



Biomethanation efficiency of para-grass in piggery wastewater in single stage and temperature phased anaerobic systems

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ABSTRACT

Effects of para-grass (PG) addition to pig manure (PM) digester were evaluated at organic loadings 0.10–3.76 gVS/L.d by different PG mixing ratios 0–8% in mesophilic single-stage (MS) and temperature-phased anaerobic digestion (TPAD, comprising T1 and M2 reactors) systems. Results showed equivalent methane production between MS and TPAD until 4%PG mix. Highest biogas yields obtained were 271.7 and 264.0 m³/ton_{dry} for MS and TPAD, respectively. Even with intense VFAs accumulation, the acidification yield in T1 was less than M2 because of continuous conversion of VFAs to CH₄. Only at higher loading (8%PG), reactor staging by temperature was justified for this co-digestion as TPAD exhibited a superior performance and lesser mass transfer impediment. Para-grass addition to PM digester shifted the domination of bacterial strains whereas archaea were steady. Higher microbial diversity and some evolving hydrolytic bacteria observed in T1 could contribute to greater system stability at high solid loading. An addition of small front-end thermophilic tank should be considered when expansion of the existing MS system is planned to process higher solid.

1. Introduction

Energy and environment have become the important elements in human activities. A large portion of energy we consume is non-renewable or renewable from food-derived origin, whose productions always cause negative impacts on the environment. Wastes derived energy can have a two-fold benefit where environmental clause is taken care of while producing clean energy. Waste produced from agro-industry is endlessly growing as to serve more food to our growing population. Due to its large quantity, extensive treatment or conversion is critically required prior to circulate it back to the environment. Swine farm is one of the major agro-businesses in Thailand involving over 180,000 farmers with, in year 2017, approximately 10,191,784 pigs raised generating an estimate of 5–6 billion kilograms of manure in a

year (DLD, 2017). This manure, rich in organics and nutrients, needs practical handling. Raising swine in a modern time is energy intensive due to the animal house cooling/heating and waste management. Coupling waste treatment with energy production posed clear advantages both in environmental and economic aspects. Anaerobic digestion (AD) of animal manure delivers biogas (containing 50–70% CH₄) for use as fuel for electricity and heat generation while the separated solid digestate can be sold to crop farmers and the liquid part could be applied on the nearby lands for grass growing.

Para-grass (*Branchiria mutica*) is a tropical weed that widely grows on wet soils in various tropical countries, which is now a good source of cow and horse feedstock. Over-growing of this grass in many areas can, however, be a burden for fire hazard control. There is an enormous potential for it as feedstock for co-digestion with swine waste in existing

List of abbreviations: AD, anaerobic digestion; ALK, alkalinity; CHONS, carbon, hydrogen, oxygen, nitrogen and sulfur; COD, chemical oxygen demand; CSTR, continuously stirred tank reactor; C:N, carbon to nitrogen ratio; DGGE, denaturing gradient gel electrophoresis; HAC, acetic acid; HPr, propionic acid; HRT, hydraulic retention time; Hbu, butyric acid; HVa, valeric acid; i-Hbu, iso-butyric acid; i-HVa, isovaleric acid; MS, mesophilic single stage reactor; M2, mesophilic 2nd stage reactor in TPAD; OLR, organic loading rate; PG, para-grass; PM, pig manure; SVFA, short chain volatile fatty acids; TPAD, temperature-phased anaerobic digestion; TS, total solids; TVFA, total volatile fatty acid; T1, thermophilic 1st stage reactor in TPAD; VFA, volatile fatty acid; VS, volatile solids; Y_a, acidification yield; Y_h, hydrolysis yield

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anaerobic digesters on farm. Previous investigations reported that significant increases in volumetric biogas production can be achieved by adding carbon rich agricultural residues to the AD treating animal manure (Wu et al., 2010), preferably at carbon to nitrogen ratio of 25–30 to 1 (Ward et al., 2008). Lansing et al. (2010) showed that co-digesting carbon-rich cooking grease waste with swine manure in low-cost digesters could double the amount of energy production. Adding the crop residue such as sugar beet tops, grass and oat straw at 30% by weight (based on volatile solid) to a CSTR digester receiving cow manure increased methane production by 16–65% (Lehtomäki et al., 2007) while the mixture of 1:3 ratio on dry weight basis of cattle manure to pretreated corn stover gave the highest biogas yield (Li et al., 2009). Optimized C:N of the total feedstock suits metabolic activities in AD and indirectly decrease ammonia inhibition (Xie et al., 2011).

