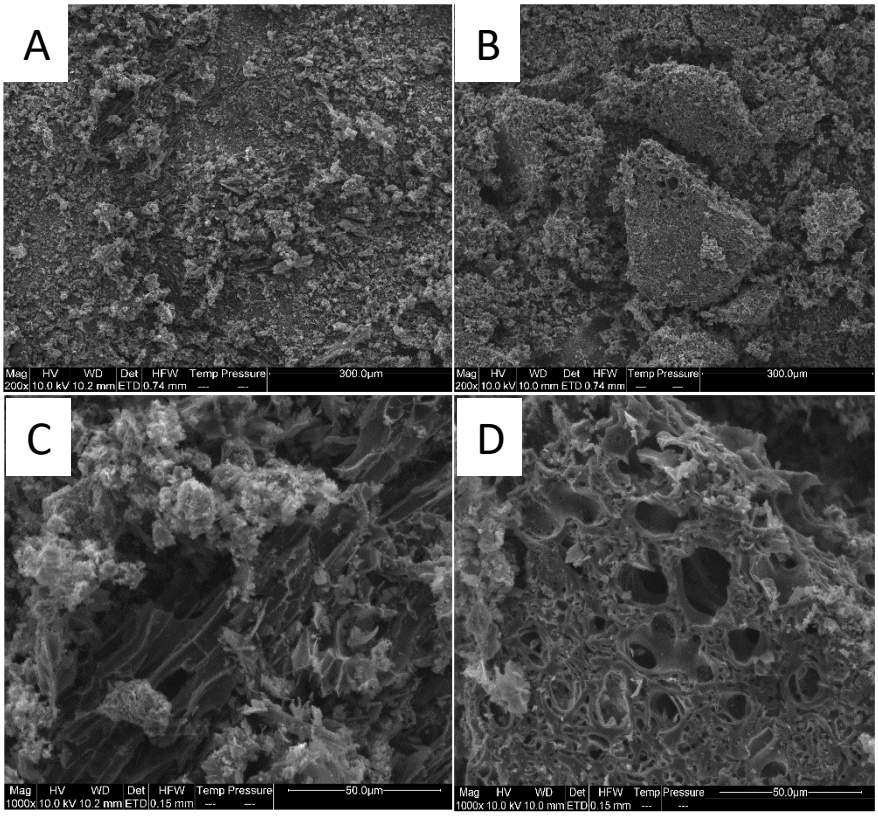
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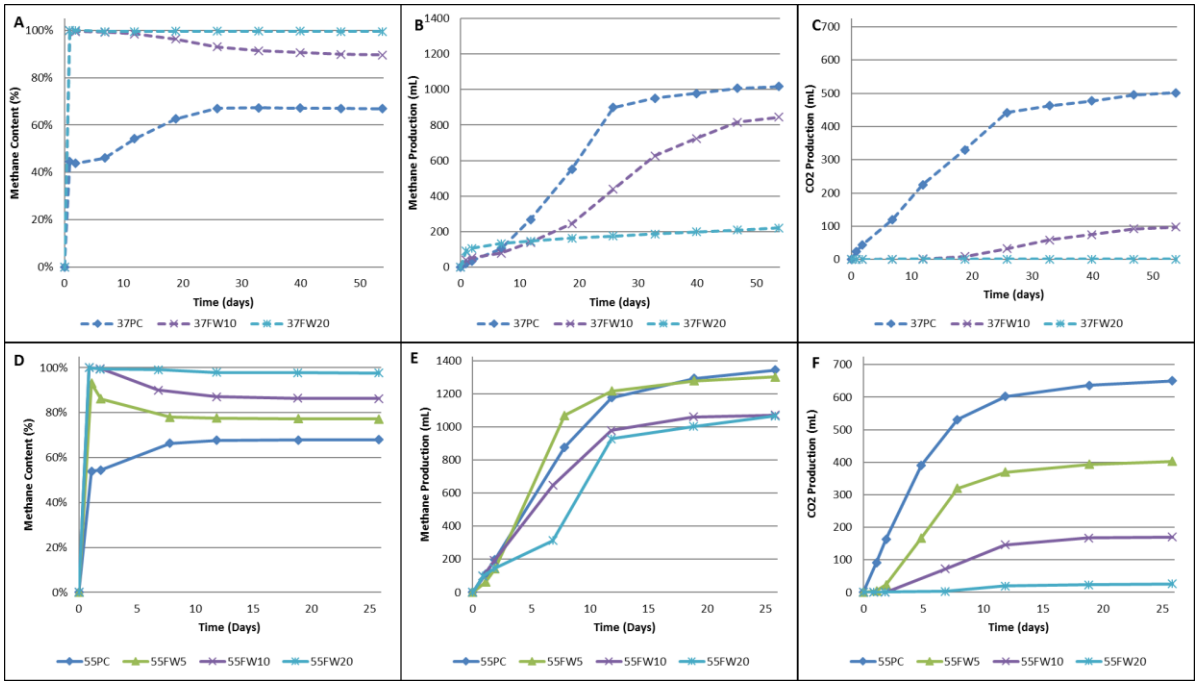

