Enhancing waste degradation and biogas production by pre-digestion with a hyperthermophilic anaerobic bacterium

Jaron C. Hansen¹, Zachary T. Aanderud², Lindsey E. Reid¹, Carson Bateman¹, Conly L. Hansen¹, L. Scott Rogers⁴, Lee D. Hansen¹,*

¹Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA.
²Department of Plant and Wildlife Science, Brigham Young University, Provo, UT 84602, USA.
³Department of Food Science and Nutrition, Utah State University, Logan, UT 84322, USA.
⁴Aqua Engineering, Bountiful, UT 84010, USA.

HIGHLIGHTS
➢ Several organic wastes successfully pre-digested with Caldicellulosiruptor bescii.
➢ Waste destruction and biogas production increased in all cases.
➢ Pre-digestion doubled biogas production from dairy manure and waste activated sludge.
➢ Optimization requires optimizing both pre-digestion and digestion retention times.

GRAPHICAL ABSTRACT

ABSTRACT

The hyperthermophilic anaerobic bacterium, Caldicellulosiruptor bescii, is effective in degrading and solubilizing lignocellulosic materials. Laboratory studies have characterized the chemistry of the process for crystalline cellulose and switchgrass, but the data are insufficient for engineering commercial plants to use C. bescii for pre-digestion of waste streams. The purpose of this study is three-fold: 1) to identify any potential toxicities in C. bescii pre-digestion and biogas production from several wastes; 2) to determine the potential enhancement of biogas production by anaerobic digestion of pre-digested dairy manure and waste activated sludge; and 3) to identify variables that must be quantified and controlled for engineering commercial, continuous-flow systems for waste disposal and biogas production incorporating C. bescii pre-digestion. Tests were run at lab-, bench- and pilot plant-scale with C. bescii pre-digestion and controls run at 75°C and pH 7-8 followed by mesophilic anaerobic digestion at 37-41°C. The lab- and bench-scale tests demonstrate that C. bescii is capable of growing on several organic wastes and pre-digestion with C. bescii increases conversion of waste into biogas, typically by a factor of 2 or more. Incorporation of C. bescii pre-digestion in an optimized commercial system is predicted to provide 75-85% volatile solids conversion to biogas with 75% methane when digesting dairy manure and sewage sludge. Achieving these results at a commercial scale requires further work to quantify C. bescii growth and enzyme production rates, as well as rates of base- and enzyme-catalyzed hydrolysis of the polymeric materials, e.g., lignocellulose, in the waste in order to optimize retention times.
1. Introduction

Transformative advancement in renewable energy production by anaerobic digestion (AD) of waste streams requires an inexpensive, simple, and scalable pretreatment to increase organic waste conversion into biogas (Atelge et al., 2020; Zanri et al., 2021). Production of biogas by AD offers a proven, readily-scalable, and well-understood mechanism for energy production and disposal of organic wastes. However, inefficient conversion of waste into biogas, typically 30-40% in mesophilic digesters without pretreatment (Rico et al., 2011; Noor et al., 2012; Atelge et al., 2020; Tabatabaei et al., 2020; Liu et al., 2021), makes it difficult for AD to be an economically viable source of renewable energy. Improving the economic viability of AD in the renewable energy market, therefore, requires a low-cost, efficient pretreatment that consistently and significantly increases the fraction of biomass converted into biogas (Carrere et al., 2016; Anukam and Berghel, 2020; Atelge et al., 2020; Cheah et al., 2020; Sevillano et al., 2021).

Pretreatment of organic wastes prior to AD by physical (e.g., mechanical pulverization, cavitation, and limited pyrolysis), physicochemical (e.g., steam explosion and ammonia fiber explosion), chemical (e.g., acid hydrolysis, alkaline hydrolysis, high-temperature organic solvent pretreatment, and oxidative delignification), biological (e.g., lignin degradation by white- and soft-rot fungi), and electrical methods, and various combinations thereof, have existed for several decades, but are energy inefficient and are often not economically viable (Lee et al., 2016; Kumar and Sharma, 2017; Vyas et al., 2018; Anukam and Berghel, 2020; Atelge et al., 2020). To date, the only economically successful pretreatment method for increasing degradation and biogas production is the thermal hydrolysis process (THP), in which the influent is heated to 130-180°C for 30-60 min. THP of sewage sludges increases biogas yield by 50%, decreases viscosity, allowing higher loading rates, decreases effluent chemical oxygen demand (COD) by 50%, improves dewatering, and provides stabilized, odor-free compost (Liao et al., 2014).

The optimum system for waste pretreatment depends on the physical and chemical characteristics of the waste being treated, and for some wastes, a pretreatment that uses a thermophilic biological component may provide many of the same advantages as THP at less cost. A biological pre-digestion process is more energy-efficient than THP because it operates at lower temperatures and pressure. However, for some wastes, the optimum pretreatment may be to add thermophilic biological post-THP, which could be done with no additional energy cost because the influent is already heated. Such a combination of compatible pretreatments may provide a significant increase in performance over THP or biological pre-digestion alone for some wastes.

Many wastes are recalcitrant for AD because the organic solids are large, polymeric molecules, e.g., lignocellulosic, that are not directly accessible to methanogens (Sayara and Sanchez, 2019; Atelge et al., 2020). Hydrolysis of these polymeric materials into small, soluble molecules or ions makes them readily accessible for methanogenesis and improves the rate and efficiency of conversion of the substrate into biogas by AD. Laboratory studies of enzyme-catalyzed hydrolysis and metabolism of the products of hydrolysis of switchgrass with Caldicellulosiruptor bescii (Kateava et al., 2013; Basen et al., 2014; Straub et al., 2019) have demonstrated a potential for substantially improving biogas yields and for increasing processing rates of lignocellulosic wastes by AD. C. bescii is a hyperthermophilic obligate anaerobe that grows at pH 7-8 and 70-80°C (Yang et al., 2010; Kataeva et al., 2013; Basen et al., 2014), and produces more than 50 different exozymes (Lochner et al., 2011), mostly hydrolyses. The wealth of exozymes produced by C. bescii suggests pre-digestion is capable of catalyzing the hydrolysis of many other polymers such as polysaccharides, esters, and amides found in waste streams. Although C. bescii may not be capable of metabolizing some of the molecules from hydrolysis of some wastes, methanogenesis may still occur. The temperature and processing time of C. bescii pre-digestion also provide a side benefit of pasteurizing influent materials, which makes bio-solid byproducts more valuable as soil amendments.
Fig. 1 The proposed commercial process occurs in three steps. First, the feedstock is mixed and heated in a hydrolysis tank to drive off $O_2$ and reach the requisite temperature and pH for the growth of C. bescii. Second, the feedstock is pre-digested in an anaerobic secretome bioreactor (ASB). Third, the predigested feedstock is anaerobically digested to produce biogas in a conventional anaerobic digestion vessel.

