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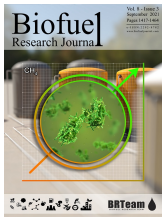

Original Research Paper

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

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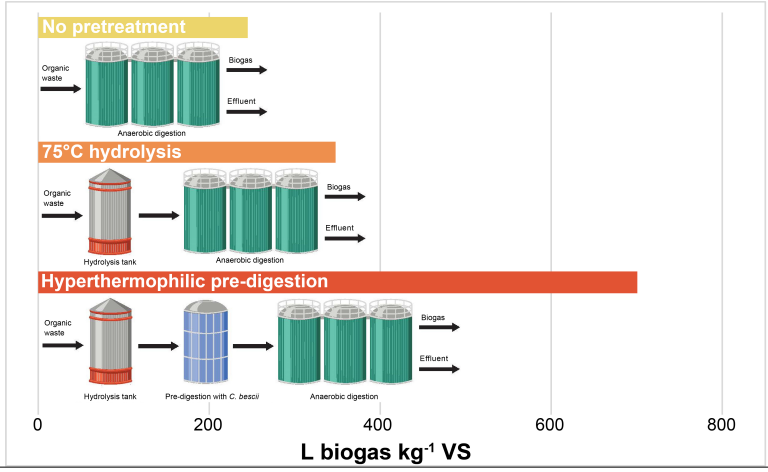
HIGHLIGHTS

- 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



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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.

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Lab-scale system

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. The ASB tanks were constructed with 10 cm iron pipe, and the AD tank was constructed from 25 cm iron pipe. 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 ASB vessels were stirred at 100 rpm with paint stirrers and maintained at 75°C with silicone rubber heaters and electronic controllers (Omegaette, CN4116-R1-R2 Temperature Process Controller). The AD vessel was maintained at 37°C with a silicone rubber heater and an electronic controller (Omegaette, CN4116-R1-R2 Temperature Process Controller). Biogas produced in the digesters was measured with Whisper series integrating flow meters (Alicat Scientific, Tucson, AZ) calibrated with NIST traceable Mesa Labs (Butler, NJ) FlexCal series flow meters.

Bench-scale system

The bench-scale system was comprised of twin systems with 30-L vessels for pre-digestion and 60-L vessels for AD. The 30-L vessels were 25.4 cm diameter steel vessels maintained at 75°C by two 1400 W silicone rubber heaters and Love Controls 16B temperature controllers that maintained the temperature to ±2°C. The pre-digestion vessels were stirred continuously at 40 rpm by paddle stirrers that extended to the bottom of the vessels. AD to produce biogas was done in 25.4 cm diameter, 60 L, unstirred, steel vessels. The AD vessels were maintained at 37°C by Love Controls 16B temperature controllers (Dwyer Instruments. Michigan City, IN) and 1400 W silicone rubber heaters. Biogas produced in the digesters was measured with the Whisper series integrating flow meters (Alicat Scientific, Tucson, AZ). The flow meters were calibrated with NIST traceable Mesa Labs FlexCal series flow meters.

Pilot plant

Figure S1 shows a schematic diagram of the pilot plant used in this work. The pilot plant was constructed in an unheated building located on Powerhouse Rd., Spanish Fork, Utah (USA). The system has two 938-L storage tanks, an 1875-L anaerobic secretome bioreactor (ASB) pre-digestion vessel, and two 1875-L AD vessels operated in parallel. One AD vessel was a CSTR, and the other is an IBR. At start-up, manure was pumped into the ASB as well as both AD tanks, after which both AD tanks were inoculated with sludge from the AD tanks at the Springville, UT wastewater treatment facility. After 48 h, the AD tanks had reached 37°C, and the ASB had reached 75°C. The system reached a steady state after two weeks, and experimental runs began. The IBR continued to operate at 37°C, but because of power input from stirring, the CSTR equilibrated at 41±2°C. Because alkalinity was depleted during periods when the system was not actively fed, KOH (50% in water) was added to the ASB tank to adjust the pH as necessary before a run was started. When the system was being fed, the pH in the ASB was stable between 7.2-7.8, and no further pH control was necessary. To inoculate the ASB tank, a *C. bescii* culture was first grown on cellobiose according to the instructions received from DSMZ and then transferred to 30 L of the feedstock, 4.5% manure at 75°C, in one of the 30-L vessels in the bench-scale system for expansion for inoculation of the ASB tank. Twenty-five liters of *C. bescii* inoculum were used to inoculate the ASB in the pilot plant.

