Co-digestion of polylactide and kitchen garbage in hyperthermophilic and thermophilic continuous anaerobic process

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Abstract

Two series of two-phase anaerobic systems, consisting of a hyperthermophilic (80 °C) reactor and a thermophilic (55 °C) reactor, fed with a mixture of kitchen garbage (KG) and polylactide (PLA), was compared with a single-phase thermophilic reactor for the overall performance. The result indicated that ammonia addition under hyperthermophilic condition promoted the transformation of PLA particles to lactic acid. The systems with hyperthermophilic treatment had advantages on PLA transformation and methane conversion ratio to the control system. Under the organic loading rate (OLR) of 10.3 g COD/(L day), the PLA transformation ratios of the two-phase systems were 82.0 and 85.2%, respectively, higher than that of the control system (63.5%). The methane conversion ratios of the two-phase systems were 82.9 and 80.8%, respectively, higher than 70.1% of the control system. The microbial community analysis indicated that hyperthermophilic treatment is easily installed to traditional thermophilic anaerobic digestion plants without inoculation of special bacteria.

Keywords: anaerobic process, co-digestion, polylactide, kitchen garbage, hyperthermophilic condition, ammonia

1. Introduction

Kitchen garbage (KG) is an organic solid waste suitable for anaerobic digestion (AD) treatment (Kim et al., 2003). A large amount of plastic materials used as boxes and bags are typically commingled with the collected KG, which makes it necessary to remove these plastics before the AD process to avoid the adverse effect on the treatment performance due to the non-biodegradability and mechanical troubles in mixers and pumps of the reactors. The extra separation cost weakens the economic benefit of the AD process; an option is to replace the non-biodegradable plastics with polylactide (PLA), one of the well-known biodegradable plastics (Armentano et al., 2010; Perepelkin, 2002), and treat PLA and KG simultaneously. An increase of biogas production by the PLA degradation is anticipated. Consequently, an effective anaerobic digestion system for co-digestion of PLA and KG is important to be developed on the basis of biodegradation characteristics of PLA.

For the last decade, numerous studies have focused on the PLA degradation under various treatment conditions including hydrolysis, hydrothermal and compost. Previous research results indicated that temperature is one of the key factors affecting the PLA biodegradation since the rate of PLA degradation increased with temperature (Copinet et al., 2004; Dunne et al., 2000; Ghorpade et al., 2001). Higher temperature also

promotes the performance of the AD process. Compared with the generally operated mesophilic (37 °C) processes, thermophilic (55 °C) and hyperthermophilic (over 55 °C) anaerobic digestion processes have the advantages of effective organic particles solubilization and higher biogas production (Nielsen and Petersen, 2000; Scherer et al., 2000). Two-phase systems employing hyperthermophilic (70 – 80 °C) and thermophilic (55 °C) AD process have been developed for KG and sewage sludge treatment (Lee et al., 2008; 2009). The hyperthermophilic AD treatment for PLA is expected to promote the hydrolysis and biodegradation performance, but generally applicable and effective anaerobic digestion methods for PLA have not been proposed yet.

Aqueous ammonia solutions of have been widely used as reaction media for the degradation of polymer materials, such as poly-ethylene naphthalate, poly-ethylene terephthalate, polycarbonate and poly-hexamethylene carbonate. These degradation processes were accomplished under hydrothermal or supercritical conditions of over 120 °C and over 10 MPa (Arai et al., 2010; Zenda and Funazukuri, 2008). Supposing that PLA is able to be degraded via enzymatic reactions under temperature and pressure acceptable for AD process with existence of ammonia, the hydrolysis product of protein, PLA is expected to be transformed to methane easily. Wang et al. (2011) performed batch experiments and demonstrated that PLA was hydrolyzed under hyperthermophilic

pretreatment with ammonia addition and converted to methane by methanogens under the following thermophilic anaerobic conditions. However, few researches focusing on PLA biodegradation with ammonia under continuous anaerobic operation have been published to date and the optimum configuration of the system including hyperthermophilic treatment is poorly understood.

In this study, three types of anaerobic digestion systems were operated continuously for 592 days with a co-substrate of PLA and KG to evaluate the PLA biodegradability. The overall treatment performances of these systems were compared in terms of PLA degradation, methane conversion, and dewatering property.

2. Material and methods

2.1 Reactor configuration and operation

One control reactor (M_0) operated under a thermophilic (55 °C) condition, and two series of two-phase systems were operated continuously fed with a mixture of PLA plastic and KG as shown in Fig.1. Both of the two-phase systems consisted of a thermophilic reactor (M_1, M_2) and a hyperthermophilic (80 °C) reactor (S_1, S_2). In the post-dissolution system of M_1 + S_1, the co-substrate was diluted by the liquor from the hyperthermophilic reactor S_1 and then fed into the reactor M_1, and a given part of the mixed liquor from M_1 was fed into S_1. In the pre-dissolution system of S_2 + M_2, the

co-substrate was diluted by the mixed liquor from the reactor M_2 and then fed into the reactor S_2. The liquor discharged from S_2 was fed into M_2. The temperature was controlled by setting the reactors in oil bath. In each reactor, a steel stirrer was used for stirring at 200 rpm. Withdrawing and feeding were conducted once a day. The seed sludge was obtained from a continuously operated anaerobic digestion system (Lee et al., 2009).

The operational conditions are summarized in Table 1. The initial organic loading rate (OLR) of the three systems was 3 g COD/(L day). After the systems got stable for biogas production, the OLRs were increased to 4.5 and 6.8 g COD/(L day) gradually. The solids of the discharged sludge from each thermophilic reactor was returned after it was concentrated to a high density with centrifuge under 3000 rpm for 10 minutes until the end of Run 3. In Run 4, the solid return operation was terminated while the OLR was unchanged and the biogas production decreased obviously. Consequently, the HRT of the systems was increased in Run 5. After the biogas production was recovered, the HRT was decreased in Run 6 and the PLA 2 was used as substrate instead of PLA 1. In Run 7, the overall OLR was increased to 10.3 COD/(L day). In Run b8 to Run b9 of S_1, and in Run c2 to Run c9 of S_2, NH_4Cl was added into the reactors to evaluate the promotion effect of ammonia on PLA degradation under hyperthermophilic condition.