Figure 1 shows a schematic diagram of how C. bescii pre-digestion would be implemented in a commercial plant for producing biogas from an organic waste stream. In the first stage or tank, an organic waste containing polymeric organic materials is suspended in water in a mixing-hydrolysis tank at 75°C or higher where partial hydrolysis of the substrate occurs, $O_2$ is removed by decreased solubility and reaction with the organic material, and the suspension is pasteurized. Note that this hydrolysis tank could be a THP tank which may be advantageous for some wastes. Pre-digestion by C. bescii takes place in a second stage or tank (termed an anaerobic secretome bioreactor, ASB) at 75°C and pH 7-8. C. bescii is a free-living organism, and at least some of the exozymes are free in the solution (Lochner et al., 2011), so the ASB tank must be well mixed to optimize contact with the substrate.

The temperature in the ASB is high enough to provide relatively fast reactions and short retention times, but not so high as to require specific materials or designs for tanks, pumps, and fittings or to incure excessive heating costs. In the last phase, AD takes place in a third vessel that could be thermophilic or mesophilic. Although it was not examined in this study, thermophilic digestion may be advantageous since the energy cost of heating the influent has already been incurred during the pre-digestion phase. The hypothesis tested in this work is that pre-digestion of wastes with C. bescii will significantly increase the amount of volatile solid (VS) destroyed and therefore increase the yield of biogas and methane.

Figure 2 shows how the biogas composition from methanogenesis of the products of C. bescii pre-digestion of cellulose depends on the mix of substrates.

The solution containing acetate, lactate, and remaining sugars is then transferred to the anaerobic digester, where the reactions are as follows (Eqs. 5-7):

$$\text{CH}_3\text{COO}^- + H_2O \rightarrow \text{CH}_3(g) + \text{HCO}_3^-(aq)$$ Eq. 5

$$2\text{CH}_3\text{CH(OH)COO}^- + 2H_2O \rightarrow 2\text{CH}_3(g) + 2\text{HCO}_3^-(aq) + \text{CO}_2(g)$$ Eq. 6

$$2\text{C}(H_2O) \rightarrow \text{CH}_4(g) + \text{CO}_2(g)$$ Eq. 7

Because bicarbonate ion is not volatile, the reaction presented by Equation 5 produces 100% CH$_4$ gas, the reaction presented by Equation 6 produces biogas with 75% CH$_4$ and 25% CO$_2$, and the reaction shown by Equation 7 produces biogas with 50% CH$_4$ and 50% CO$_2$. Thus, because of the production of acetate and lactate from the metabolism of sugars, pre-digestion with C. bescii has the potential to increase methane concentration in the biogas.

Please cite this article as: Hansen J.C., Aanderud Z.T., Reid L.E., Bateman C., Hansen C.L., Rogers L.S., Hansen L.D. Enhancing waste degradation and biogas production by pre-digestion with a hyperthermophilic anaerobic bacterium. Biofuel Research Journal 31 (2021) 1433-1443. DOI: 10.18331/BRJ2021.8.3.3
The thermodynamics of the pre-digestion process is very favorable if there is a sufficient supply of base (alkalininity) in the pre-digestion vessel. The Gibbs energy change (∆G) for hydrolysis of cellulose to glucose at the optimum temperature for growth of *C. bescii*, 75°C, is negative and large (Popovic et al., 2019), but the uncatalyzed reaction is extremely slow under most conditions. The enzymes from *C. bescii* catalyze rapid hydrolysis of cellulose and hemicellulose to glucose and other sugars. The *C. bescii* cells metabolize the sugars to acetic and lactic acids, but ∆G for the reaction is approximately zero, so the bacteria do not gain any energy for growth and other activities until the reaction with base occurs. Neutralization of acetic and lactic acids by bicarbonate ion, hydroxide ion, and aqueous ammonia at 25°C. Note that these values are more negative at higher temperatures.

Table 1. ∆G° (kJ mol⁻¹) for the reaction of acetic and lactic acids with bicarbonate ion, hydroxide ion, and aqueous ammonia at 25°C. Note that these values are more negative at higher temperatures.

| Base       | HCO₃⁻ | OH⁻ | NH₃  |
|------------|-------|-----|------|
| Acetic acid| -17.5 | -52.8| -25.8 |  
| Lactic acid| -22   | -58  | -31  |

The three specific purposes of this work are: 1) to identify any potential toxicities associated with *C. bescii* pre-digestion and AD of pre-digested substrates from several different wastes, 2) to determine the potential enhancement of biogas production by the AD of pre-digested dairy manure and waste activated sludge (WAS), and 3) to identify variables that must be quantified for engineering commercial, continuous-flow systems for waste disposal and biogas production by *C. bescii* pre-digested followed by AD. Potential toxicities in pre-digestion and methanogenesis were tested by determining the enhancement of biogas production in a bench-scale batch process. Pre-digestion and AD of WAS was further characterized in a lab-scale semi-continuous flow system, and dairy manure was further characterized in a bench-scale batch system. The commercial feasibility of *C. bescii* pre-digestion of dairy manure followed by AD for methane production was further examined in a semi-continuous flow pilot plant. In this study, *C. bescii* pre-digestion was compared with the low temperature hydrolysis process (75°C) (LTHP) (Lu et al., 2008; Ferrer et al., 2008) that was used as a control.

2. Materials and Methods

2.1. Caldicellulosiruptor bescii cultivation

*C. bescii* was obtained as lyophilized inactivated culture from DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, accession #6725. A culture was first grown on cellobiose in media 516 according to the instructions from the DSMZ (https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium516.pdf) and used to inoculate the ASB vessels for pre-digestion, regardless of feedstock type.