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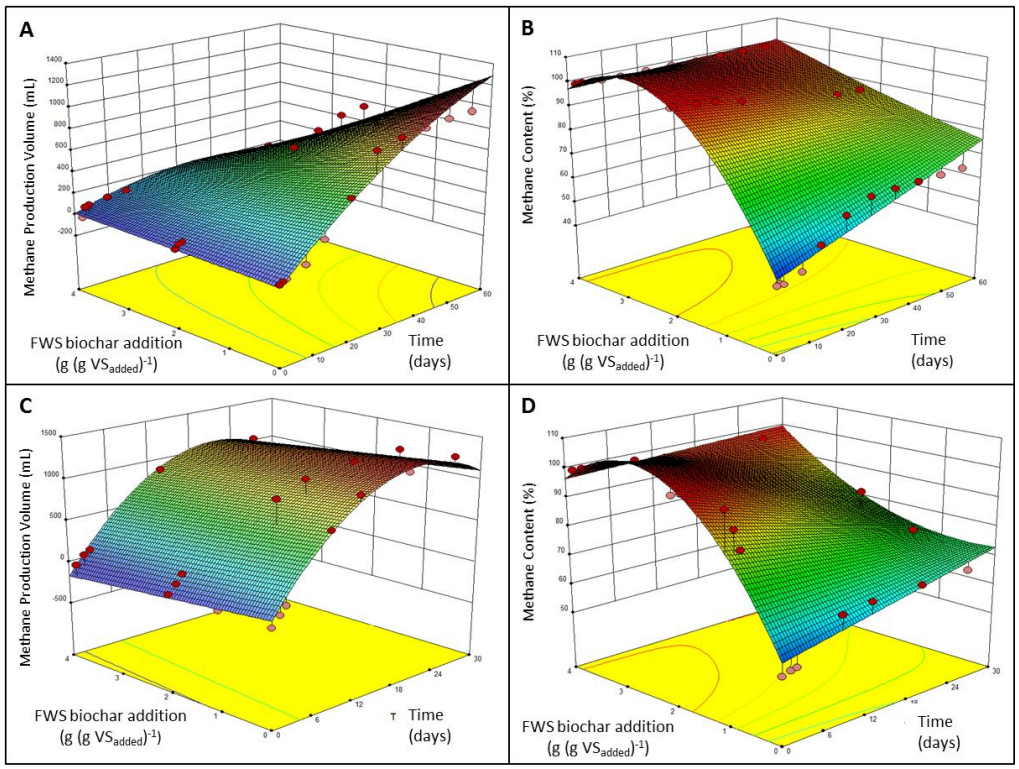