S_1 was operated without pH control while pH in S_2 was maintained at 7.5 by automatic titration of 10 N KOH in Run c2 to Run c9 to promote the PLA degradation as pH drops by degradation of PLA to lactic acid, and it was indicated that higher pH of aqueous solution was favorable for the PLA hydrolysis (Karst et al., 2008; Tsuji and Ikarashi, 2004).

2.2 Characteristics of PLA and KG

Two kinds of plastic bags (PLA 1 and PLA 2) were used. PLA 1 was composed entirely of polylactide, while PLA 2 had a polylactide content of 70%. Both of the two plastics were 0.1 mm thick and were cut into small pieces (2 × 2 mm) using a shredder (M-450Cs, Fellowes, Japan) before conducting the experiments. The COD and VS of the two types of plastics were 1.38 ± 0.07 (PLA 1) and 1.40 ± 0.09 (PLA 2) g COD/g, and 0.99 ± 0.02 (PLA 1) and 0.98 ± 0.01 (PLA 2) g VS/g. The artificial KG consisted of 14 types of food items (Lee et al., 2008; 2009) on the basis of a survey conducted in Tokyo Metropolitan City, Japan (Tanigawa et al., 1997). The average COD value of the raw KG was 230 g/L and in Runs 1 to 4, it was diluted with tap waster and then mixed with PLA based on a COD ratio of 4: 1. In other runs, the raw KG without dilution was mixed with PLA based on the same COD ratio. The characteristics of the substrate in each run are summarized in Table 2.

2.3 Chemical analysis

Once every two or three days, sample was taken from the effluent of each reactor for chemical analysis. Total solids (TS), volatile solids (VS), suspended solids (SS), volatile suspended solids (VSS), total COD (TCOD), soluble COD (SCOD), total ammonia (TAN) and pH were measured according to the Standard Methods (AWWA and WEF, 1998). Free ammonia (FAN) was calculated from the TAN as follows (Hansen et al., 1998):

$$[FAN] = [TAN] \times \left\{ 1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}} \right\}^{-1} \qquad (1)$$

Where T (K) is temperature of Kelvin.

Seven kinds of main organic acids during AD process (lactate (HLa), acetate (HAc), propionate (HPr), *iso*-butyrate (*i*-HBu), *n*-butyrate (*n*-HBu), iso-valerate (*i*-HVa) and n-valerate (*n*-HVa)) were measured using HPLC (CDD-10Avp, Shimadzu, Japan) with the column of Shim-pack SCR-102H (Shimadzu, Japan), and soluble phase of 5 mM p-toluenesulfonic acid monohydrate under the column temperature of 43°C. The produced gas was collected in a gasbag and was analyzed for composition and volume using gas analyzers (GC-14B with thermal conductivity detector, and CGT-7000, Shimadzu, Japan). The column of GC-14B was SHINCARBON ST and carrier-gas was

helium. In Run a3 to a7, b3 to b9 and c3 to c9, the dewatering characteristic of the sludge was evaluated using the Capillary Suction Time (CST) test (AWWA and WEF, 1998).

2.4 Microbial analysis

In Runs a-2, b-2 and c-2, the systems got stable with PLA degradation after 100 days operation, which indicated the acclimation of the microbes to PLA and sample was taken from M_0, M_1 and M_2 reactor for microbial analysis. DNA was extracted using a Dneasy Tissue Kit (Oiagen, Hilden, Germany). A primer set of UNIV519F (5'-CAGCMGCCGCGGTAATWC-3'; Lane, 1991) and UNIV1406R (5'-ACGGGCGGTGTGTRC-3'; Lane, 1991) was used to amplify approximately 900 and 700-bp fragments, respectively. DNA extraction, PCR amplification, gel extraction and cloning operation were described by Lee et al. (2009). The sequencing of 16S rRNA was conducted by TaKaRa Bio Dragon Genomics Center in Japan. Obtained sequence data were compared with similar sequence in National Center for Biotechnology Information data using the BLAST program (Altschul et al., 1990).

3. Results and discussion

3.1 Hydrolysis in the hyperthermophilic reactors

Fig. 2 shows the PCOD dissolution ratio and the PLA transformation ratio in the two hyperthermophilic reactors, S_1 and S_2. The solubilization ratio of particulate COD (PCOD) were calculated as follows:

$$PCOD\ solubilization\ ratio\ (\%) = [(P_{inf} - P_{enf})/P_{inf}] \times 100 \quad (2)$$

Where, P_{inf} and P_{enf} are the influent and the effluent concentrations of PCOD (g/L), respectively. The definition of "soluble" in this study was any material passing through filter with pore size of 1 μm (ADVENTEC).

The PLA transformation ratio for each single reactor or system was calculated as follows:

$$PLA\ transformation\ ratio\ (\%) = [(C_{inf} - C_{enf})/C_{inf}] \times 100 \quad (3)$$

Where, C_{inf} (g/L) and C_{enf} (g/L) are the PLA concentration in the influent and the effluent for one single reactor or system as shown in the Fig. 1, respectively. C_{enf} (g/L) was calculated as follows:

$$C_{enf} = C_{total\ lactic} - C_{lactic} \quad (4)$$

Where $C_{total\ lactic}$ (g/L) is the total lactic acid concentration in the effluent after chemical dissolution, and C_{lactic} is the lactic acid concentration in the soluble phase. To measure $C_{total\ lactic}$, the sample (2 mL) and 5 N NaOH (5 mL) were mixed to dissolve all proportions of PLA into lactic acid, and the lactic acid concentration in the filtered

solution was measured by HPLC. This ratio was determined by our preliminary examination by comparing 0.1 – 10 N NaOH, and the linier correlation between added PLA and measured lactic acid with R^2=0.996 was obtained.

