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Performance and microbial community dynamics in a two-phase anaerobic co-digestion system using cassava dregs and pig manure

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HIGHLIGHTS

- The two-phase anaerobic co-digestion of CD with PM was evaluated using 4 SBRs and a CSTR.
- Co-acidification of PM and CD promoted the production and accumulation of VFAs, SCOD and NH_4–N.
- Methanogenic fermentation of the acidification products was efficient and steady.
- Co-digestion of PM and CD supported higher quantity and diversity of methanogens.

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ABSTRACT

The two-phase anaerobic co-digestion of cassava dregs (CD) with pig manure (PM) was evaluated using four sequencing batch reactors (SBRs) and a continuously stirred tank reactor (CSTR). The effect of seven different PM to CD volatile solid ratios (10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10) on the acidification phase was investigated. Results indicated the concentrations of soluble chemical oxygen demand, NH_4–N and volatile fatty acids increased substantially at seven ratios. Co-acidification of PM and CD performed well. Methanogenic fermentation of the acidification products at seven ratios was steady in CSTR. The highest methane yield and VS removal of 0.352 m^3/kg VS_{added} and 68.5% were achieved at PM:CD (4:6). The microbial population in CSTR was analyzed using molecular methods. Findings revealed that bacteria such as *Firmicutes* and *Bacteroidetes*, archaea such as *Methanobacteriales* and *Methanomicrobiales* were advantageous populations. Co-digestion of PM and CD supported higher quantity and diversity of methanogens.

1. Introduction

Approximately 950,000 tons of cassava dregs (CD) are produced during cassava starch-processing each year in China (Yang et al., 2011). At present, at least some of these dregs are used for animal feed, or in the production of ethanol fuel (Tang and Xie, 2006; Yang et al., 2011). However, a large percentage of these dregs are discarded as solid waste, creating problems with land use allocation, foul odors, and ground water pollution (Garcia-Peña et al., 2011). Anaerobic digestion (AD) process is considered as an alternative method for disposal of large quantities of these wastes (Bouallagui et al., 2005). A primary advantage of AD is the resulting production of a methane-rich biogas, which can be subsequently used to generate heat and electricity, or refined as an automotive fuel.

Previous studies have examined the feasibility of using CD as a substrate for anaerobic digestion (Panichnumsin et al., 2010; Pu and Liu, 2009). Similar to fruit and vegetable wastes (FVW), CD contains large quantities of biodegradable organic fractions (50–60% starch and 30–40% crude fiber) (Panichnumsin et al., 2010; Yang et al., 2011), making them a viable alternative substrate for biogas production. In most digestion processes, hydrolysis is considered to be the rate limiting phase (Mata-Alvarez et al., 2000). However, in the anaerobic digestion of biodegradable substances, the rate of reaction is limited by methanogenesis, rather than by hydrolysis (Bouallagui et al., 2005). This is due to the rapid acidification of these wastes to volatile fatty acids (VFAs) resulting in a rapid decrease in process pH, and subsequent inhibition of necessary methanogens activity. This is further exacerbated, if the feedstock is not buffered adequately, and the lowering pH tends to inhibit the conversion of organic fractions to VFAs. The constant hydrolysis rate depends on pH rather than the total VFAs concentrations (Veeken et al., 2000).

When large amounts of biodegradable substances are used for AD, these limitations can be overcome by co-substrates, and by dividing the digestion process into an acidification phase and a methanogenic fermentation phase. The addition of co-substrate

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(e.g., pig manure) to CD could improve the stability of the anaerobic digestion process providing the additional nutrients and maintaining the buffering capacity. The benefits of co-digesting pig manure and cassava dregs were described by Panichnumsin et al. (2010). Furthermore, two-phase AD systems are demonstrated an attractive alternative for conventional one-phase anaerobic digestion since they (1) allow for the selection and enrichment of different bacterium in each phase, and (2) enable the first phase to potentially protect methanogens from substantial VFAs production (especially acetic acid), which results in rapid acidification of the reactor (Demirel and Scherer, 2008). These two-phase AD systems have been demonstrated to increase methane production and COD removal efficiencies when compared to conventional one-phase digesters (Bouallagui et al., 2005). Nevertheless, the two-phase AD system is not often used to treat CD when mixed with PM.

AD occurs via microbial action in four main stages: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis (Demirel and Scherer, 2008). Yet, the characteristics and microbial dynamics involved in these four stages are not well understood, primarily due to the complexity of microbial interaction within these systems (Supaphol et al., 2011), Thus the success of the efforts to maximize methane production efficiency of such systems, relies on better understanding of these interactions and controlling environmental conditions.

The aim of this study was to evaluate the performance of two-phase anaerobic co-digestion of PM and CD in four sequencing batch reactors (SBRs) and a continuously stirred tank reactor (CSTR). The effects of seven different PM to CD volatile solid (VS) ratios volatile solid ratios (10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10) on the acidification phase and methanogenic fermentation phase were evaluated. In order to further understand microbial ecology within such systems, the microbial community in CSTR when operating at PM to CD ratios of 10:0, 4:6, and 0:10 were evaluated and its relationships with process performance were assessed.