2.2. Feedstock descriptions

Diverse feedstocks containing various amounts of cellulose and other polymers were evaluated in the initial part of the study. Giant king grass (GKG, African *Pennisetum purpureum* Schumach [Poaeeae]) was grown at the BYU Life Sciences Greenhouse from corms obtained from an experimental agricultural plot in California. GKG leaves were separated from stems, after which the material was chopped, shredded, and screened to 0.65 cm. Kentucky Bluegrass (*Poa pratensis* L. [Poaeeae]) clippings were obtained from a local resident’s lawn in Provo, UT. Mixed municipal green waste was obtained from the compost yard of the Springville, UT Wastewater Treatment Facility. The mixed green waste consisted of a chopped mixture of lawn clippings, leaves, branches, other plant materials, dirt, and pebbles. The raw material was sifted through a 0.65 cm screen and the fine material used in tests. Corn mash was obtained from a brewery in Colorado. Grass silage was obtained from Roeslein Alternative Energy. Dairy manure was obtained from the Bateman Dairy in Mosida, UT. Waste activated sludge (WAS) was collected from the DAF unit, and anaerobically digested waste activated sludge (DWAS) was collected from the digesters at the Orem, UT Wastewater Treatment Facility. The feedstock for the wastewater digesters was 50% primary sludge with 4-5% total solids (TS), and 50% WAS from the DAF containing 4% TS. The first digester was thermophilic at 55.6°C, and the second was mesophilic at 37.8°C with a total retention time of 21 d. All feedstocks were dried at 105°C and weighed to determine water content, and dried samples were analyzed for C, N, and P by the Brigham Young University Environmental Analytical Laboratory (https://pws.byu.edu/eal).

2.3. Experimental systems for pre-digestion and anaerobic digestion

Three systems were used in the experiments. One system was a flow-through lab-scale system, with two 1-L pre-digestion anaerobic secretome bioreactor (ASB) vessels connected in series and connected to a 10-L anaerobic digester. Another system was a bench-scale system with a 30-L pre-digestion (ASB) vessel and a 60-L digester that were not connected, so this system could only be used for batch operations. Both of these systems were twinned, with one inoculated with *C. bescii* and the other operated at the same conditions serving as a control. The third system was a pilot-scale, semi-continuous feed, 7500-L total volume system with a mixing tank, pre-digestion in an ASB, and two AD tanks. This system differed from the system detailed in Figure 1 because the mixing tank was not heated. Details of the construction of these systems are given in the Supplementary Data; only relevant operational information is provided here.

2.3.1. Lab-scale system and experiments with WAS and anaerobically digested WAS (DWAS)

The lab-scale system was comprised of twin systems with two 1-L ASB vessels connected in series for pre-digestion and a 10-L vessel for AD. All three tanks were connected with 2.5 cm iron tubing from the top of one tank to the bottom of the next. The 1-L vessels were stirred at 100 rpm with paint stirrers and maintained at 75°C. The AD vessel was maintained at 37°C. Biogas was measured with Whisper series integrating flow meters calibrated with NIST traceable Mesa Labs FlexCal series flow meters. WAS and DWAS were evaluated in the lab-scale system. Waste was fed into the system twice a day with 200 mL portions from a stock that was kept in a refrigerator at 4°C to prevent reaction outside the system. The actual volumes used in the ASB tanks were 800 mL each and 5 L in the AD tank. Retention time in the ASB was 4 d and retention time in the AD was 12 d. *C. bescii* was acclimated for at least two days after the inoculation before data collection began. Pre-digestion of WAS with *C. bescii* noticeably reduced the viscosity but not sufficiently to allow complete transfer of solids so only total suspended solids (TSS), volatile suspended solids (VSS), and COD were also determined in the input and AD effluent.

2.3.2. Bench-scale system and experiments with dairy manure, WAS, and various other wastes

The bench-scale system was comprised of twin systems with 30-L vessels for pre-digestion and 60-L vessels for AD. The pre-digestion vessels were stirred continuously at 40 rpm by paddle stirrers that extended to the bottom of the vessels. The AD vessels were maintained at 37°C and were not stirred. Biogas was measured with Whisper series integrating flow meters calibrated with NIST traceable Mesa Labs FlexCal series flow meters. Because the ASB and AD vessels were not connected, experiments were performed as batches. Feedstocks were mixed with sufficient deionized water to make a 29-L suspension of 3-5% solids and poured into the top of the 30-L vessels, and after the temperature reached 75°C, one liter of *C. bescii* culture was added to one of the 30-L vessels and one liter of distilled water was added to the other 30-L vessel. The control system was operated at the same temperature as the *C. bescii* vessel. The pH in the vessel with *C. bescii* was measured with a Cole-Palmer double junction electrode and pH meter calibrated with standard pH 4 and 7 buffers. The
Table 2. Properties of feedstocks and ratios of methane content, total methane, and total biogas from the digester along with the pre-digested material to maintain the methanogen culture in the digesters. Gas production was monitored until the rate dropped to <200 mL/h, at which point the digestion was considered complete.

The second set of experiments in the bench-scale system evaluated dairy manure in more detail with a 2-d retention time in the ASB. TS, VS, and COD were determined in the suspensions before loading into the 30-L vessels. In these experiments, dairy manure from pre-digestion was not screened, and a 5-L representative sample from the 30-L vessels was pumped into the digesters. Because the AD tanks were not stirred, TS, VS, and COD values for digester effluent could not be obtained.

2.3.3. Pilot plant and experiments with dairy manure

Figure S1 in the Supplementary Data is a schematic diagram of the pilot plant. The system has two 938-L storage tanks, an 1875-L ASB pre-digestion vessel, and two 1875-L AD vessels operated in parallel. One AD vessel is a continuously stirred tank reactor (CSTR), and the other is an induced bed reactor (IBR). The ASB was operated at 75°C. The IBR was operated at 37°C, and the CSTR was operated at 41°C. The pH in the ASB was stable at 7.2-7.8. To inoculate the ASB tank, one liter of C. bescii was first grown on cellulbiose, transferred to 30 liters of 4.5% manure at 75°C, and twenty-five liters was used to inoculate the ASB.

The feedstock for the pilot plant was obtained from the Bateman Dairy in Mosida, UT. Fresh manure was obtained after passage over a slope screen to remove sand and excess water, hauled to the pilot plant in storage tanks, and used within 72 h. The manure suspension in the feed tank was continually mixed using a paddle-type mixer rotating at 60 rpm. Biogas was collected in Tedlar (Sigma-Aldrich, St. Louis, MO) or Mylar (SKC Quality) gas sampling bags for analysis for CH₄ and CO₂ with Edinburgh Sensors gas analyzers (Livingston, United Kingdom) calibrated with Airgas (Radnor, PA) standard mixtures of CH₄ and CO₂.