As shown in Fig. 2 (a), the PCOD dissolution ratio in the two reactors varied in the range of 10.8 – 15.7% (S_1, Runs b1 to b6) and 12.6 – 16.8% (S_2, Runs c1 to c6), respectively. The PCOD dissolution ratio in S_1 and S_2 was comparable to that of Lee et al. (2008), who employed a two-phase system consisting of a hyperthermophilic (80 °C) reactor and a following thermophilic (55 °C) methane fermentation reactor to treat the KG with same composition as that used in this study. HRT of that hyperthermophilic reactor was longer than 4 days (Lee et al., 2008), while HRT of S_1 and S_2 was 1 day in this study.

Both of the PCOD dissolution ratio and PLA transformation ratio in the hyperthermophilic reactors were linearly related with the ammonia concentration as shown in Runs b7 to b9 and Runs c7 to c9. These results accorded with the previous research conducted with batch experiments (Wang et al., 2011). The pretreatment with ammonia solution can promote the dissolution of biomass waste such as lignin and hemicellulose before the subsequent fermentation (Kurakake et al., 2001; Lee et al., 2010). Aqueous ammonia was used as reaction media for polymer depolymerization

under hydrothermal conditions (Arai et al., 2010; Zenda and Funazukuri, 2008). In this study, ammonia was effective for PLA transformation under the biologically acceptable temperature and pressure. This is a mild reaction condition compared with supercritical or hydrothermal treatment for other polymer materials and the reaction rate was higher than that of compost treatment.

3.2 Effect of ammonia on methane production

Fig.3 shows the ammonia concentration, methane production rate and organic acid concentrations under the OLR of 10.3 g COD/(L day) in the three methane fermentation reactors. The average TAN in Run a7 of the M_0 reactor was 1210 mg N/L and the average methane production rate was 2.7 L CH_4 /(L day). NH_4Cl was added into S_1 in Runs b8 and b9, and the ammonia concentration in M_1 was correspondingly increased. The average methane production rate was 2.9, 3.1 and 3.3 L CH_4 /(L day) in Runs b7, b8 and b9, respectively. The increase in methane production was attributed to the improvement of PCOD dissolution and PLA transformation caused by ammonia addition in S_1 since anaerobic digestion of solid wastes is rate-limited by the hydrolysis step; and promotion of solubilization can improve the overall system performance (Mata-Alvarez et al., 2000). The ammonia concentration in M_2 increased from 824 mg N/L in Run c7 to 871 mg N/L in Run c8. The methane production rate of M_2 increased

slightly from 3.0 CH_4/(L day) in Run c7 to 3.1 L CH_4/(L day) in Run c8. However, the ammonia concentration in Run c9 increased to 2010 mg N/L, and the methane production rate decreased to 2.8 L CH_4 /(L day). This was due to an inhibiting effect of ammonia on methane fermentation (Kadam and Boone, 1996). The average FAN concentration in M_1 was 148 (Run b7), 209 (Run b8) and 229 (Run b9) mg N/L, respectively. The FAN in M_2 was 270 (Run c7), 274 (Run c8) and a much higher value of 422 (Run c9) mg N/L. FAN is the active component which causes inhibition (Hansen et al., 1998; Sterling et al., 2001). Except in Run c9 (reactor M_2), the lactic acid concentration in the three reactors was lower than 1 g COD/L, indicating that the lactic acid generated from KG and PLA hydrolysis was transformed to methane gas. In all runs, the total organic acid concentrations in M_1 were lower than those in the control reactor and M_2. An accumulation of acetic acid was observed in M_2 and this was the premier indicator of an ammonia inhibition as acetate-utilizing methanogens are most easily inhibited (Angelidaki and Ahring, 1993). The deterioration of M_2 performance corresponded with the fact that ammonia was a significant inhibitor of methane production (Sung and Santha, 2003). Addition of ammonia in the hyperthermophilic reactors can promote the PLA hydrolysis rate, however the ammonia concentration should be controlled with caution to avoid the adverse effect on methane production in

the methane fermentation reactors.

3.3 Dewatering property of the sludge in thermophilic reactors

Dewatering property of sludge is a factor affecting the economic benefit of practical AD processes. Fig. 4 shows the results of CST measurement tests in the three methane fermentation reactors. There was a linear relationship between VS/TS and CST/TS. The CST/TS ratio of M_1 and M_2 was lower than that of the control reactor, which indicated that the dewatering property was improved after hyperthermophilic treatment was introduced. The deterioration in dewatering properties is associated with the accumulation of proteins and polysaccharides in the colloidal size fraction (Bivins and Novak, 2001). Consequently, reduction of the colloidal materials may improve the dewatering property. There are two possible reasons for the improvement of dewatering property with hyperthermophilic treatment. One is the increase of the overall system performance such as organic proportion removal; and the efficient removal of larger proportion of colloidal materials. The other is that more filamentous bacteria can be destructed in a higher treatment temperature (Marneri et al., 2009).

3.4 Performance comparison of different digestion systems

The performance comparisons of the three systems are summarized in Fig 5. The

methane conversion ratio was calculated as follows:

$$Methane\,conversion\ ratio\ (\%) = \left(COD\ of\ methane/COD\ of\ substrate\right) \times 100 \quad (5)$$

In each system, the PLA transformation ratio (Fig. 5 (a)) gradually increased, which indicated the acclimation of the microbes to PLA. The two-phase systems had obvious advantage of the overall PLA transformation ratio compared with the control reactor (Fig. 5 (a, b)). This accorded with the previous researches results that higher temperature was favorable for PLA biodegradation (Copinet et al., 2004; Itavaara et al., 2002; Wang et al., 2011).