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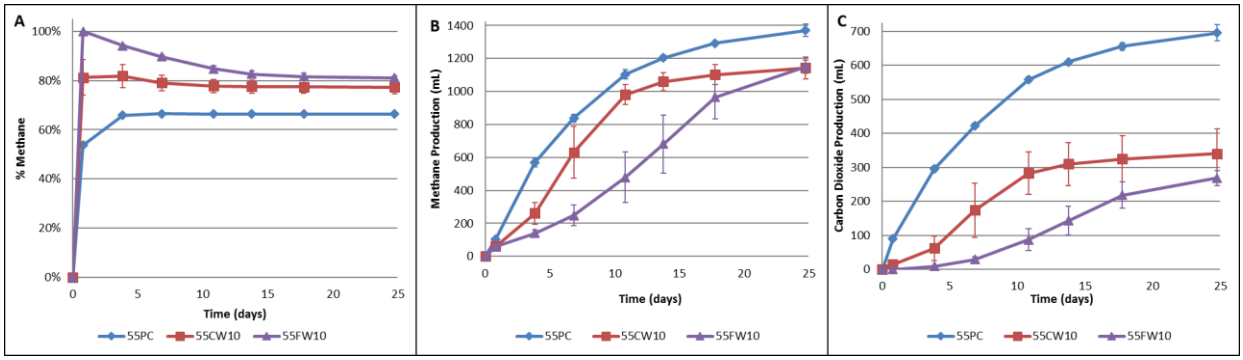

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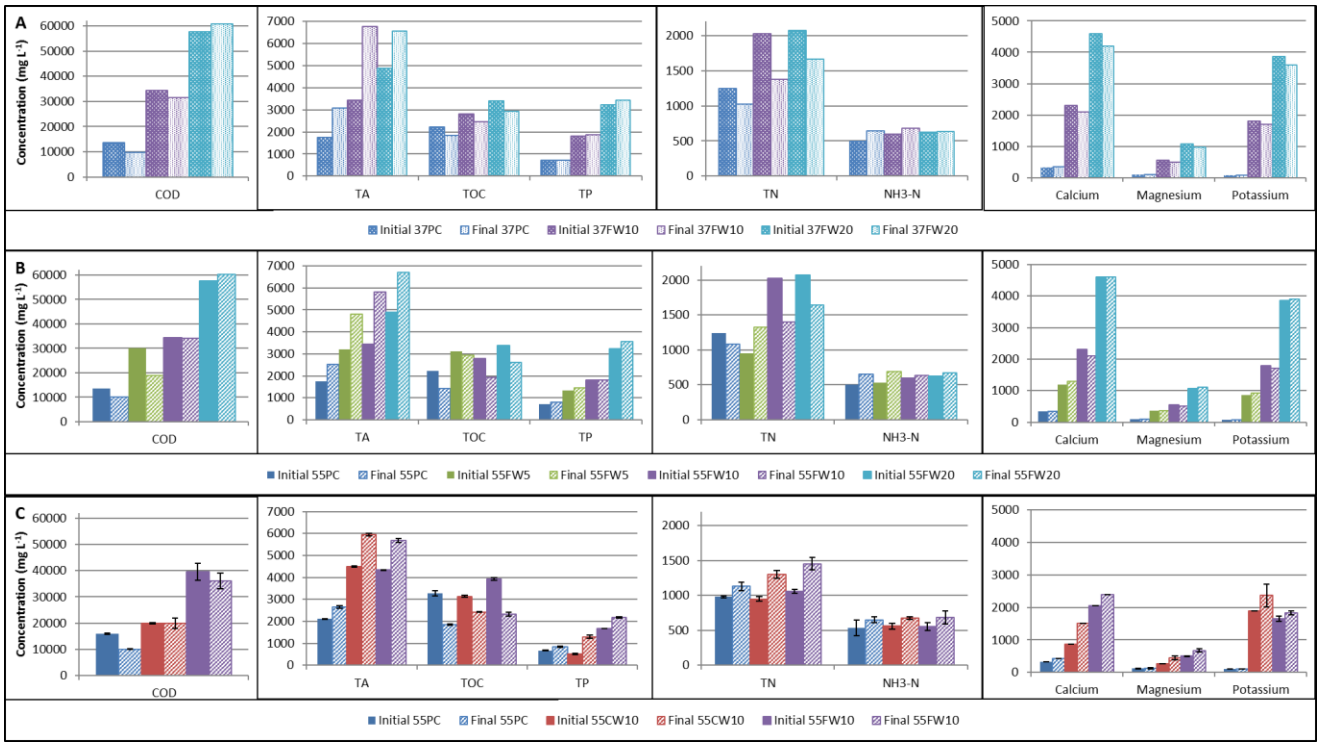