2.5. Stoichiometric relations between biogas yield or methane yield and VS destruction

The following stoichiometric relations relate the fraction of carbon in VS to the amount of biogas and methane that can be obtained from the conversion of VS to biogas.

\[
\text{biogas/kg VS destroyed} = 0.030 \frac{\% \text{C in VS}}{\text{VS}} - 0.79
\]

As a reference point, the calculated biogas yield from cellulose is 0.747 m³ biogas/kg VS destroyed, and the biogas has 50% methane, so the estimated amount of methane produced is 0.272 m³ CH₄/kg VS destroyed. These relations were used to estimate VS destruction, biogas yield, and methane yield when VS destruction or biogas and methane yield could not be measured.

3. Results and Discussion

3.1. Results of preliminary experiments on various wastes

The properties of the feedstocks, increases in biogas methane content, methane produced, and biogas produced by C. bescii during pre-digestion relative to the controls for the preliminary experiments done in the bench-scale system are provided in Table 2. The feedstocks tested produced on average 87% more biogas and 95% more methane than the controls, with a concomitant increase in VS destruction. Feedstocks with higher...
cellulose/lignin ratios (i.e., GKG leaves, grass silage, and corn mash) produced more biogas from pre-digested materials compared with controls than feedstocks with lower cellulose/lignin ratios (i.e., dairy manure and GKG stems). The methane content (%) in the biogas also increased for all the tested wastes except green waste 1. No toxicities to either C. bescii or methanogens were apparent, as demonstrated by the increase in biogas production and observations of live C. bescii in the effluent after pre-digestion.

3.2. Results of experiments on WAS and DWAS

VSS from WAS was reduced by 52% in the ASB tank in the lab-scale system by solubilizing solids. Also, as a consequence of C. bescii pre-digestion, the viscosity of WAS was noticeably reduced, probably because of enzyme-catalyzed hydrolysis of the polyhydroxyalkanoates (biodegradable polyesters) synthesized by filamentous bacteria. Experiments on WAS diluted to 4% TS in the bench-scale system with a 2-d retention in the ASB and 17 d in the AD showed 85% conversion into biogas with 70% methane. The control, hydrolyzed at 75°C for 2 d, showed 49% conversion into biogas with 69% methane. These data show C. bescii could be used to double biogas production by pre-digesting the influent to a WAS digester.

Table 3 shows in the lab-scale system, AD of DWAS after C. bescii pre-digestion gave approximately 69% reduction in VSS and 81% more biogas than the control. Although the total amount is relatively small, i.e., 33 L biogas kg⁻¹ VSS, the data on DWAS in Table 3 show that even after lengthy thermophilic AD, there is still some material that can be hydrolyzed by C. bescii and made available for AD.

Table 3.
Properties of DWAS used in the lab-scale system, biogas produced, biogas composition, and waste destruction.

| Analysis | C. bescii DWAS | Control DWAS |
|----------|----------------|--------------|
| %C in TS | 32.8           | 32.8         |
| %N in TS | 4.53           | 4.53         |
| %P in TS | 3.92           | 3.92         |
| TSS, g L⁻¹ | 12.9           | 12.9         |
| VSS, g L⁻¹ | 8.26           | 8.26         |
| COD, g O₂ L⁻¹ | 14.5           | 14.5         |
| Biogas, L kg⁻¹ VSS | 32.9           | 13.3         |
| CHO/CO₂ in biogas | 2.65 (73% CH₄) | 2.48 (71% CH₄) |
| %VSS destruction | 8.80           | 3.56         |

Table 4.
Properties of dairy manure used in the bench-scale system, biogas produced, biogas composition, and waste destruction.

| Analysis | C. bescii COW | Control COW |
|----------|---------------|-------------|
| %C in TS | 36.3          | 36.2        |
| %N in TS | 3.03          | 3.02        |
| %P in TS | 0.78          | 0.78        |
| TSS, g L⁻¹ | 40.7±10.7⁻¹  | 37.7±4.7⁻¹  |
| VS, g L⁻¹ | 25.3±6.3⁻¹ (58% C) | 23.0±2.7⁻¹ (59% C) |
| COD, g O₂ L⁻¹ | 52.2           | 44.7        |
| Biogas, L kg⁻¹ VS | 476            | 265         |
| Methane, L kg⁻¹ VS | 362            | 182         |
| CHO/CO₂ in biogas | 3.18 (76% CH₄) | 2.22 (69% CH₄) |
| %VS destruction | 44¹           | 24¹         |

¹ Note: The values represent the difficulty of obtaining a representative sample of the suspension, not the uncertainty in the measurement method.

3.3. Results of experiments on dairy manure

Table 4 provides the properties of dairy manure, as well as biogas produced, biogas composition, and waste destruction in the more detailed experiments on dairy manure run in the bench-scale system. Figure 3 shows biogas production, the rate of biogas production, and the methane content of biogas from dairy manure as functions of time. C. bescii pre-digestion of dairy manure nearly doubled the amount of biogas per kg VS, and on average, methane content of

Fig. 3. (a) Representative plot of biogas production as a function of time from dairy manure in the bench-scale, batch system. Black circle and line are from C. bescii pre-treated substrate, red circle and line are from the control. The grey vertical line is 4-d retention time, and the blue vertical line is 8-d retention time; (b) Rates of biogas production in a; (c) Methane content of biogas as a function of time from four runs in the bench-scale, batch system. Black circles are C. bescii pre-treated substrate, and red triangles are from the control.
the biogas was about 10% greater than in the control hydrolyzed at 75°C for the same time. The higher methane content in biogas from pre-digested dairy manure was primarily caused by very high methane, 85%, at the beginning of digestion (Fig. 3c), which could only be produced from a significant concentration of acetate in the AD feedstock (see Fig. 2). Also, note that the highest rate of biogas production (Fig. 3b), occurs at the same time as the high methane content indicating digestion of acetate by acetoclastic methanogens.