2. Methods

2.1. Material and inoculum

Approximately 200 kg of dried cassava dregs (CD) were obtained from a cassava starch plant in Guangxi Province, China and 200 kg of pig manure (PM) were collected from a pig farm in Guangxi Province, China (Table 1). Following collection all feedstock were frozen at −20 °C to prevent further biological decomposition, and subsequently thawed overnight at 4 °C just prior to addition to the reactor. Anaerobic activated sludge (pH 7.3, 21 g/L of volatile solid, and 35 g/L of total solid) from UASB in a cassava starch plant, was used as the reactor inoculum.

Table 1
The characteristics of PM and CD.

Composition	CD	PM
Moisture (% of waste)	11.05	76.82
TS (% of waste)	88.94	23.18
VS (g/kg TS)	940	783
Ash (g/kg TS)	60	217
Soluble sugar (g/kg TS)	316	31
Protein (g/kg TS)	22	247
Cellulose (g/kg TS	158	128
Hemicellulose (g/kg TS)	230	184
Lignin (g/kg TS)	62	22
TKN (g/kg TS)	3.58	40
TC (g/kg TS)	547	432
C/N ratios	152	11

2.2. System design and operation

The SBRs-CSTR system combined four sequentially batch reactors (each with 8-L feedstock capacity) and a continuously stirred tank reactor (CSTR) with a 30-L working volume. The acidification of the feedstock occurred in four sequentially batch reactors (SBRs) and the methanogenic fermentation took place in the CSTR. In order to achieve the acidification for 4 days, the four SBRs were fed in sequence as follows: Reactor 1 was fed on day 0, Reactor 2 on day 1, Reactor 3 on day 2, and Reactor 4 on day 3; Reactor 1 was subsequently emptied and refilled on day 4, the acidified products from Reactor 1 were transferred to the CSTR; The rest was done in the same manner. The CSTR was operated at a constant organic loading rate of 4 kg VS/m^3 d with a withdraw/feed method per day. Temperature of the SBRs and the CSTR were maintained at 25 ± 2 °C and 37 °C, respectively. The feedstock of different PM to CD volatile solid (VS) ratios (10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10) were introduced into this system. The SBRs was fed with the CD and PM mixtures (volatile solid content of 6%) daily while CSTR was fed with the acidified products from SBRs (adjusted pH to 6.8–7.2). The system was started up using at PM to CD ratio of 4:6 as the initial substrate for 36 days. After the CSTR reached the steady state at the designed OLR, characterized by stable pH, methane yield and TVFA concentration, it was fed with various ratios of PM to CD as described above.

2.3. Analytical methods

Total solid (TS), volatile solid (VS), total nitrogen (TKN) and ammonium-nitrogen (NH_4–N) were analyzed according to the Standard Method (APHA, 2005). Protein content was calculated from total N using a multiplier of 6.25. Cellulose, hemicelluloses and lignin were determined by Goering and Van Soest (1970). Samples were centrifuged at 800*g* for 3 min, and the resulting supernatant was used to determine soluble chemical oxygen demand (SCOD) using a water quality monitor (LOVIBOND99731COD, Germany). Sample pH was determined using a HORIBA Compact pH meter (Model B-212, Japan). Biogas production was determined using wet gas flow meter (LML, china) with the percentage of methane and carbon dioxide within determined using biogas analyzer (BioGas, Geotec, UK). Individual VFA concentration (acetic, propionic, butyric and valeric acids) was analyzed using the Shimadzu Gas Chromatograph (GCMS-2010).

2.4. DNA extraction and conventional PCR

Effluent sludge samples were collected at 0 d (seed), 51 d (PM to CD ratios (10:0)), 111 d (PM to CD ratios 4:6) and 141 d (PM to CD ratios 0:10) from the bottom of the reactor. The 3–4 ml sludge samples were centrifuged at 13,000*g* for 20 min, and the supernatant was decanted carefully to obtain the sediment sample (300 mg net weight) for DNA extraction. The VS concentration of the sediment was used to estimate the amount of biomass to be used for DNA extraction. Genomic DNA was extracted using the Fast DNA Spin Kit for soil following the manufacturer's instructions (Bioteke Biotech Co., Ltd., China). Extracted DNA was eluted with 40 μL of Tris–HCl buffer (pH 8.0) and stored at −20 °C until used in further analysis. PCR amplification (95 °C for 10 min, 25 cycles of denaturation at 93 °C for 1 min, annealing at 48 °C for 1 min, and elongation at 72 °C for 1 min 30 s, followed by a final elongation phase at 72 °C for 5 min) was performed using a GeneAmp PCR System (Model 9700, Applied Biosystems, USA) for DGGE. The thermal profile of archaeal PCR for clone library analysis was as described above except that 30 cycles were applied for amplification. The products were examined by electrophoresis on 2% agarose gel before being subjected to further analysis.