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588 **Supplemental Information**

589 ***In-situ* biogas upgrading during anaerobic digestion of food waste amended with walnut shell**

590 **biochar at bench-scale**

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592 **Urgun-Demirtas***

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Table S1: Characteristics of FW substrate

Parameter	Value
TS (%)	12.4%
VS as % of TS	95.5%
TOC (g C/ L)	22.1
TN (g N/ L)	1.3
TP (g P/ L)	0.24
C:N	19.8

Figure S1. A) Particle size distribution of fine walnut shell biochar (FWSB) and coarse walnut shell biochar(CWSB), and visual comparison of B) FWSB and C) CWSB showing the difference in the particle size distribution.

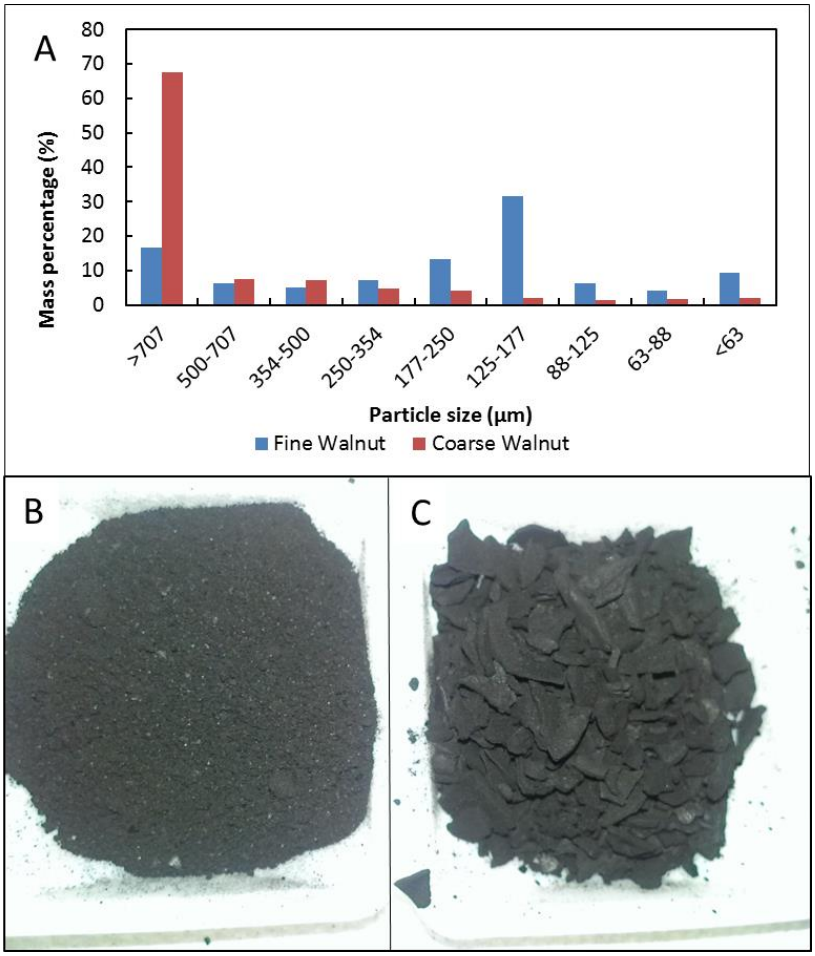
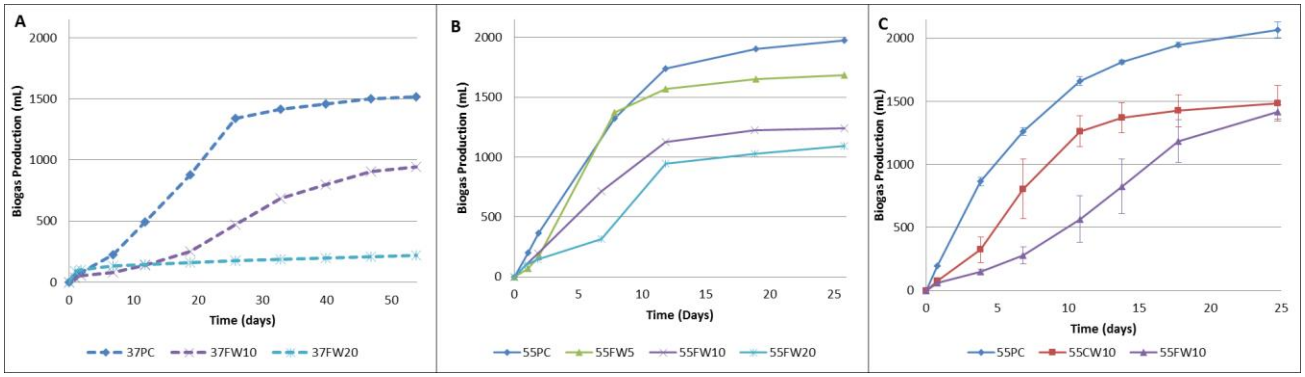