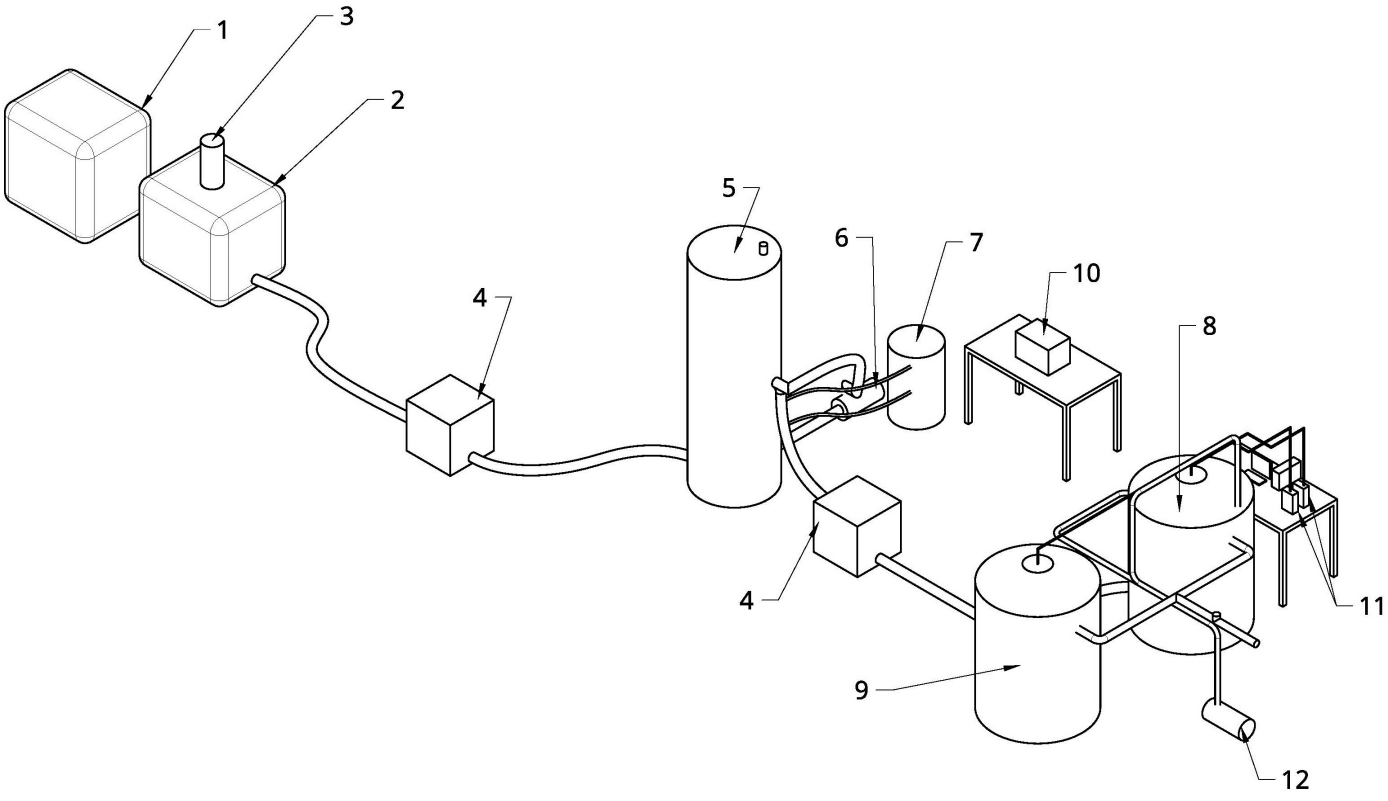

Fig. S1. Schematic diagram of the pilot plant used in our work. The pilot plant has a 1.1 m^3 storage tank (#1); a 1.1 m^3 stirred storage tank for feeding the system (#2); a stirrer for continuously mixing of the feed tank (60 rpm Sew-Eurodrive) (#3); a diaphragm pump for feeding the anaerobic secretome bioreactor (ASB) tank and for pumping from the ASB tank (#4); a 1.9 m^3 ASB (#5); a pump for continuous mixing of ASB (AMT, 0.8 m^3 min^{-1}) (#6); a water heater for maintaining the ASB at 75°±5°C (#7); a 1.8 m^3 continuously stirred digester (CSTR) (#8); a 1.8 m^3 induced bed reactor (IBR) for anaerobic digestion (#9); a digital control, measurement, and data recording system (#10); two gas flow meters (Alicat Whisper) (#11); and a pump for continuously mixing the CSTR (0.8 m^3 min^{-1}) (#12).

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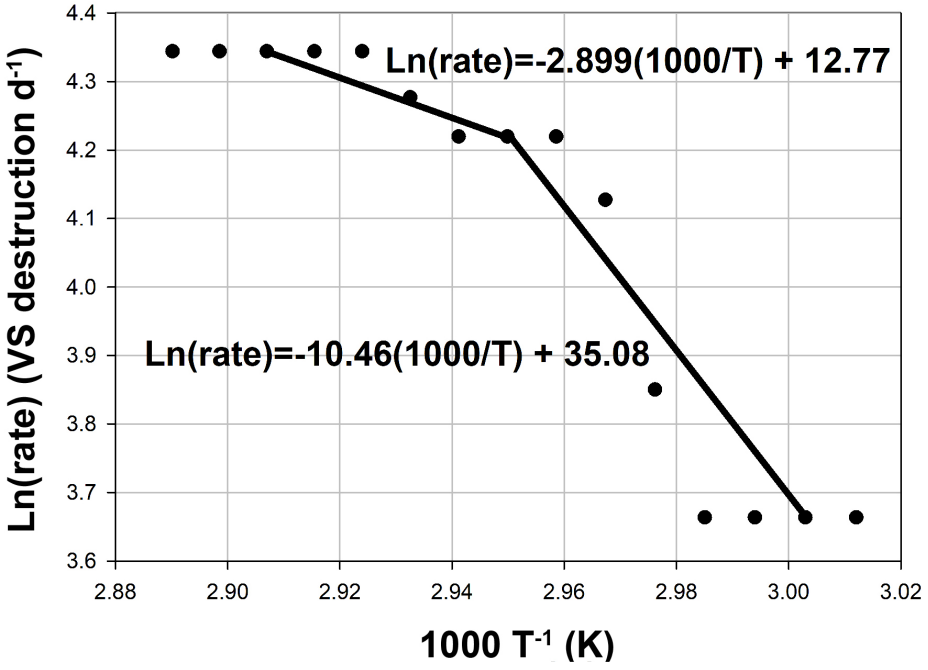

Fig. S2. An Arrhenius plot of the data in **Figure 5**. The rate of hydrolysis was assumed to be directly proportional to the %VS destruction.

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