CHAPTER 6

General discussion

Anaerobic co-digestion has been widely used to enhance biogas production. Most studies focus on the effect of parameters such as substrate concentration, feeding composition, type of reactor and organic loading rate (OLR) on performance of co-digestion and biogas production. These factors have impacts on the microbial community in the reactor (Akarsubasi *et al.*, 2005; Pholchan *et al.*, 2010). Therefore, successful biogas production is based on stable, adaptation and activity of these microbial communities which depends on environmental conditions in the reactor (Gerardi, 2003). However, microbial community structure has recently been studied. Thus, the knowledge about microbial community structure remained lacking and uncertain, particularly in semi-continuous mode which is widely used in farm scale. This study reported the microbial community structure in anaerobic co-digestion of 70% pig manure with 30% Napier grass and 60% pig manure with 40% food waste based on volatile solid (VS) under different hydraulic retention time (HRT) of 10, 20 and 30 days in channel digester-upflow anaerobic sludge blanket (CD-UASB) and completely stirred tank reactor (CSTR) by denaturing gradient gel electrophoresis (DGGE).

The results of DGGE profile and the unweighted pair group method with arithmetic mean (UPGMA) dendrogram generated from bacterial DGGE profile by Jaccard similarity coefficient method clearly demonstrated the change in bacterial population during operation. In the study using Napier grass as co-substrate, the bacterial population in CD-UASB seemed to be more stable in the beginning and middle of operation than those of CSTR in all HRT and also similar to seed. As the seed was collected from CD-UASB reactor, the bacterial population in CD-UASB adapted to the system better than CSTR. However, after 45 days bacterial population structure was shifted to another pattern and became stable. While those of CSTR, an obvious shift of bacterial population was observed in the first 36 days and then also stable. This observation showed that CSTR needed shorter time to establish a stable bacterial population. A stable bacterial population was also established during the steady state of methane production (Rerkkriangkrai *et al*., 2009; Muenjee, 2010). This may indicate the relationship between bacterial population and methane production. Therefore, anaerobic digestion process could be predicted by monitoring bacterial population shift throughout the operation process. The study using food waste as co-substrate showed that bacterial population in both CD-UASB and CSTR in all HRT was generally stable throughout the process even though some bands were faded. Because food waste and pig manure contained easily degradable compounds so bacterial population could adapt and utilize these organic materials easily.

The UPGMA dendrogram based on Jaccard similarity was generated from bacterial DGGE profile in steady state of system to investigate the effect of HRT and reactor type on bacterial population structure (Figure 6.1). It was found that HRT affected bacterial population structure in co-digesting with Napier grass, which had similar structure at long HRT. Those of co-digesting with food waste were more affected by type of reactor than HRT. These results implied that HRT had more influence on hard degradable compounds than easily degradable material because bacteria need longer time to breakdown such material within the reactor. Moreover, these results were in line with the average methane yield during steady state of system (Table 6.1) that reactor co-digested with Napier grass gave similar yield at long HRT. However, the methane yield at HRT 20 days of CSTR (0.372 m^3/kg VS_{add}) was lower than those from HRT 10 and 30 days (0.629 and 0.637 m^3/kg VS_{add}, respectively). However, the DGGE profile of bacterial population structure at HRT 20 days during steady state of was stable and similar to those of HRT 30 days. This observation indicated that low methane yield at HRT 20 days was unlikely to be a result from bacterial population but may be caused by system problem. A report of Muenjee (2010) confirmed that biogas was leaked from a reactor at HRT 20 days in certain day of operation resulted in low average methane yields which was lower than yield from HRT 10 and 30 days. Nevertheless, CD-UASB showed better efficiency in the removal of chemical oxygen demand (COD) than CSTR (Table 6.1). For food waste co-digestion, the methane yield in each HRT was not significantly different (Rerkkriangkrai *et al*., 2009; Muenjee, 2010) which reflected in similar bacterial population structure in all HRT. Moreover, the COD removal efficiency of both reactor types was also not different as shown in Table 6.1. Methane yield from CSTR co-digested with Napier grass was higher than CD-UASB reactor. The methane yields of both reactor type co-digested with food waste were not different. This indicated that mixing has less effect on degradation of easily degradable material compared to hard biodegradable compounds such cellulose and lignin. Mixing increased intimate contact between microorganisms and organic substance including degradation rate (Anderson *et al*., 2003).