Figure S2. Time-course profiles of cumulative biogas production volume (mL) for: A) FWSB amended AD experiment at mesophilic temperature, B) FWSB amended AD experiment at thermophilic temperature, and C) FWSB and CWSB amended AD experiment at thermophilic temperature



The 37PC has a statistically higher biogas production volume (p-value <0.005) compared to the 37FWS10 and 37FWS20 digesters (Figure 2A). The 55PC has a statistically higher biogas production volume (p-value <0.025 for all results) compared to the 55FWS5, 55FWS10 and 55FWS20 digesters (Figure 2B). For the second experiment, 55PC had a statistically higher biogas production volume (p-value 0.0013) than 55CWS10 digesters, and 55CWS10 digesters had a statistically higher biogas production volume (p-value 0.0211) than 55FWS10 digesters (Figure 2C).

RSM Modeling

Digestion time (T) and FWSB dosage (BD) had a complex effect on the CH_4 production volume and CH_4 content as indicated by the interaction between the terms in the models (Table 3). Per the coefficient of each factor, T had the greatest influence on CH_4 production volume at both temperatures. The BD had a negative effect on CH_4 production volume at thermophilic temperature like the hypothesis and experimental results showing the inhibition at high biochar doses (≥ 10g FWSB). At mesophilic temperature, BD had a positive effect on CH_4 production volume which was offset by the negative coefficient for the interactive term T*BD. Conversely, BD had the greatest effect on CH_4 content followed by T which supports the hypothesis of FWSB addition causing CO_2 removal. At both temperatures, the negative coefficients for the interactive term T*BA and BA^2 in the CH_4 content models indicate the consumption of FWSB active sites during the experiment. The model's coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}), and F-value are consistent (and higher in some cases) with previously reported RSM models (Gonzalez-Fernandez et al. 2011, Jimenez et al. 2014, Jin et al. 2013, Linville et al. 2016) indicating an acceptable model accuracy (Figure S3 and Table S2).

Table S2: Statistical analysis of the two evaluated parameters (Time (days) and FWSB addition (g/g VS_{added})) with regard to the two responses (CH_4 production volume (mL) and CH_4 content (%)) at mesophilic and thermophilic temperature.