The methane conversion ratio in the three thermophilic reactors showed a decline in Runs a4, b4 and c4. This was possibly caused by the reduction of SRT because in these runs the effluent was discharged without centrifuge and the solid was not returned to the reactors. The SS concentrations were 30.3 g/L, 20.1 g/L and 18.4 g/L in Runs a3, b3 and c3, respectively, and the values decreased to 15.8, 13.7 and 17.6 g/L in Runs a4, b4 and c4, respectively. After the HRT of the three reactors were increased (OLR was kept constant), the SS concentrations recovered to 25.5, 21.0 and 23.4 g/L in Runs a5, b5 and c5, respectively. Climenhaga and Banks (2008), Nges and Liu (2010) showed that SRT is one of the key factors to keep the high treatment performance. In this study, most methanogens were inactivated in the hyperthermophilic reactors; hence the SRT should

be controlled with more caution in the methanogenic reactor than in traditional two-phase mesophilic or thermophilic treatment. The $M_1 + S_1$ and $S_2 + M_2$ systems had also higher COD removal ratio than the control system. However, the total organic acid and ammonia concentrations in M_2 were higher than those in M_1 and M_0 (Fig. 5 (c, d)). The reactor configuration of the $M_1 + S_1$ system had the advantage over the $S_2 + M_2$ system because the liquor from S_1 with high ammonia concentration was diluted by the fresh substrate. This operation can avoid deterioration of methanogens activity caused by the instant loading of substrate with a high ammonia concentration as that in M_2. In the control reactor, there was no obvious organic accumulation; consequently the lower treatment performance of the control reactor was possibly due to lower hydrolysis efficiency under the thermophilic condition.

Fig. 6 shows the COD balance of the three systems under the same OLR (Runs a7, b9 and c8). The COD balance of the three systems was closed to 100% and the difference between the fresh substrate COD and the total effluent COD due to measurement error was less than 5%. The methane production in hyperthermophilic reactors was less than 1% of the substrate COD and this demonstrated the successful separation of the two phases. The PLA transformation ratio and the methane conversion ratio of the control system were lower than those of the two-phase systems.

In a series of batch experiments conducted in the previous research (Wang et al., 2010), the same plastics were pretreated under the hyperthermophilic condition with ammonia addition and the hydrolysis product was converted to methane gas in the following thermophilic reactor with the ratios of 81.8% (PLA 1) and 77.0% (PLA 2). The present results demonstrated that the continuous anaerobic digestion processes were applicable for PLA degradation with same range of transformation ratio and methane conversion ratio.

3.5 Microbial analysis

The microbial diversity of the three methane fermentation reactors is summarized in Table 3. In each reactor, 96 clones were analyzed. All clones having a sequence similarity of more than 97 % with each other were grouped into an operational taxonomic unit (OTU). The detected OTUs with an occupation ratio over 3 % in each reactor are listed in this table. In the control reactor and M_1, two detected OTUs with the highest occupation were closely related to *Thermotogaceae bacterium FR850164* and *Geobacillus sp. MJU 148-2*. They were reported in thermophilic anaerobic digesters treating excess sludge and cheese whey. In the two reactors, an OTU closely related to *Clostridium sp. PR67* was also detected. In M_2, an OTU closely related to *Geobacillus*

sp. MJU 148-2 dominated (12.5% of the total colons). The other OTUs with occupation ratios over 3% were related to *Thermotogaceae bacterium FR850164* (4.2 % of the total colons), *Clostridium sp. 5-8* (3.1% of the total colons), and *Geobacillus sp. NBM49* (3.1 % of the total colons). The dominant microbes were bacteria which are reported to be surviving in thermophilic anaerobic conditions. Comparing the microbial distribution in the three reactors, there was no obvious difference. This implies that the microbial communities were not affected by the reactor configuration of the three systems markedly. HRT in the hyperthermophilic reactors for single pass was set at 1 d, and this did not possibly affect the microbial diversity. Wang et al. (2011) showed that PLA degradation was partially contributed by biological and/or enzyme activity in the hyperthermophilic treatment by the batch experiments. This hyperthermophilic treatment does not require special bacteria adapted to this extreme temperature condition, but bacteria in the thermophilic condition can be utilized, so the hyperthermophilic solubilization can be easily installed to traditional thermophilic anaerobic digestion plants.

4. Conclusion

The results of this study showed that PLA was degraded to methane in the continuous anaerobic digestion process. The hyperthermophilic treatment promoted the PLA

transformation and methane conversion of the two-phase systems compared with the control reactor. The highest PCOD dissolution ratio and PLA transformation ratio were 25.0 % and 54.9 %, respectively. Under the OLR of 10.3 g COD/(L day), the highest methane conversion ratios in the two-phase systems were 82.9 % and 80.8 %, respectively, higher than that of 70.1 % in the control reactor. The systems including the hyperthermophilic reactors also had the advantage on dewatering property. The microbial analysis indicated that hyperthermophilic treatment does not require special bacteria adapted to this extreme temperature condition, and can be easily installed to traditional thermophilic anaerobic digestion plants.

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Figure captions

Fig.1 Configuration of three systems in the continuous operation. S_1, S_2: Hyperthermophilic reactors; M_0, M_1, M_2: Thermophilic reactors; Q: Substrate flow rate; r: recirculation ratio; $C_{inf,\ X}$ and $C_{enf,\ X}$: PLA concentration in the influent and the effluent of reactor or system X.

Fig. 2 PCOD dissolution and PLA transformation in the hyperthermophilic reactors, (a) PCOD dissolution ratio; (b) PLA transformation ratio. Runs b7 to b9 and c7 to c9 were operated under the same condition except for the addition amount of ammonia in the hyperthermophilic reactors, respectively.

