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The fate of antagonistic microorganisms and antimicrobial substances during anaerobic digestion of pig and dairy manure

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HIGHLIGHTS

- We studied the effects of anaerobic digestion (AD) on the characteristics of pig manure and dairy manure that are related to disease suppressiveness.
- AD had no significant impact on the numbers of fluorescent pseudomonads and *Bacillus* sp.
- Total phenolic, VFA and total sugar in manures were decreased by AD, while the contents of humic substance and ammonia were increased.
- FTIR spectra of the manures are similar to humin while spectra of anaerobically digested slurry (ADS) are similar to humic acid and fulvic acid.
- AD had different influences on the content of amino acid in pig manure and dairy manure, results obtained indicated that the key inhibitory factors of ADS might be attributed to ammonia and humic substances.

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ABSTRACT

The goals of the present study were to evaluate the suppressive capability of anaerobically digested slurry (ADS) against *Phytophthora capsici* and to determine the key factors of disease control in ADS. This was achieved by the investigations of the changes in microbial populations and the levels of antimicrobial compound during anaerobic digestion (AD). AD had no significant impact on the numbers of antagonistic fluorescent pseudomonads or *Bacillus* sp. The contents of total phenolics, volatile fatty acids and sugar fed with the raw slurries to the reactors were decreased by AD. However, the bioreactor effluents had higher concentrations of humic substances and ammonia than the feedstocks. Moreover, AD had a different influence on the content of amino acid in the pig manure compared to the dairy manure. The results obtained indicated that the key inhibitory factors of ADS might be attributed to ammonia and humic substances.

1. Introduction

With the intensive and concentrated development of animal husbandry, the livestock and poultry manure slurry production has reached approximately 3.19 billion tons, and more than 70% was directly discharged to the environment (Tian et al., 2012). The overproduction of and current practices for managing animal manure have caused detrimental effects on the environment and are potentially hazardous to human and animal health. Anaerobic digestion (AD) is widely used for organic waste treatment. In China, there are more than 3800 large-scale biogas plants treating animal manure, and more than 1 billion tons of digested slurry is produced annually by AD plants (Jin and Chang, 2011).

The literatures on various uses of anaerobically digested slurry (ADS) to promote plant growth, increase soil fertility and preserve the environment are voluminous (Forge et al., 2005). Recently, an increasing body of experimental evidence indicates that application of ADS from heterogeneous wastes could suppress plant diseases caused by plant-parasitic nematodes and a variety of phytopathogens (Jothi et al., 2003; McBride et al., 2000).

Nematode control by the use of ADS has been attributed to its components such as ammonia and butyic acid (Jothi et al., 2003), and to stimulation of naturally occurring antagonists against nematodes and/or changes of soil nematode community structure (Oka et al., 2007). However, research on the mechanisms by which ADS inhibits plant fungal pathogens is limited. Most studies dealt mainly with compost or watery fermented compost teas. Apparently, compost teas and ADS are different from each other in terms of preparation methods. However, they are both organic amendments and are similar in respect to the complexity of components including organic carbon and crop nutrients as well as abundant microbial populations. It is therefore possible that ADS could control plant disease through similar mechanisms, including microbial interactions and physico-chemical properties of organic amendments, namely ammonia, organic acid, humic or phenolic

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compounds, which may suppress plant diseases through direct toxicity towards the pathogen or induced systemic resistance (Hoitink et al., 1997).

Although a number of studies have shown that ADS may have potential for the control of plant diseases in numerous agricultural systems, inhibitory effects on plant pathogens are variable, depending on various factors including the initial feedstocks, the preparation methods and the physico-chemical composition of the wastes (Hoitink et al., 1997). It has been noted that the mechanisms responsible for the suppressive effects on plant disease control by ADS are not universal. For example, many authors reported that sterilization negated suppressiveness (El-Masry et al., 2002). Conversely, others have concluded that filtration or autoclave did not negate plant disease reduction in most cases (Elad and Shtienberg, 1994). Indeed, although most studies examined the physio-chemical and biological characteristics of ADS, very few studies focused on the changes in the physical, chemical and biological characteristics of feedstocks after AD. In fact, despite the presence of toxic compounds such as ammonia and volatile fatty acids and antagonistic microorganisms in raw manures, few experimental findings indicated suppression of plant diseases by application of raw manures. In the current work, in order to evaluate the potential use of ADS in suppressing plant diseases and to identify the dominant factors in ADS that affect disease suppression, raw pig slurry (RPS) and dairy slurry (RDS) were used as feedstock in continuous stirred-tank reactors (CSTR) at 37 °C for 130 d. Various physico-chemical and biological properties related to disease control in both the raw slurries and the digested slurries were analyzed. This information will be useful to produce ADS with higher bio-control efficacy against plant diseases.