	Mesophilic Temperature		Thermophilic Temperature	
Y	CH_4 Production Volume (mL)	CH_4 Content (%)	CH_4 Production Volume (mL)	CH_4 Content (%)

	Coefficient (p-value)	Coefficient (p-value)	Coefficient (p-value)	Coefficient (p-value)
β_0	-20.81 (<0.0001)	+46.45 (<0.0001)	+77.85 (<0.0001)	+57.40 (<0.0001)
β_1	+31.96 (<0.0001)	+0.50 (<0.0001)	+119.88 (<0.0001)	+0.52 (<0.0001)
β_2	+9.14 (<0.0001)	+42.17 (<0.0001)	-60.46 (0.0011)	+36.26 (<0.0001)
β_{12}	-5.00 (<0.0001)	-0.62 (<0.0001)	n.a.	-1.27 (0.1512)
β_{11}	-0.17 (0.0078)	n.a.	-2.84 (<0.0001)	n.a.
β_{22}	n.a.	-7.37 (<0.0001)	n.a.	-6.62 (0.0020)
β_{122}	n.a	+0.13 (<0.0001)	n.a.	+0.29 (<0.0001)
R^2	0.9448	0.9832	0.9509	0.9257
R^2_{adj}	0.9369	0.9800	0.9448	0.9088
F-value	119.71	315.10	154.91	54.82

n.a.: regression coefficients were not significant based on p-value

Figure S3. Actual vs. predicted values for RSM models of A) CH_4 production volume (mL) and B) CH_4 content (%) at mesophilic temperature; and C) CH_4 production volume (mL) and D) CH_4 content (%) at thermophilic temperature.