Fig. 3 Methane production rates, ammonia concentration and organic acid concentrations in methane fermentation reactors, (a) Control system; (b) Post-dissolution system; (c) Pre-dissolution system.

Fig.4 Relationship between VS/TS and CST/TS.

Fig. 5 Performance comparison of three digestion systems, (a) PLA transformation ratio; (b) Methane conversion ratio; (c) Total organic acid concentrations; (d) Ammonia concentration.

Fig. 6 COD balance (Runs a7, b9, c8; OLR=10.3 g COD/(L· d)), (a) Control system; (b) Post-dissolution system; (c) Pre-dissolution system

Table 1 Operation condition

Period		0-16	17-170	171-213	214-325	326-464	465-524	525-553	554-568	569-592
Control		Run a1	Run a2	Run a3	Run a4	Run a5	Run a6	----------Run a7----------		
HRT (d)		25	25	25	25	41	30	30		
OLR (g COD/(L day))		3	4.5	6.8	6.8	6.7	6.7	10.3		
(M_1 + S_1)		Run b1	Run b2	Run b3	Run b4	Run b5	Run b6	Run b7	Run b8	Run b9
Overall	HRT (d)	25	25	25	25	41	41	30	30	30
system	OLR (g COD/(L day))	3	4.5	6.8	6.8	6.7	6.7	10.3	10.3	10.3
	r*	1.5	1.5	1.5	1.5	1	1	0.5	0.5	0.5
M_1	HRT (d) **	9.4	9.4	9.4	9.4	20	20	19.7	19.7	19.7
S_1	HRT (d) **	1	1	1	1	1	1	1	1	1
	NH_4Cl addition (g N/L)	No	No	No	No	No	No	No	2	3
(S_2 + M_2)		Run c1	Run c2	Run c3	Run c4	Run c5	Run c6	Run c7	Run c8	Run c9
Overall	HRT (d)	25	25	25	25	41	41	30	30	30
system	OLR (g COD/(L day))	3	4.5	6.8	6.8	6.7	6.7	10.3	10.3	10.3
	r*	1.5	1.5	1.5	1.5	1	1	0.5	0.5	0.5
M_2	HRT (d) **	9	9	9	9	19.5	19.5	19	19	19
S_2	HRT(d) **	1	1	1	1	1	1	1	1	1
	pH control	No	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
	NH_4Cl addition (g N/L)	No	1.5	1.5	1.5	1.5	1.5	1.5	2	3

*: r is defined as the ratio of the recirculation flow rate from S_1 or M_2 to the influent flow rate of the fresh substrate;

**: HRT for single pass.

Table 2 Mixed substrate characteristics

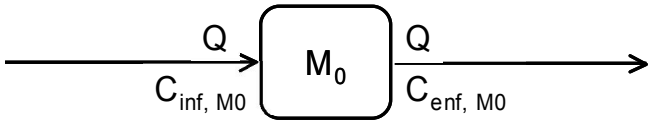
Control	Run a1	Run a2	Run a3	Run a4	Run a5	Run a6	Run a7		
$M_1 + S_1$	Run b1	Run b2	Run b3	Run b4	Run b5	Run b6	Run b7	Run b8	Run b9
$S_2 + M_2$	Run c1	Run c2	Run c3	Run c4	Run c5	Run c6	Run c7	Run c8	Run c9
TS (g/L)	60.6	90.9	136		216	216	248		
VS (g/L)	58.8	78.4	132		210	210	239		
COD of mixture (g/L)	75.1	113	169		276	276	310		
COD of KG (g/L)	60.1	90.4	135		221	221	248		
PLA type	1	1	1		1	2	2		
PLA concentration (g/L)	10.9	16.3	24.5		40	27.6	31.0		

Table 3 Results of clones sequence in three methane fermentation reactors

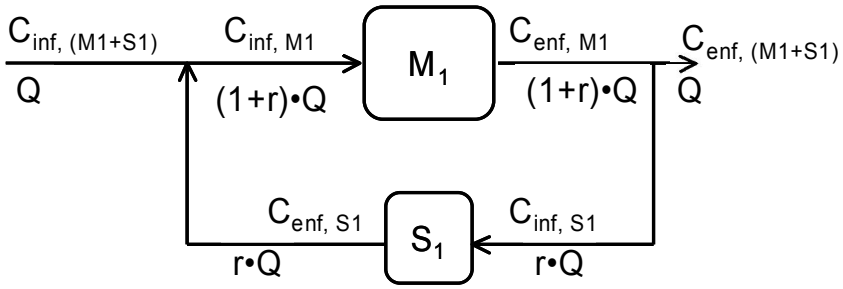
Reactor	OTU	Comment	Similarity (%)	Cloning no.	Occupation ratio* (%)
M_0	FR850164	*Thermotogaceae bacterium*	99.4	13/96	13.5
(Run a-2)	EU093964	*Geobacillus sp. MJU 148-2*	95.9	7/96	7.3
	AB174828	*Clostridium sp. RR67*	95.9	4/96	4.2
M_1	FR850164	*Thermotogaceae bacterium*	99.2	11/96	11.5
(Run b-2)	EU093964	*Geobacillus sp. MJU 148-2*	96.0	10/96	10.4
	AB174828	*Clostridium sp. RR67*	95.9	3/96	3.1
M_2	EU093964	*Geobacillus sp. MJU 148-2*	96.0	12/96	12.5
(Run c-2)	FR850164	*Thermotogaceae bacterium*	99.6	4/96	4.2
	FJ808605	*Clostridium sp. 5-8*	94.0	3/96	3.1
	HQ703944	*Geobacillus sp. NBM49*	96.0	3/96	3.1

