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Anaerobic digestion in mesophilic and room temperature conditions: Digestion performance and soil-borne pathogen survival

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ABSTRACT

Tomato plant waste (TPW) was used as the feedstock of a batch anaerobic reactor to evaluate the effect of anaerobic digestion on *Ralstonia solanacearum* and *Phytophthora capsici* survival. Batch experiments were carried out for TS (total solid) concentrations of 2%, 4% and 6% respectively, at mesophilic (37 ± 1°C) and room (20–25°C) temperatures. Results showed that higher digestion performance was achieved under mesophilic digestion temperature and lower TS concentration conditions. The biogas production ranged from 71 to 416 L/kg VS (volatile solids). The inactivation of anaerobic digestion tended to increase as digestion performance improved. The maximum log copies reduction of *R. solanacearum* and *P. capsici* detected by quantitative PCR (polymerase chain reaction) were 3.80 and 4.08 respectively in reactors with 4% TS concentration at mesophilic temperatures. However, both in mesophilic and room temperature conditions, the lowest reduction of *R. solanacearum* was found in the reactors with 6% TS concentration, which possessed the highest VFA (volatile fatty acid) concentration. These findings indicated that simple accumulation of VFAs failed to restrain *R. solanacearum* effectively, although the VFAs were considered poisonous. *P. capsici* was nearly completely dead under all conditions. Based on the digestion performance and the pathogen survival rate, a model was established to evaluate the digestate biosafety.

Introduction

Large amounts of fruit and vegetable wastes (FVWs) are produced in China, and the amount has increased rapidly in recent years (Shen et al., 2013). Because of the high organic and water content, anaerobic digestion has been the most promising alternative method for treatment of FVW (Bouallagui et al., 2003). The main advantages of anaerobic digestion for FVW treatment are the production of renewable energy in the form of biogas, and the effluent or digestate, which can be used as soil conditioner or organic fertilizer (Viswanath et al., 1992; Holm-Nielsen et al., 2009). However, FVW derived from disease-infected or decomposed plants contain pathogens, which might remain after anaerobic digestion. This leads to high application risk of digestate toward plants, and consequently to animals and human health (Chen et al., 2012).

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Anaerobic digestion can inactivate plant pathogens effectively under certain conditions (Kumar et al., 1999; Engeli et al., 1993). However, anaerobic digestion was developed primarily as a stabilization process rather than for disinfection. This means that it is difficult to evaluate the survival of pathogens if the environment changes. There are many predominant factors controlling the reduction of pathogens during the digestion process (Bochmann and Montgomery, 2013), such as TS concentration, temperatures, retention time, and reactor type, which should be primarily optimized for anaerobic digestion. We should evaluate the survival status of pathogens when these parameters are given. Also, environmental factors may present toxicity, such as pH, VFA concentration, NH_4^+-N concentration, and redox potential, of which the VFA concentration has been widely studied (Salsali et al., 2006; Smith et al., 2005). Meanwhile other factors such as the storage time of substrates, type of substrates, pathogen species and so on may have effects on pathogen survival (Bandte et al., 2013). The complex environment makes finding a predominant factor for controlling the reduction of pathogens challenging, and the mechanism of pathogen inactivation is still not clear.

Some researchers have built models predicting pathogen survival (Popat et al., 2010; Salsali et al., 2006; Smith et al., 2005), providing inspiration for the present study. Digestion parameters can affect the survival of pathogenic bacteria directly and indirectly through changing the fermentation environment, including environmental factors. Digestion conditions could influence the digestion performance and pathogen survival simultaneously. Therefore, we hypothesize that the inactivation of pathogens is affected by a combination of environmental variables and that there might be a connection between the pathogen survival rate and anaerobic digestion performance.