In addition, the influence of substrate types on the bacterial population structure was also investigated by using cluster analysis (Figure 6.1). Two different groups can be observed. The first group contained bacterial DGGE profiles of reactors co-digested with food waste and the second group contained bacterial DGGE profiles of reactors co-digested with Napier grass. This result indicated that the substrate type affected bacterial population structure. Regueiro *et al*. (2013) studied the influence of five different agro-industrial wastes on the microbial community structure and they concluded that the type of substrate affected the population structure in anaerobic reactors. The nutrient composition and physico-chemical characteristic of substrate, leaded to a change in bacterial population structure (Song *et al*., 2014).

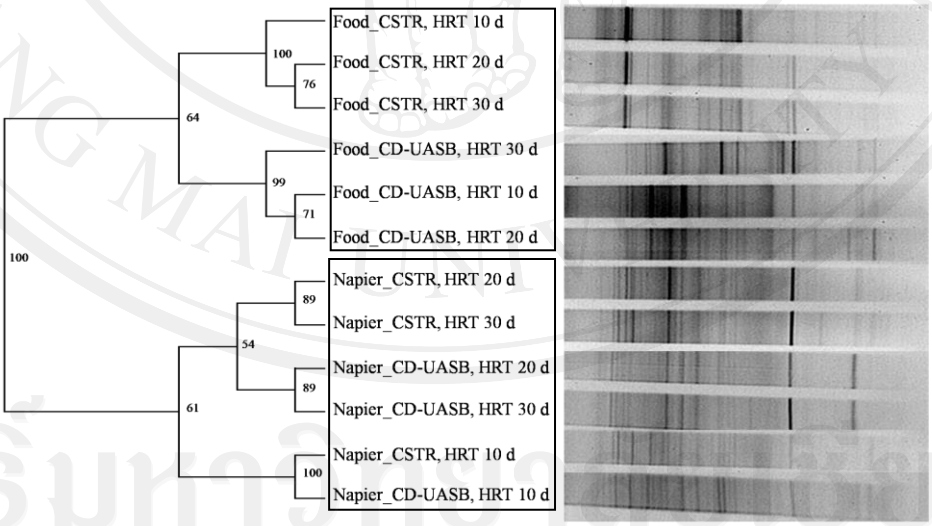

Figure 6.1 Cluster analysis of bacterial banding pattern at steady state from reactors co-digested with Napier grass or food waste under different HRT. The UPGMA algorithm was used to cluster pattern based on Jaccard similarity coefficients. Bootstrap values (>50%) based on 1000 replicates are given at each node.

Bacterial diversity in each reactor was compared by counting the numbers of DGGE bands on the gel, where each band was considered to correspond to a different taxon. Although this approach is not the most accurate, the diversity could be estimated (Pholchan *et al*., 2010). The bacterial diversity was affected by substrate type supplied to the reactor. The diversity of bacteria was high in the reactor co-digested with Napier grass as shown in Table 6.1. This finding was corresponded with Hiibel *et al*. (2011), who found higher overall bacterial diversity in lignocellose-based reactor than ethanol-based reactor due to the complexity of lignocellulosic substrate that required broader group of bacteria for it breakdown.

Table 6.1 Species richness, methane yield and COD removal efficiency in all the reactors studied.