* detected clones to total clones

(1) Control system



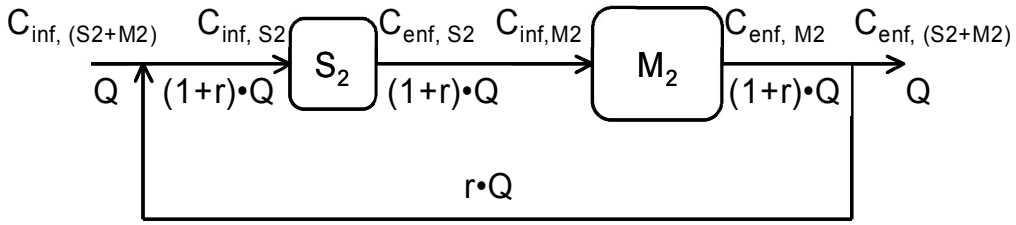
(2) Post-dissolution system (M_1+S_1)



$C_{enf, M1} = C_{enf, (M1+S1)} = C_{inf, S1}$

$C_{inf, M1} = [C_{inf, (M1+S1)} \bullet Q + C_{enf, S1} \bullet r \bullet Q] / [(1+r) \bullet Q]$

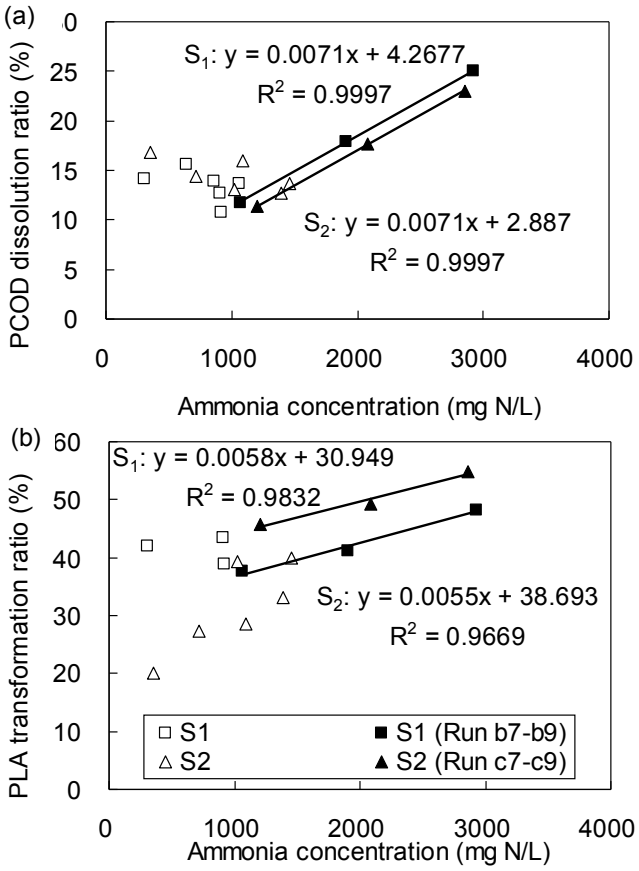
(3) Pre-dissolution system (S_2+M_2)