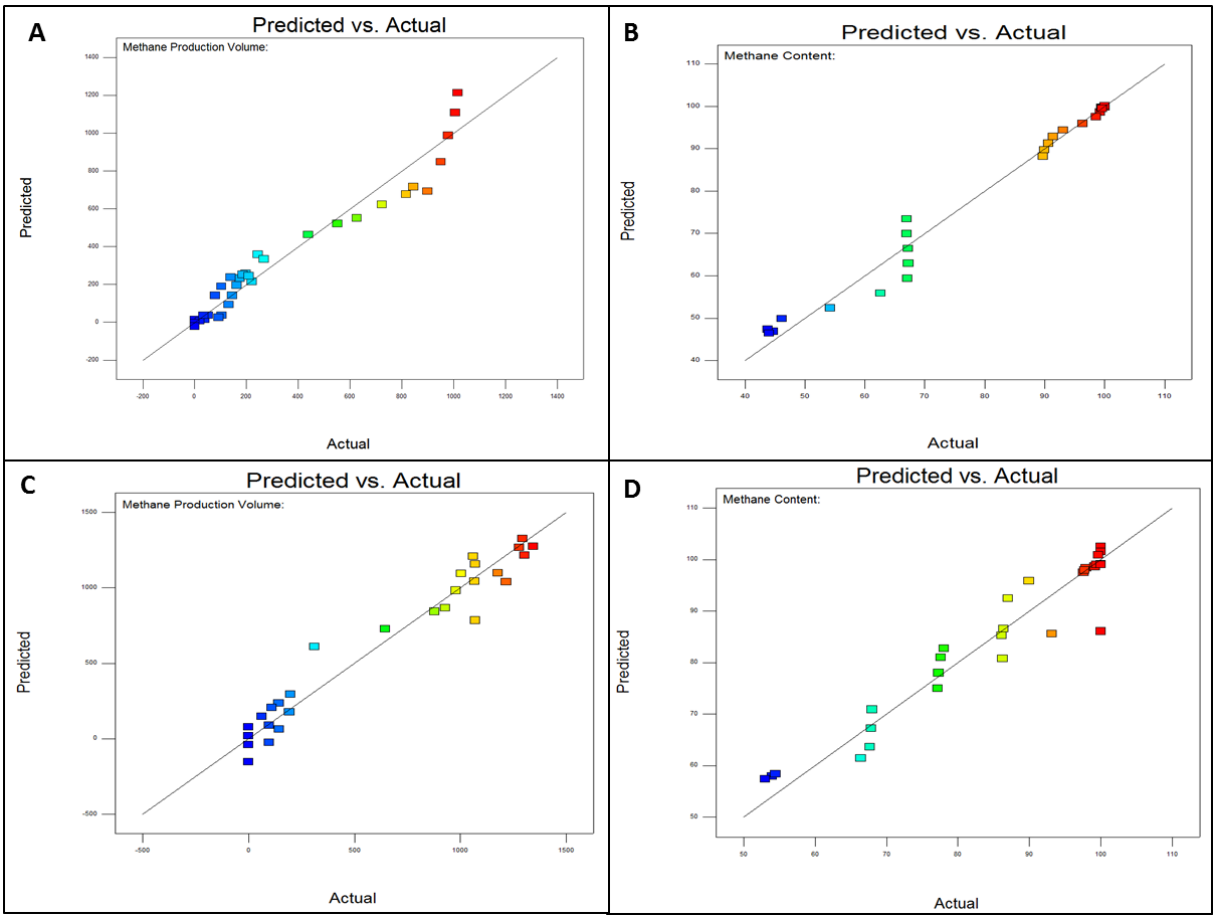


Figure S4. Digestate characteristics before and after AD experiments; A) total and volatile solids and B) pH for FWSB experiment at mesophilic temperature; C) total and volatile solids and D) pH for FWSB experiment at thermophilic temperature; and E) total and volatile solids and F) pH for FWSB and CWSB comparison.