Characteristics of the co-substrate in both physical and chemical composition play a vital role on how it can be mixed and to what limit for a particular reactor configuration. Among factors important to successful AD operation such as pH, mixing, pretreatments, and organic loading, temperature is proven to have a great impact on solid digestion. These well-established figures of 35 and 55 °C are typical and proven optimal temperatures for major groups of microorganisms; mesophiles and thermophiles, in anaerobic digestion (Li and Khanal, 2016). Higher temperature increases substrate degradation, and biogas production rate could rise substantially (Yu et al., 2002). However, operating AD at thermophilic temperature is challenging since the system becomes quite sensitive at high temperatures, not to mention a greater energy requirement (Speece, 2008). For conventional anaerobic digestion, acid forming and methane forming organisms reside together in a single reactor. Both groups differ in physiology, growth kinetic, and nutritional and environmental condition requirements (Elbeshbishy et al., 2012). Two-stage reactor configuration is believed to offer a physical separation of acid and methane forming, and optimum environmental condition of each group. Temperature-phased anaerobic digestion (TPAD) is a two-stage AD process that aims to enhance hydrolysis and pathogen destruction in the first stage at thermophilic (55 °C) condition while the second mesophilic stage (35 °C) efficiently polishes a more soluble and digestible pretreated substrate. These combined with its unique microbial diversity could give a remarkable process stability (Montañés Alonso et al., 2016; Riau et al., 2010; Wu et al., 2015). Nonetheless operating two reactors, especially one at a thermophilic temperature poses more difficulty beside the energy expenditure. Simple operation and configuration of the single stage digester system may come in to play when the performance and benefit of the two-stage are not sufficient to overcome the sacrifice it will take. This depends largely on the substrate variety and operating regimen. Organic loading rate (OLR), representing the amount of waste treatable per unit reactor volume, is one critical operational parameter regulated either by feedstock concentration and hydraulic retention time (HRT). While increasing OLR could increase the volumetric methane production, a risk of system failure due to the fast evolution of VFAs or microbial washout of AD if hydraulic retention time is shortened (Xie et al., 2012). Increasing feed concentration seems to be a better way to raise the OLR. It is, in retrospect, up to the limitation of each type of system configuration and operations to handle the more viscose feedstock.

Therefore, the objective of the study was to evaluate the effects of substrate concentration which was used as a means to increase OLR on co-digestion of para-grass in piggery wastewater. Comparison between the single stage mesophilic anaerobic digester and the two-stage TPAD system was carried out in continuous operation over 330 days in order to determine the biomethanation performance, which eventually helped identify the necessity and the operating regime that the temperature phased two stage system could be more effective than the single stage one. Microbial analysis was also conducted to explain the shift of community and dominance of different bacteria and archaea in both systems regarding the feed alteration to lignocellulosic substrate

and temperature staging.

2. Materials and methods

2.1. Inoculum

The inoculum used in this work was from anaerobic digester treating piggery wastewater from the unit of finishing (fattening) barn in Songkhla Province, Thailand. The inoculant sludge, which was dispersive, was sieved to remove large particulate impurity. It was then measured for total solids (TS) and volatile solid (VS) concentration. Its specific methanogenic activity (SMA) was measured to ensure active microbial inoculant for the system startup. The methanogenic activity found was 33.0 ± 0.2 mLCH₄/gVS which showed high concentration of active methanogenic organisms. The sludge was then inoculated to the reactors within 72 h after field collection.

2.2. Substrates

Pig manure (PM) was obtained from excretions in a finishing unit of a pig farm in Pattalung Province, Thailand. It was dried at 60 °C and ground in mortar. Fresh green para-grass (PG), *Branchiria mutica*, was randomly harvested from the field receiving liquid digestate from the pig farm where PM was collected. It was chopped with a cutting machine to approximately 2 cm, shredded to size of less than 6 mm and then dried at 60 °C until constant weight. Both substrates were kept at 4 °C in plastic bags until use. Each substrate was homogenized and characterized for total solid (TS), volatile solid (VS), moisture, fiber contents and elemental composition (CHONS). Analytical methods of samples are described in Section 2.4.

The prepared PM was taken to mix with tap water at concentration around 2.5 gTS/L or 2.0 gVS/L to imitate the piggery wastewater that contained a COD of approximately 3000–4000 mg/L. The introduction of PG as co-substrate to the prepared liquid feed were tested at 4 levels 0%, 2%, 4% and 8%TS (w/w) designated as operating conditions 0% PG, 2%PG, 4%PG, and 8%PG, in order. The substrates were homogenized to slurry before feeding to the reactors. Characteristics of PM and PG used in the experiment are shown in Table 1.

2.3. Reactor systems and operations

There were two anaerobic digestion systems evaluated; single-stage mesophilic anaerobic digester (MS) system, and two-stage temperature-phased anaerobic digestion (TPAD) system which consisted of first stage thermophilic reactor (T1) followed by second stage mesophilic digester (M2). Both mesophilic reactor vessels were made of cylindrical glass at 5 L volume and 182 mm in diameter, while the thermophilic glass reactor has a total volume of 1 L. Each system possessed a total effective volume of 4 L, that TPAD system is divided into 0.4 L in T1 and 3.6 L in M2. The reactors were inoculated with an active sludge at 30% of the effective volume yielding an initial sludge concentration of 2.5

Table 1
Chemical and elemental compositions of pig manure and para-grass.

Composition	Unit	Pig manure (PM)	Para-grass (PG)
Total solids, TS	g/kg wet wt	248.1 ± 4.5	190.6 ± 6.4
Volatile solids, VS	g/kg dry wt	806.3 ± 3.0	914.9 ± 6.2
Cellulose	% dry wt	12.7 ± 0.2	38.8 ± 2.4
Hemi-cellulose	% dry wt	25.7 ± 3.9	29.5 ± 1.5
Lignin	% dry wt	9.0 ± 5.0	8.0 ± 2.0
Carbon, C	% dry wt	38.1	41.6
Hydrogen, H	% dry wt	5.4	5.3
Oxygen, O	% dry wt	22.3	27.3
Nitrogen, N	% dry wt	3.0	1.3
Sulfur, S	% dry wt	0.3	0.3
C:N ratio		12.7	32.2

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