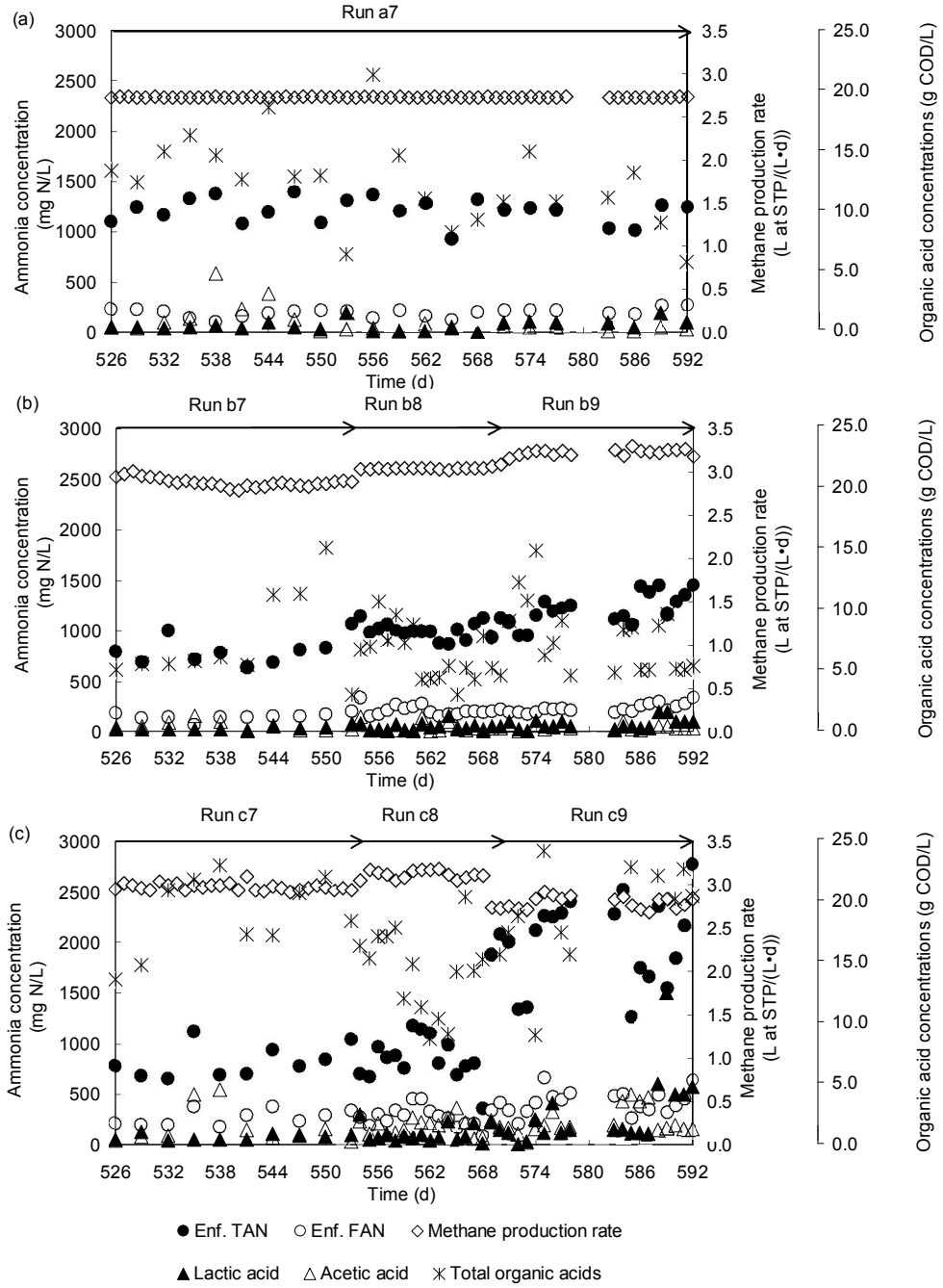


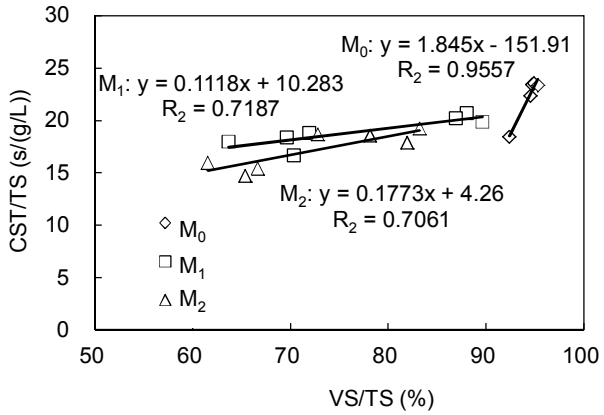
$C_{enf, S2} = C_{inf, M2}$

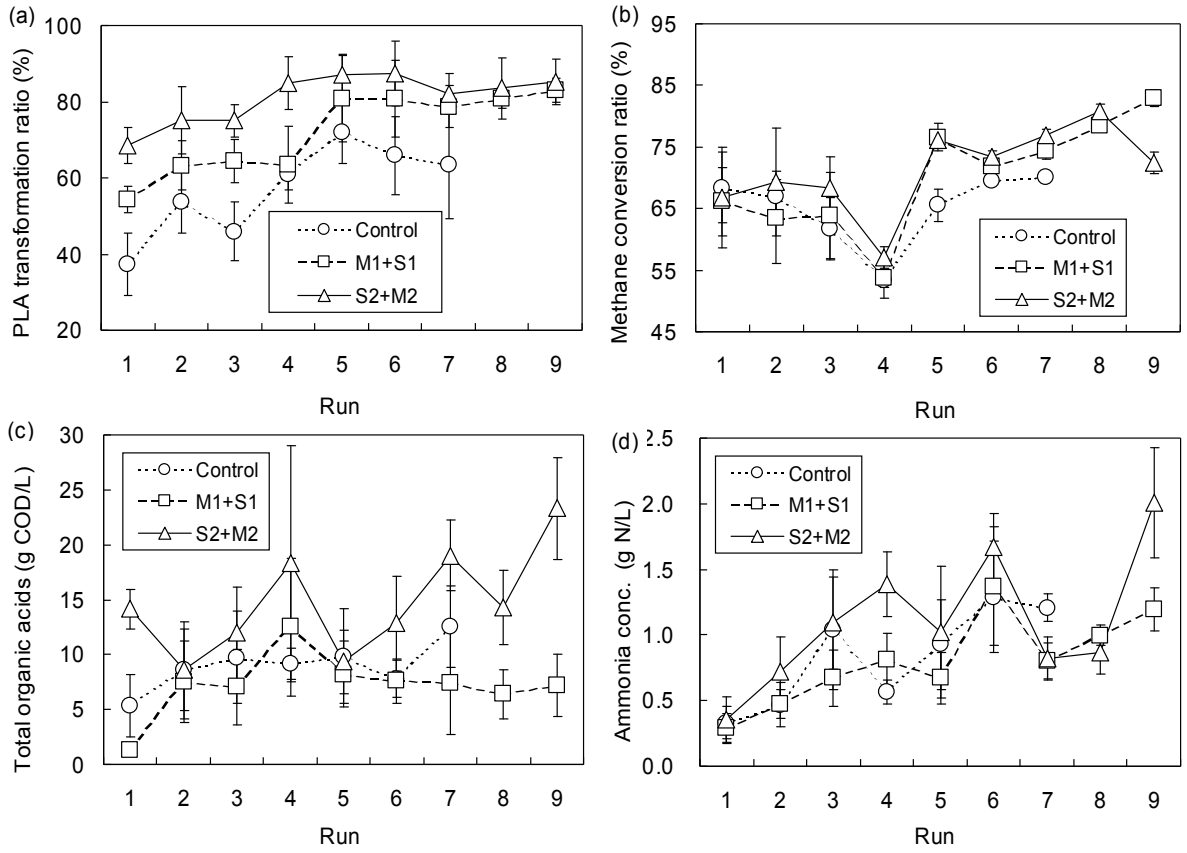
$C_{enf, M2} = C_{enf, (S2+M2)}$

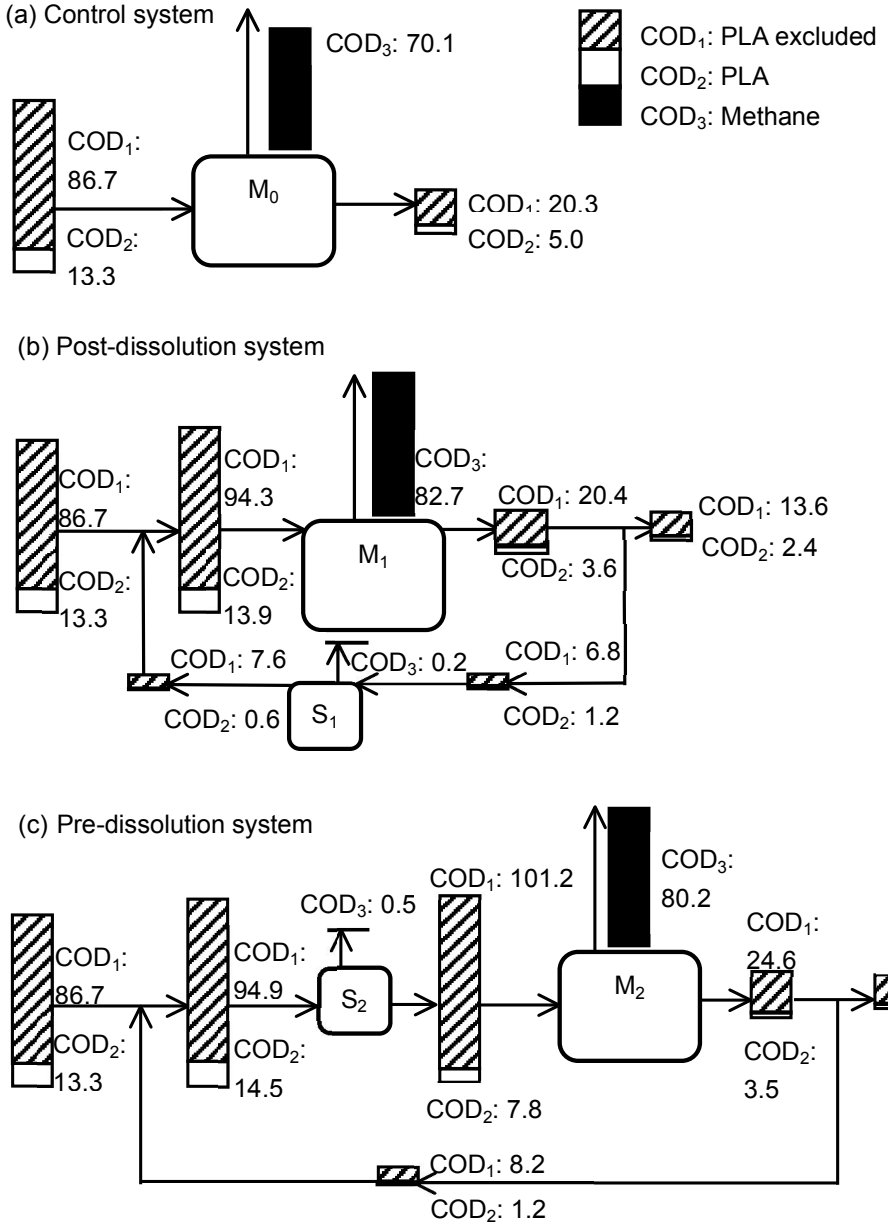
$C_{inf, S2} = [C_{inf, (S2+M2)} \bullet Q + C_{enf, M2} \bullet r \bullet Q] / [(1+r) \bullet Q]$


(a)
PCOD dissolution ratio (%)
S1: y = 0.0071x + 4.2677
R2 = 0.9997
S2: y = 0.0071x + 2.887
R2 = 0.9997
Ammonia concentration (mg N/L)
(b)
PLA transformation ratio (%)
S1: y = 0.0058x + 30.949
R2 = 0.9832
S2: y = 0.0055x + 38.693
R2 = 0.9669
S1
S2
S1 (Run b7-b9)
S2 (Run c7-c9)
Ammonia concentration (mg N/L)


(a)
Run a7
(b)
Run b7
Run b8
Run b9
(c)
Run c7
Run c8
Run c9
Ammonia concentration (mg N/L)
Methane production rate (L at STP/(L•d))
Organic acid concentrations (g COD/L)
Time (d)
Enf. TAN
Enf. FAN
Methane production rate
Lactic acid
Acetic acid
Total organic acids


M0: y = 1.845x - 151.91
R2 = 0.9557
M1: y = 0.1118x + 10.283