In this study, TPWs were used for the feedstock of anaerobic digestion. Tomato wastes were chosen because tomatoes are widely cultivated in multi-span plastic greenhouses in China (Jiang et al., 2015), which result in soil-borne diseases caused by pathogens like *R. solanacearum* and *P. capsici* (Jiang et al., 2015; Remenant et al., 2010). Plants were killed and large amounts of TPW needed to be treated, and the biological safety of the wastes should be evaluated. Total solids (TS) and digestion temperature were the variables considered in the present study. Three TS concentrations (2%, 4% and 6%) were used in batch anaerobic digestion experiments at room temperature (20–25°C) and mesophilic (37 ± 1°C) conditions, respectively. VFAs, NH_4^+-N, pH value, ORP (oxidation/reduction potential) variations and concentrations of *R. solanacearum* and *P. capsici* were investigated during the digestion. The pathogens were quantified by real-time quantity PCR (qPCR), which is able to detect viable but non-culturable (VBNC) bacteria. According to the results, we built a model regarding the relationship between digestion performance and pathogen survival. The mechanism of pathogen inactivation was also discussed preliminarily.

1. Methods

1.1. Substrates, inoculum and pathogen strains

The TPWs (*Solanum lycopersicum* L Su Feng No. 6) were derived from greenhouses in Liuhe, Nanjing, China. The TPWs were crushed to about 0.3 cm size and stored at −20°C before use. Anaerobic digested sludge from an anaerobic treatment plant was used as inoculum. *R. solanacearum* and *P. capsici* were provided by Jiangsu Academy of Agricultural Science. The characteristics of the TPW and inoculum are listed in Table 1. *R. solanacearum* was inoculated in NA media (Glucose 10 g/L, Tryptone 5 g/L, Yeast Extract 0.5 g/L, Beef Extract 3 g/L) for 12 hr. *P. capsici* was inoculated in V8 media plates (V8 juice of Campbell Soup Company 100 mL/L, 0.2 g/L $CaCO_3$) to induce spores (Granke and Hausbeck, 2010). The bacterium suspension was centrifuged and suspended using sterile distilled water (dH_2O) to remove the media. The final concentration of *R. solanacearum* was about 10^7–10^8 cfu/mL (OD_{600} = 0.2485) and the concentration of *P. capsici* spores as counted under a microscope was about 10^4–10^5/mL.

1.2. Batch reactor set-up and opera condition

Flasks of 1 L volume were used as the reactors. The mixed substrate, which was based on different ratios, was directly added to flasks with 0.6 L active volume. The temperature was controlled at 37 ± 1°C in the water bath or at room temperature (20–25°C). Anaerobic conditions were established by flushing the flasks with nitrogen for 2 min, and flasks were sealed immediately with butyl rubber stoppers. The reactors were equipped with an outlet for sampling and a port for collecting gas. The experimental design is summarized in Table 2. Reactors having the same TS level were run in triplicate, one with no pathogens added and two others with added *R. solanacearum* and *P. capsici* respectively. Reactors only containing inoculum with pathogens were used as a control to measure the background gas production and reduction of pathogens.

1.3. Sampling and chemical analysis methods

Liquid samples were collected from the reactors every two days. The biogas production was measured daily by the drainage method and the composition was analyzed using a gas chromatograph (GC 9890A, RENHUA, Nanjing) equipped with a TCD (thermal conductivity detector), a TDC-01 column (Φ4 mm × 1 m, Shimadzu, Japan) and hydrogen as the carrier gas. The injector, oven and detector temperatures were 100, 150 and 120°C, respectively. The flow rate of the carrier gas was 50 mL/min, and the injection volume of gas sample was 0.5 mL. The VFA concentration and composition were determined by a gas/liquid chromatograph (Model GC-2014, Shimadzu, Japan) fitted with an FID (flame ionization detector), a TCD and a 30 m × 0.53 mm × 1 μm Stabilwax DA column. The injector and detector temperatures were 150°C and 240°C. NH_4^+-N concentrations were measured by a NH_4^+-N Rapid Moisture Tester (5B-6D (V8), Lian-hua Tech. Co., Ltd., China). TS, VS, SCOD (soluble chemical oxygn demand) were measured according to the standard methods of the American Public Health Association (APHA, 1998). The pH value was directly measured from liquid samples with a digital pH meter (FE20K, Mettler-Toledo, Switzerland). Redox potential was measured with a METTLER TOLEDO pH 2100e (Mettler Toledo, Switzerland) and a redox potential electrode (P + 4805-SC-DPAS-K8S/225 Redox, Mettler Toledo, Switzerland).