An initial test run in the pilot plant with C. bescii pre-digestion of dairy manure demonstrated a major decrease in TSS (Fig. 4) and an 89% reduction in COD from the feedstock to the AD effluents. The C/N ratio of dairy manure in the ASB feedstock was approximately 12. Total N and total P were 1160 and 300 mg L\(^{-1}\) in the feedstock and in the effluents, respectively. After the initial test runs, the four sets of data shown in Table 5 were collected during continuous, steady-state operation for 2-3 weeks each. Table 5 gives the average results for runs with C. bescii pre-digestion, control runs, and literature data for mesophilic digestion with no pretreatment in a very similar AD system (Rico et al., 2011). C. bescii pre-digestion increased VS reduction compared with 75°C hydrolysis control runs by 19% with a 2-d ASB retention time and by 55% with a 4-d retention time. C. bescii pre-digestion gave a negligible change in COD with a 2-d ASB retention time and a 15% decrease in COD with a 4-d retention time. Although it was not possible to do the experiment in the pilot plant, Figure 3 indicates increasing AD retention time to 15 to 20 d would significantly increase VS reduction to >75% with a concomitant increase in biogas. Figure 3 shows C. bescii pre-digested manure continued to produce biogas at a significant rate after 14 d in the bench-scale system.

A decrease in alkalinity in the ASB is a relative measure of the metabolic activity of C. bescii (see Eqs. 2-4). In the runs with C. bescii pre-digestion, alkalinity in the ASB averaged 20±5 mM, and alkalinity in the digesters averaged 40±5 mM. The alkalinity in the ASB is 20-30 mM lower than that in the digesters because C. bescii metabolism produced acetic and lactic acids that were neutralized to anions by bases in the feedstock. AD of acid anions produces an equivalent amount of bicarbonate, HCO\(_3^-\), in the digesters, thus accounting for the increased alkalinity (Kateava et al., 2013).

**Figure 5** provides the VS data from a run where the heaters on the ASB were turned down to separate the effects of enzyme-catalyzed and base-catalyzed hydrolysis and to determine the temperature dependence of the two processes. The initial temperature was 73°C, and over the next 23 d, the ASB cooled to 59°C. Cooling was primarily due to the injection of unheated feedstock, so the temperature decrease was approximately linear. Retention time in the ASB was 2 d and retention time in the digesters was 4 d. The ASB and digester effluents were sampled approximately daily. The initial VS reduction was near 75% when the ASB was operating at 73-69°C, just below the optimum temperature for C. bescii growth and enzyme activity of C. bescii (see Fig. 5).

### Table 5

Average values of TS, VS, %VS reduction, % COD reduction, biogas production, and biogas composition in pilot plant runs with dairy manure with C. bescii pre-digestion and control runs without C. bescii.

| Feedstock TS, g L\(^{-1}\) | Feedstock VS, g L\(^{-1}\) | % VS reduction | % COD reduction | Biogas, L kg\(^{-1}\) VS\(^b\) | Biogas, % CH\(_4^b\) |
|-----------------------------|-----------------------------|----------------|----------------|---------------------------|----------------|
| **C. bescii**               |                             |                |                |                           |                |
| Control                     | 40.8                        | 25.1           | 64±4           | 57±13                     | 584            | 78          |
| Control                     | 42.7                        | 26.1           | 54±5           | 60±3                      | 493            | 75          |
| C. bescii                   | 33.8                        | 20.7           | 54±5           | 58±4                      | 493            | 76          |
| Control                     | 35.8                        | 21.5           | 51±5           | 55±5                      | 283            | 74          |
| Rico et al. (2011)\(^a\)    | 57.6                        | 45.0           | 36             | 38                        | 289            | 69          |
| Rico et al. (2011)\(^b\)    | 57.6                        | 45.0           | 43             | 46                        | 304            | 69          |

\(^a\) VS and COD reduction in the IBR are higher than in the CSTR because the IBR is not stirred and retains solids for a longer period of digestion.

\(^b\) Because of incomplete measurements, total biogas was calculated from the reduction in VS in the CSTR with Equation 8. The VS was 48.9% carbon.

\(^c\) Run 15 d with 2-d retention in the ASB and 4 d in AD.

\(^d\) Run 23 d with 2-d retention at 75°C and 4 d in AD.

\(^e\) Run 20 d with 4-d retention in the ASB and 8 d in AD.

\(^f\) Run 14 d with 4-d retention at 75°C and 8 d in AD.

\(^g\) Experiment done with no pretreatment and 10-d retention in a 1500-L digester very similar to the CSTR used in this study.

\(^h\) Experiment done with no pretreatment and 20-d retention in a 1500-L digester very similar to the CSTR used in this study.
activity. The approximate activation energies found from an Arrhenius plot (Fig. S2 in Supplementary Data) of the data in Figure 5, assuming %VS destruction is proportional to the rate of hydrolysis reactions in the e ASB, are 24 kJ mol⁻¹ for the exozyme catalyzed reaction and 87 kJ mol⁻¹ for the base-catalyzed reaction.

3.4. Summary of results

Pre-digestion with C. bescii increased biogas production from all the feedstocks tested. All of the feedstocks produced more biogas after C. bescii pre-digestion than the 75°C hydrolyzed controls, apparently because exozymes increased hydrolysis of polymeric materials to produce soluble, small molecules that were readily digested in AD. The data on dairy manure suggests an optimized commercial system using C. bescii pre-digestion would produce ~600 L of biogas per kg VS with 75% methane and would destroy 75% of VS. As reference points, LTHP is estimated to increase VS reduction in dairy manure to 50-60% giving ≈450 L biogas per kg VS, and mesophilic AD of cow manure without pretreatment typically achieves 30-40% waste destruction and ≈300 L of biogas per kg VS with 60% methane (Rico et al., 2011; Nasir et al., 2012; Ateție et al., 2020). Mesophilic digestion of WAS without pretreatment achieves a similar level of waste destruction, biogas production, and biogas composition (Xu et al., 2020).

The increase in biogas methane content observed in nearly all experiments was most likely a consequence of the metabolism of some of the hydrolysis products to acetate and lactate by C. bescii. This increase in methane content can only be accounted for by digestion of acetate and/or lactate produced by C. bescii metabolism or by digestion of organic material with a low oxidation state, e.g., fats and oils. These results are in agreement with the results presented in Straub et al. (2019).

Pre-digestion of dairy manure with C. bescii resulted in increased VS destruction, increased biogas production, and a higher ratio of CH₄/CO₂ in the biogas. Optimizing the ASB retention time requires maintaining C. bescii in an actively growing state to maximize metabolism and enzyme activities (Lochner et al., 2011; Straub et al., 2019). But because the pilot plant was built with available parts and very little funding, equipment limitations prevented optimizing retention times in the ASB and AD for either the C. bescii or control runs. Also, ASB and AD retention times could not be changed independently. The results in Table 5 show that decreasing retention time in the ASB from 4 d to 2 d did not significantly change VS reduction even though retention time in AD was decreased from 8 d to 4 d. Experiments in the batch process used in the bench-scale system showed 15-20 d AD retention time would be required to maximize biogas production. With four days in the ASB, C. bescii most likely reached a near stationary state that limits growth, metabolism, and exozyme production. With two days in the ASB, washout of C. bescii cells and exozymes is likely. Therefore, we conclude that the optimum time for pre-digestion of dairy manure is between 2 and 4 d.