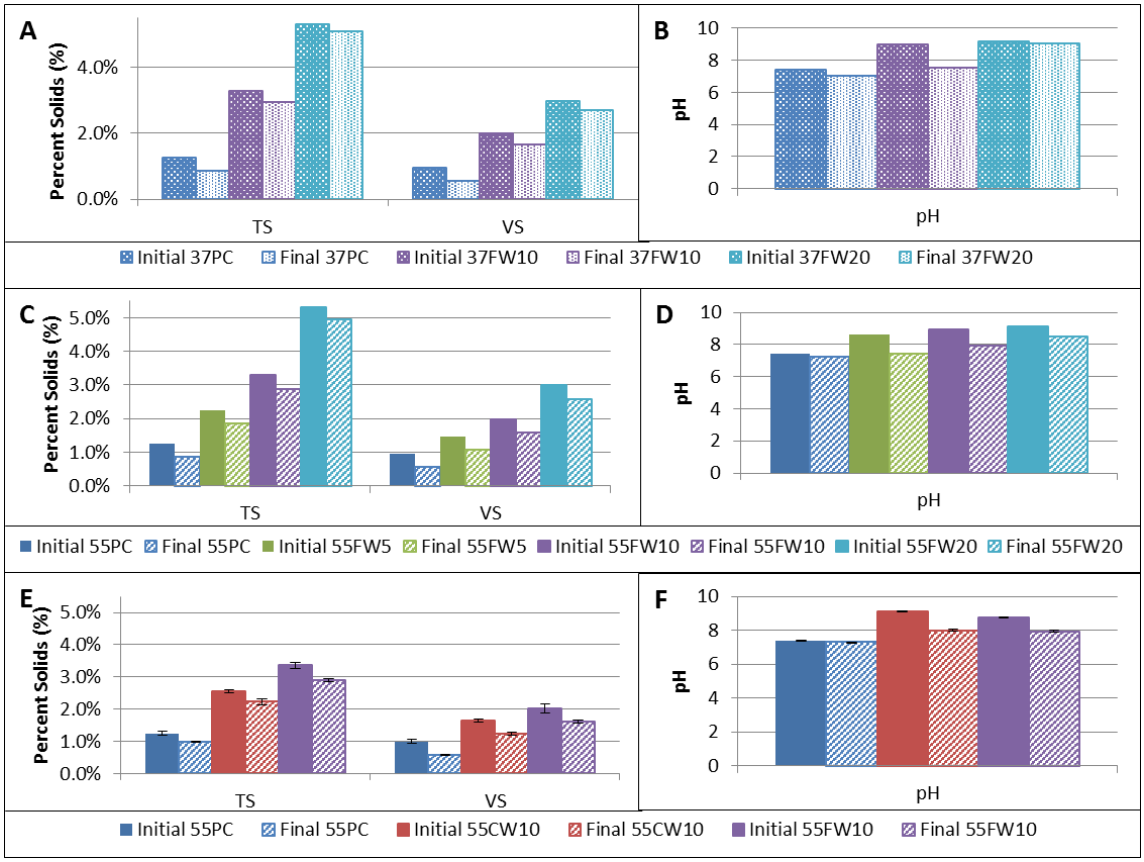
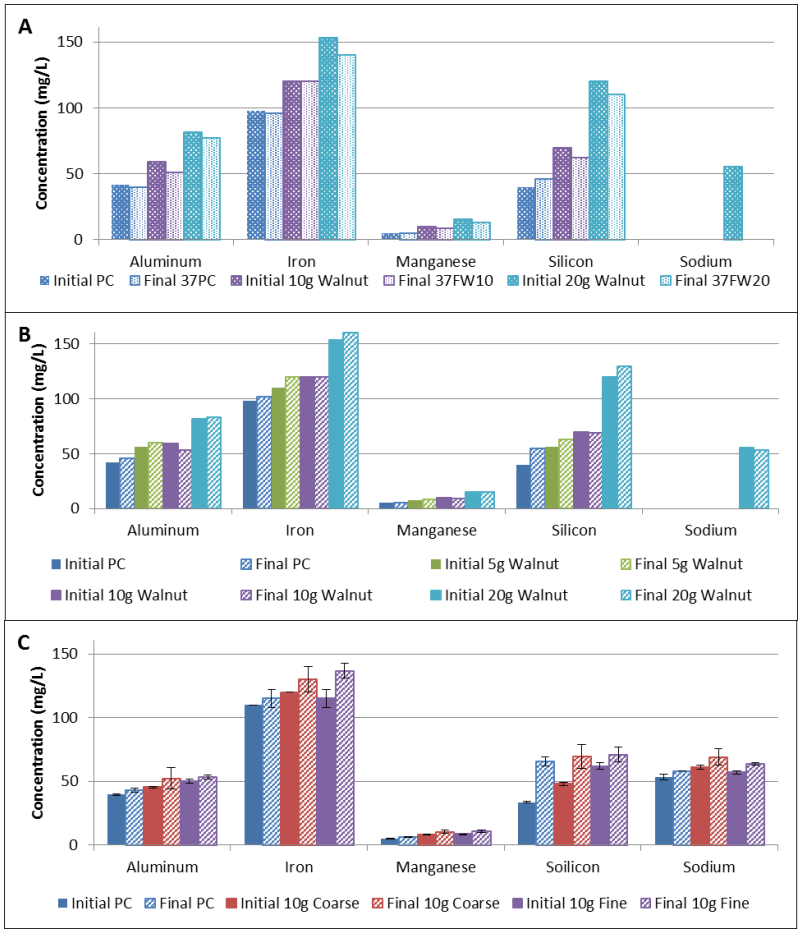


Figure S5. Metal Concentrations before and after AD experiments for; A) FWSB experiment at mesophilic temperature; B) FWSB experiment at thermophilic temperature; and C) for FWSB and CWSB comparison.


A
Concentration (mg/L)
150
100
50
0
Aluminum
Iron
Manganese
Silicon
Sodium
Initial PC
Final 37PC
Initial 10g Walnut
Final 37FW10
Initial 20g Walnut
Final 37FW20
B
Concentration (mg/L)
150
100
50
0
Aluminum
Iron
Manganese
Silicon
Sodium
Initial PC
Final PC
Initial 5g Walnut
Final 5g Walnut
Initial 10g Walnut
Final 10g Walnut
Initial 20g Walnut
Final 20g Walnut
C
Concentration (mg/L)
150
100
50
0
Aluminum
Iron
Manganese
Soilicon
Sodium
Initial PC
Final PC
Initial 10g Coarse
Final 10g Coarse
Initial 10g Fine
Final 10g Fine

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