2. Methods

2.1. Feed characteristics

Pig manure and dairy manure were collected from a livestock farm near Jiangsu agricultural academy of sciences. All substrates were obtained in one batch and stored at −18 °C and the frozen substrates were thawed at 4 °C for 3 d before use. The characteristics of the substrates were listed in Table 1. The inoculum used was digested dairy manure taken from a lab-scale reactor operated at 37 °C. The content of total solids (TS) was 3% (w/w) and the content of volatile solids content (VS) was 78% of TS (w/w).

2.2. Reactor experiments

Anaerobic digestion of manures was tested in two 13.2 L biogas reactors with a working volume of 10.5 L. The CSTR digester consisted of an optical plastic cylindrical fixed vessel. The reactor temperature was kept at 37 ± 2 °C by water circulation in the water bath surrounding the reactors. Inside, the digesters had a slow vertical stirring blade (10 r/min), which was activated for 3 min every 6 h. Biogas left the digester by its own pressure and was measured by a mass flow meter.

The CSTR experiment ran in two main phases. The first phase (initialization phase) lasted 28 d. The second phase (stablization phase) lasted 130 d. Steady state was defined as a situation where methane yield, pH and EC were constant for at least 7 d. The initialization phase started from Aug 1, 2011 and the reactors were filled with 9 L of inoculum and 1 L of dairy manure. The initial total solids (TS) of pig manure and dairy manure was 4%. The dairy manure was filtered using a 20-mesh sieve to remove large particles prior to feeding into the reactor. The reactors were fed 700 g substrates manually once a day and the digested slurry was simultaneously discharged. Methane yield, gas compostion (CH_4, CO_2 and N_2), pH and EC of the digested slurry were measured every day.

The biogas reactor reached steady state after 28 d and lasted until Jan 5, 2012, so the whole experiment ran for 130 d. Preliminary studies showed that anaerobically digested slurry obtained from the digester in which stabilized gas production was observed for more than 3 months had more stable suppressive effects on plant pathogen. Consequently, when the biogas reactor had run stably for 100 d, the influent and effluent were collected each day and stored at 0–4 °C. The daily collected samples were mixed separately every 10 d. Thus, three mixed samples were obtained for physico-chemical and biological analysis. Methane yield was measured every 5 d to ensure stable operation of the reactors.

2.3. Physico-chemical analyses

The pH of all samples was determined using a pH meter (model PHS-2F, Shanghai Precision Scientific Instrument Ltd., China). Total nitrogen and ammonium nitrogen was analyzed by a continuous flow analyzer (FIAstar™ 5000 Systems, FOSS, USA). The free ammonia concentration was calculated from Henderson–Hasselbalch equation (Anthonisen et al., 1976):

$$NH_3 = \frac{TAN \cdot 10^{pH}}{e^{\frac{6344}{273.15+T}} + 10^{pH}}$$

For the analysis of volatile fatty acid (VFA), the samples were centrifuged at 12,000 rpm for 10 min and the supernatant was filtered through a 0.45 μm Millipore membrane. VFA in the filtrate was measured using a gas chromatograph (GC-2014, Shimadzu, Japan).

The contents of total humic substances (HS) were extracted from freeze dried samples. The freeze dried samples were mixed with 0.1 M $Na_4P_2O_7$ and 0.1 M NaOH at a ratio of 1:25 (w/v). The mixture was kept in boiling water for 30 min and then filtered after cool. The total HS in the extract was determined using $K_2Cr_2O_7$ tiltration method (Bao, 2000). For the extraction of HS, freeze dried anaerobically digested slurry was mixed with 0.1 M NaOH at a ratio of 1:10 (w/v) under a nitrogen atmosphere, stirred for 24 h and then centrifuged at 7000 rpm for 20 min. The HS was separated from solution by settling at pH 1. The extraction was repeated three times until a yellow extract was obtained. The HS was purified by 0.1 M HCl + 0.3 M HF, washed with distilled water and finally freeze-dried. The Fourier transform infrared (FT-IR) spectra of HS were recorded on a Nicolet Nexus 870 spectrophotometer on pellets obtained by pressing a mixture of 1 mg of HS obtained in homogenized, freeze-dried H^+-form and 400 mg of dried KBr. The scan range was set at 4000–400 cm^{-1} at a resolution of 2 cm^{-1}.

Total phenolic acids in fresh samples were determined by Folin–Ciocalteu's reagent method (Santosa et al., 2012). One milliliter of Folin–Ciocalteu's reagent and 5 ml of sodium carbonate (1 M) were added to 4 ml 10^{-1} diluted fresh samples. Each mixture was

Table 1
Physical and chemical characteristics of raw manures fed to the CSTRs.

	Total solids (%, w/w)	COD (mg/L)	TN (mg/L)	TP (mg/L)	TK (mg/L)
Pig manure	4	38304	650	328	189
Dairy manure	2.43	35884	1109	489	344