3.5. Optimizing pre-digestion

Our work highlights the need to optimize a C. bescii pre-digestion/AD system to achieve maximum benefits. Optimization requires quantifying the kinetics of the three processes that occur in the ASB tank as well as the rate of methanogenesis in the AD tank. Because an optimized continuous flow system should operate at a steady state near the inflection point in the growth curve, the growth rate of C. bescii can be described with a simple first-order equation (Eq. 10):

\[
d[n]/dt = k[n] = k_b[n] \text{ where } [n] \text{ is the concentration of } C. \text{bescii} \tag{10}
\]

Operating at this condition maximizes the rate of growth and exozyme production (Straub et al., 2019). The rate of enzyme-catalyzed hydrolysis of the substrate can be described with the Michaelis-Menten/Briggs-Haldane equation (Eq. 11):

\[
d[\text{products}]/dt = k_{\text{cat}}[\text{exozymes}][\text{accessible sites}] \tag{11}
\]

\[
(k_M + [\text{accessible sites}])
\]

Base-catalyzed hydrolysis occurs both in the hydrolysate tank (Fig. 1) and in the pre-digestion tank in parallel with enzyme-catalyzed hydrolysis. The rate of base-catalyzed hydrolysis is described by a second-order rate law as shown in Equation 12:

\[
d[\text{products}]/dt = k_b[\text{base}][\text{accessible sites}] \tag{12}
\]

In Equations 10, 11, and 12, brackets indicate concentrations, and t is time. In Equation 10, [n] is the number concentration of C. bescii, and \( k \) is the rate constant for growth. In Equations 11 and 12, [products] is the sum of the concentrations of hydrolysis products, \( k_{\text{cat}} \) is the rate constant for enzyme-catalyzed hydrolysis, [exozymes] is the concentration of active hydrolyases, [accessible sites] is the concentration of sites on the polymers that are accessible by the hydrolyases, and \( K_M \) is the Michaelis-Menten constant. The rate constant for base-catalyzed hydrolysis is \( k_b \) and [base] is the concentration of bases in the solution. Accessible sites are the places on the polymeric materials where exozymes and bases catalyze the addition of water to split off small molecules. Equation 11 indicates the rate of hydrolysis increases with the increasing concentration of exozymes, which is directly proportional to the growth rate of C. bescii (Lochner et al., 2011). Hydrolysis rates also increase with the accessibility of exozymes and bases to sites on the hydrolyzable material, i.e., the smaller the particles, the faster the reaction. The exozymes are only produced when C. bescii is actively growing, so to optimize the pre-digestion process, the culture must be continuously fed and diluted to prevent the culture from reaching a stationary state (Lochner et al., 2011; Straub et al., 2019). Metabolism of the products of hydrolysis to acetate and lactate also obviously depends on maintaining an actively growing C. bescii culture.

The exozyme-catalyzed hydrolysis of polymeric wastes creates small, soluble molecules and organic anions that are readily digested by methanogens in anaerobic digesters. Because AD of acetate and lactate from C. bescii metabolism produces nonvolatile bicarbonate as well as CO₂ and CH₄, digestion of pre-digested material produces biogas with reduced CO₂ content. Because the relative concentrations of sugar, lactate, acetate, and other digestible material in the effluent from pre-digestion depends on the relative rates of exozyme- and base-catalyzed hydrolysis and C. bescii metabolism, the methane content of biogas and the amount of biogas produced depend on the pre-digestion time and concentration of feedstock. Figures 3b and c indicate the higher rate of biogas production and methane content observed with pre-digested feedstock is likely because the small molecules and anions in pre-digested material favor free-living acetoclastic methanogens over biofilm-forming oxidative methanogens.

The 20 mM decrease in alkalinity and the difference in VS destruction between operating the ASB at 69-73°C and 67-65°C both suggest base-catalyzed hydrolysis at 75°C accounted for 80-90% of the hydrolysis products, and C. bescii metabolism accounted for 10-20% of the hydrolysis products being digested in the pilot plant with a 2-d ASB retention. Table 6 summarizes the data on dairy manure from Tables 2, 3, and 5, comparing results from pre-digested and control experiments. The batch experiments done in the bench-scale system demonstrated a 37 and 82% increase in VS destruction in pre-digested manure compared with the LTHP control. Comparing the 4+8 d run with the 2+4 d run in the pilot plant shows C. bescii pre-digestion was more significant at a 4-d ASB retention than at a 2-d retention. However, there is very little difference in the VS destruction at the different ASB retention times even though the AD retention time was shorter. Therefore, VS destruction may be greater with the 2-d ASB retention. The COD ratios in Table 6 show the same trends as the ratios of VS although the COD values are less accurate. The ratio of enzyme-catalyzed to base-catalyzed products being digested in the batch experiments is not subject to the washout expected at short AD retention times in the pilot plant. Washout of compounds that are more slowly digested by AD is likely the cause of the trend seen in Table 6. Unfortunately, this complication makes it impossible to identify an optimum AD retention time, although it is clear that longer retention times in AD are necessary.

Equations 1, 2, 10, 11, and 12 identify four variables that must be considered in designing the system in Figure 1 for a particular feedstock: 1) concentration of volatile solids (VS) in the feedstock, 2) retention time in the hydrolysate tank, 3) retention time in the ASB, and 4) retention time in AD. Optimizing engineering design and operation for a given feedstock thus requires determination of the growth rate of C. bescii, exozyme activity, and the base-catalyzed hydrolysis rate as a function of feedstock.
concentration. Since it provides the food for *C. bescii* growth, the rate of hydrolysis has to be faster than the growth rate, but hydrolysis may continue after *C. bescii* has reached a stationary state. Equation 11 demonstrates that exozyrne-catalyzed hydrolysis likely requires longer ASB retention times with increasing substrate concentration. However, the exozymes may become saturated, a condition set by the upper limit of the rate of exozyme production and VS concentration. For base-catalyzed hydrolysis, the rate depends on the concentrations and identity of the bases present in the system, e.g., HCO₃⁻ and VS concentration. For base-catalyzed hydrolysis, the rate depends on the saturated, a condition set by the upper limit of the rate of exozyme production after a point in the growth curve where growth rate and enzyme production rate are in Lochner et al. (2011) for *C. bescii* growing on Avicell, a crystalline cellulose, in standard media. Assuming the optimum conditions are near the inflection point, the growth curve where growth rate and enzyme production rate are maximal, growth rate, \( \frac{dV}{dt} = 5 \times 10^{3} \text{ cells mL}^{-1} \text{ h}^{-1} \) and the rate of exozyme production = \( \frac{d[\text{exozymes}]}{dt} = 6 \mu \text{g mL}^{-1} \text{ h}^{-1} \). The optimum concentration of *C. bescii* is \( \approx 5 \times 10^{3} \text{ cells mL}^{-1} \) and the optimum concentration of exozymes is \( \approx 100 \mu \text{g mL}^{-1} \). The retention time is given by Equation 13:

\[
\text{Retention time} = \frac{\text{tank volume}}{\text{influent rate}} = \frac{V}{\frac{dV}{dt}} \tag{13}
\]

The optimum retention time from the growth rate, \( \frac{V}{(\frac{dV}{dt})} = 5 \times 10^{3} \text{ cells mL}^{-1} / 5 \times 10^{3} \text{ cells mL}^{-1} \text{ h}^{-1} = 10 \text{ h} \) and from the exozyme production rate, \( V/\frac{dV}{dt} = 100 \mu \text{g mL}^{-1} / 6 \mu \text{g mL}^{-1} \text{ h}^{-1} = 17 \text{ h} \). So, the optimum retention time for growth and production of simple sugars from Avicell is between 10 and 17 h at the concentration of Avicell used in Lochner et al. (2011), Determining the dependence of optimum retention time on Avicell concentration would require data obtained at different Avicell concentrations.

The effects of substrate concentration on the rate of conversion of polymeric VS into small soluble molecules is described by the Michaelis-Menten/Briggs-Haldane equation (Equation 14). At the optimum exozyme concentration from Lochner et al. (2011), and assuming the concentration of accessible sites is directly proportional to the VS concentration:

\[
d[\text{products}]/dt = \frac{k_4[100 \mu \text{g mL}^{-1}][VS]/(K_M + [VS])}{100 \mu \text{g mL}^{-1} / [VS]} \tag{14}
\]

**Equation 14** shows the rate increases linearly with enzyme concentration, but because [VS] appears in both the numerator and denominator, the rate increases nonlinearly with increasing VS concentration. The rate reaches a maximum that depends on the value of \( K_M \) relative to the VS concentration. The effect of increasing solids content of the feedstock was not evaluated in this study, but 8-10% solids will be required to take full economic advantage of *C. bescii* pre-digestion.

### 4. Conclusions and future directions

The focus of this study was to gain an understanding of the commercial potential of the *C. bescii* pre-digestion process. However, our study shows that taking advantage of thermophilic pre-digestion at a commercial scale requires further work to quantify the kinetics of the pre-digestion process. Characterizing the non-linear effects of feedstock concentration will be necessary to realize the full economic potential. The extent of base-catalyzed hydrolysis at the relatively low temperature used in this study was surprisingly high and needed further characterization. The products of *C. bescii* pre-digestion are very different than feedstocks typically used in AD, including those from other pretreatments, and as a consequence, further work is required to optimize the AD process.

Although more work is required to optimize engineering design and operation, this work demonstrates that pre-digestion with *C. bescii* has the potential to significantly increase biogas production from organic wastes at minimal additional operating cost. For example, an optimized system with *C. bescii* pre-digestion is expected to convert 75 to 85% of the VS in dairy manure and WAS into biogas with 75% methane. *C. bescii* pre-digestion thus has the potential to greatly increase the application of AD for the production of renewable energy.

**References**

[1] Anukam, A., Berghel, J., 2020. Biomass pretreatment and characterization: a review. Biomass.

[2] Atelge, M.R., Atabania, A.E., Banu, J.R., Krisae, D., Kayaf, M., Eskicioglu, C., Kumarg, G., Lee, C., Yildizi, Y.Ş., Unalana, S., Mohanasundaram, R., Dumank, F., 2020. A critical review of pretreatment technologies to enhance anaerobic digestion and energy recovery. Fuel, 270, 117494.

---

**Table 6.**

Results of VS destruction in dairy manure expressed as the ratio of percent destruction in pre-digested to control.

| System | Ratio \( \frac{\text{AVS}_{\text{pre}}}{\text{AVS}_{\text{cont}}} \) IBR | Ratio \( \frac{\text{AVS}_{\text{pre}}}{\text{AVS}_{\text{cont}}} \) CSTR | Ratio \( \frac{\text{ACOD}_{\text{pre}}}{\text{ACOD}_{\text{cont}}} \) IBR | Ratio \( \frac{\text{ACOD}_{\text{pre}}}{\text{ACOD}_{\text{cont}}} \) CSTR |
|--------|----------------|----------------|----------------|----------------|
| 30/60 L batch | 1.37 | NA<sup>a</sup> | NA<sup>a</sup> | NA<sup>a</sup> |
| 30/60 L batch | 44/24 = 1.83 | NA<sup>a</sup> | 52.2/44.7 = 1.17 | NA<sup>a</sup> |
| Pilot 4+8 d | 69/51 = 1.35 | 54/31 = 1.74 | 66/52 = 1.27 | 58/35 = 1.66 |
| Pilot 2+4 d | 75/63 = 1.19 | 64/54 = 1.19 | 69/70 = 0.99 | 57/60 = 0.95 |

<sup>a</sup> Not available
methods for enhanced production of biogas and for the degradation of
Hansen et al. / Biofuel Research Journal 31 (2021) 1433-1443

obsidiansis

Pre-treatment methods for lignocellulosic biofuels production: current advances, challenges and future prospects. Bioresour. Technol. 199, 386-397.

Ferre, I., Ponsáb, S., Vázquez, F., Font, X., 2008. Increasing biogas production by thermal (70 °C) sludge pretreatment prior to thermophilic anaerobic digestion. Biochem. Eng. J. 42(2), 186-192.