R2 = 0.7187
M2: y = 0.1773x + 4.26
R2 = 0.7061
M0
M1
M2
CST/TS (s/(g/L))
VS/TS (%)
30
25
20
15
10
5
0
50
60
70
80
90
100


(a)
PLA transformation ratio (%)
100
80
60
40
20
Control
M1+S1
S2+M2
1 2 3 4 5 6 7 8 9
Run
(b)
Methane conversion ratio (%)
95
85
75
65
55
45
Control
M1+S1
S2+M2
1 2 3 4 5 6 7 8 9
Run
(c)
Total organic acids (g COD/L)
30
25
20
15
10
5
0
Control
M1+S1
S2+M2
1 2 3 4 5 6 7 8 9
Run
(d)
Ammonia conc. (g N/L)
2.5
2.0
1.5
1.0
0.5
0.0
Control
M1+S1
S2+M2
1 2 3 4 5 6 7 8 9
Run


(a) Control system
COD3: 70.1
COD1: PLA excluded
COD2: PLA
COD3: Methane
COD1: 86.7
COD2: 13.3
M0
COD1: 20.3
COD2: 5.0
(b) Post-dissolution system
COD1: 86.7
COD2: 13.3
COD1: 94.3
COD2: 13.9
COD3: 82.7
M1
COD1: 20.4
COD2: 3.6
COD1: 13.6
COD2: 2.4
COD1: 7.6
COD2: 0.6
COD3: 0.2
S1
COD1: 6.8
COD2: 1.2
(c) Pre-dissolution system
COD1: 86.7
COD2: 13.3
COD1: 94.9
COD2: 14.5
COD3: 0.5
S2
COD1: 101.2
COD2: 7.8
COD3: 80.2
M2
COD1: 24.6
COD2: 3.5
COD1: 16.4
COD2: 2.3
COD1: 8.2
COD2: 1.2