Reactor	**Species richness** [a]		**CH_4 yield** [b]	**COD removal** [c]
	Bacteria	**Archaea**	**(m^3/kg VS_{add})**	**(%)**
1	23	5	0.229	88
2	21	5	0.334	91
3	29	5	0.360	99
4	26	5	0.629	77
5	30	5	0.372	87
6	29	5	0.637	88
7	18	5	0.370	97
8	13	5	0.533	96
9	16	5	0.430	96
10	17	11	0.486	95
11	10	12	0.484	97
12	19	11	0.329	97

[a] Total number of DGGE band

[b, c] The results from prior study by Rerkkriangkrai *et al*. (2009) and Muenjee (2010)

1, 2 and 3: The CD-UASB reactor co-digested with Napier grass at HRT 10, 20 and 30 days, respectively

4, 5 and 6: The CSTR co-digested with Napier grass at HRT 10, 20 and 30 days, respectively

7, 8 and 9: The CD-UASB reactor co-digested with food waste at HRT 10, 20 and 30 days, respectively

10, 11 and 12: The CSTR co-digested with food waste at HRT 10, 20 and 30 days, respectively

Identification of dominant bands obtained from bacterial DGGE profile revealed that most 16S rDNA sequences of dominant bacteria belonged to uncultured bacteria. Phyla *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Chloroflexi* and *Cloacamonas* were found in both types of substrate. Moreover, bacterial member in phylum *Spirochaete* were also found when co-digested with Napier grass, whereas *Flavobacteria* and *Acidobacteria* were found in food waste co-digestion. The dominant bacteria which found throughout the process in reactor co-digested with Napier grass was the phylum *Firmicutes* while phyla *Firmicutes* and *Bacteroidetes* were found for reactor co-digested with food waste. Microorganisms within class *Clostridia* and *Bacteroidia* are known as hydrolytic and fermentative bacteria. They are commonly found in anaerobic habitats such as rumen and digesters. They have ability to degrade various complex organic matters, including protein, carbohydrate and lipid. Especially, class *Clostridia* which is also capable of utilizing the cellulose. Moreover, some of these dominant bacteria were originated from seed (inoculum). This result clearly showed crucial role of inocula for anaerobic digestion process.

The DGGE analysis of archaeal population showed lower diversity than bacteria with stable pattern throughout the reactor operation in all experiment even though some bands were faded, except for CSTR co-digested with food waste due to improper influent pH (Muenjee, 2010) which affected the growth of methanogens within reactor. This observation indicated that HRT, reactor and substrate type had less influence on the archaeal population.

Identification of dominant bands obtained from archaeal DGGE profile showed that members of order *Methanomicrobiales* and *Methanosarcinales* were dominated in the reactors. Hydrogenotrophic *Methanomicrobiales* were dominated in the reactor followed by acetoclastic *Methanosarcinales*. This finding suggested that hydrogenotrophic methanogenesis may be the major pathway of methane formation and most methane may be produced from the conversion of H_2 and CO_2.

Analysis using principle coordinates analysis (PCA) or canonical correlation analysis (CCA) are needed in future study to determine the relationship between operation parameters which have stronger influence on bacterial population in the reactor in order to efficiently control the operation of system. Moreover, long adaptation time of bacterial population in the anaerobic co-digestion of Napier grass with pig manure in both CD-UASB and CSTR was observed. Future study should try to enrich these bacteria before feeding to reactor to reduce start up time. Our results also showed the important role of seed for anaerobic digestion process. Thus, selection of effective original seed from various sources is a potential way to enhance reactor efficiency. Furthermore, most sequences showed <97% similarity to known species indicating that the majority of bacterial sequences were related to uncultured bacteria. They are a potentially new bacterial species which have never been reported. The cultivation, determination of microbiological characteristics, metabolic activity and growth factor of these bacteria are also important in order to understand their role during anaerobic digestion.

Recommendation

DGGE profile in this study indicated that bacterial populations in anaerobic co-digestion are very complex and difficult to evaluate. Design and application of primers for amplification of 16S rDNA fragments from specific bacterial groups in mixed communities are needed to investigate and monitor shift of certain microbial groups. In addition, analysis of mRNA will also help to determine active bacteria and archaea in anaerobic digestion process.