Kataeva, I., Foston, M.B., Yang, S.J., Pattathil, S., Biswal, A.K., Poole II, F.L., Basen, M., Rhaesa, A.M., Thomas, T.P., Azadi, P., Olman, V., Saffold, T.D., Mohler, K.E., Lewis, D.L., Doepke, C., Zeng, Y., Tschaplinski, T.J., York, W.S., Davis, M., Mohnen, D., Xu, Y., Ragauskas, A.J., Ding, S.Y., Kelly, R.M., Hahn M.G., Adams, M.W.W., 2013. Carbohydrate and lignin are simultaneously solubilized from unpretreated switchgrass by microbial action at high temperature. Energy Environ. Sci. 6(7), 2186-2195.

Kumar, A.K., Sharma, S., 2017. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresour. Bioprocess. 4(1), 7.

Lee, J.-S., Saddler, J., Binod, P., 2016. Pretreatment of biomass. Bioresour. Technol. 199, 1.

Liao Z., P.K., Peot, C., Lancaster R., Mills N., Kleiven M., 2014. Thermal hydrolysis pretreatment for advanced anaerobic digestion for sludge treatment and disposal in large scale projects. In: DSD International Conference, Hong Kong.

Liu, X., Wang, Q., Tang, Y., Pavlović, S.G., 2021. Hydrothermal pretreatment of sewage sludge for enhanced anaerobic digestion resource: transformation and energy balance. Chem. Eng. J. 410, 127430.

Lochner, A., Giannone, R.J., Rodriguez Jr, M., Shah, M.B., Mielzen, J.R., Kellar, M., Antranikian, G., Graham, D.E., Hettich, R.L., 2011. Use of label-free quantitative proteomics to distinguish the secreted cellulolytic systems of Caldicellulosiruptor bescii and Caldicellulosiruptor obsidiansis. Appl. Environ. Microbiol. 77(12), 4042-4054.

Liu, J., Gaval, H.N., Skiaďas, I.V., Mladenovska, Z., Ahrling, B.K., 2008. Improving anaerobic sewage sludge digestion by implementation of a hyper-thermophilic prehydrolysis step. J. Environ. Manage. 88(4), 881-889.

Profi. Jaron C. Hansen is a Professor of Chemistry and Biochemistry at Brigham Young University and co-founder of Verde. He earned his PhD in chemistry from Purdue University (2002) and did his postdoctoral work at the California Institute of Technology/Jet Propulsion Laboratory (2002-2005). His research involves improving the understanding of atmospheric and environmental chemical processes through focused laboratory, field, and computational studies as well as the development of improved anaerobic digestion methods for enhanced production of biogas and for the degradation of hazardous pollutants.

Prof. Lee D. Hansen is Prof. Emeritus in the Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT. During his career, he has published over 300 papers in peer-reviewed journals on a wide range of topics. His specialties are calorimetry, thermodynamics, and the kinetics and energetics of biological systems.

Please cite this article as: Hansen J.C., Aanderud Z.T., Reid L.E., Bateman C., Hansen C.L., Rogers L.S., Hansen L.D. Enhancing waste degradation and biogas production by pre-digestion with a hyperthermophilic anaerobic bacterium. Biofuel Research Journal 31 (2021) 1433-1443. DOI: 10.18331/BRJ2021.8.3.3
Prof. Zachary T. Aanderud is an Associate Professor of Microbial Ecology and Biogeochemistry at Brigham Young University. He earned his PhD in Biogeochemistry from the University of California, Davis (2006) and did his postdoctoral work at Michigan State University. His research links temporal fluctuations in resources to microorganism responses and ecosystem services. This overarching theme has inspired questions relating to the impacts of human-induced disturbances on aquatic and terrestrial bacterial dormancy, dominance, and species interactions; and, more recently, the use of hyperthermophilic bacteria to digest waste streams prior to methane production.

Mr. L. Scott Rogers, P.E., has over 39 years’ experience as a civil engineer, primarily in water and wastewater treatment. He earned his Master of Engineering (ME) in Civil Engineering from Brigham Young University in 1983. President and Founder of AQUA Engineering, Mr. Rogers’ expertise involves Renewable Energy, Resource Recovery, Water & Wastewater Treatment, Industrial Water Treatment, Water Reuse, Biosolids Reuse & Disposal, Instrumentation, Pump Stations, Process Control, and Performance-based Contracting. As a Professional Engineer, Mr. Roger’s passion is in water and wastewater treatment, renewable energy projects, anaerobic digestion, RNG projects, dairy and swine farm manure processing, fertilizer facilities, and nutrient recovery.

Carson J. Bateman is a research assistant in Professor Jaron Hansen’s lab at Brigham Young University (BYU) in Provo, Utah. Carson started researching with Professor Hansen in collaboration with Professor Zach Aanderud in September of 2018. Carson works as project manager heading up the full-scale testing facility at the BYU farm in Spanish Fork, Utah. Carson is majoring in Chemistry and minoring in Global Women’s Studies.

Lindsey E. Reid is a research assistant in Professor Jaron Hansen’s lab at Brigham Young University (BYU) in Provo, Utah. Lindsey started researching with Professor Hansen in collaboration with Professor Zach Aanderud in August of 2019. Lindsey works as a lead sample analyst in their biofuel research. In addition to studying biofuels, Lindsey is actively researching in other environmental fields. Since April of 2021, she has worked as a research assistant in Plant Pathology with Professor Bradley Geary at BYU where she studies secondary metabolites and biopesticides. Lindsey is majoring in Environmental Science and minoring in Chemistry. Her research interests include biofuel, thermophilic anaerobic cellulolytic bacteria, secondary metabolites, and biopesticides.

Prof. Conly L. Hansen’s research, commercialization, and philanthropic efforts have set a firm foundation to develop a cluster of research and business in Utah centered on effective management of agricultural waste. Dr. Hansen was a registered professional engineer in Ohio and Utah, and his research works are widely recognized for quality and innovation. His publications include 3 books, 6 book chapters, and 60 refereed journal articles. He holds 10 patents, with two pending. He has consulted on teaching and professional projects in 12 countries, and has chaired the Food and Process Engineering Institute. He directed the Center for Profitable Uses of Agricultural Byproducts that was established to help agriculturists be financially competitive while meeting increasingly stringent environmental regulations. Dr. Hansen is the inventor of the Induced Blanket Reactor (IBR) that allows for anaerobic digestion of agricultural wastes into biogas.

Please cite this article as: Hansen J.C., Aanderud Z.T., Reid L.E., Bateman C., Hansen C.L., Rogers L.S., Hansen L.D. Enhancing waste degradation and biogas production by pre-digestion with a hyperthermophilic anaerobic bacterium. Biofuel Research Journal 31 (2021) 1433-1443. DOI: 10.18331/BRJ2021.8.3.3