土木学会論文集G（環境），Vol.67，No.7，III_267-III_275，2011.

(32) Biodegradation of polylactide under thermophilic and hyperthermophilic anaerobic digestion condition

Feng WANG[1*], Taira HIDAKA[1], Hiroshi TSUNO[1] and Jun TSUBOTA[2]

[1] Department of Environmental Engineering, Kyoto University
(Kyoto-Daigaku-Katsura, Nishikyo-ku, Kyoto 615-8540, Japan)
[2] Energy Engineering Department, Osaka Gas Co., Ltd.
(11-61, Torishima 5-Chome, Konohana-ku, Osaka 554-0051, Japan)
* E-mail: wang.feng.7s@kyoto-u.ac.jp

The biodegradation of polylactide (PLA) under an anaerobic digestion condition was evaluated by batch experiments. It was found that transformation of PLA to lactic acid was obviously promoted and more existence of ammonia enhanced the transformation dramatically. When the PLA was used as the only organic substrate for thermophilic (55°C) anaerobic digestion, it was difficult for microbes to utilize the PLA to produce methane gas. After 60 days treatment, the net methane conversion ratio of the PLA was 11.7%. Co-digestion of the PLA and kitchen garbage (KG) promoted the biodegradation of the PLA with the net methane conversion ratio of 49.8% after 60 days treatment. After hyperthermophilic (80°C) pretreatment with anaerobic digestion sludge and ammonia of 4 g N/L, the biodegradation of the PLA was promoted greatly. The net methane conversion ratio of the PLA was 81.8 % after 22 days thermophilic anaerobic digestion.

***Key Words :** polylactide, thermophilic, hyperthermophilic, anaerobic digestion, biodegradation*

1. INTRODUCTION

Organic wastes such as kitchen garbage (KG) have been suggested as a substrate favorable for anaerobic digestion to generate methane gas [1, 2]. During the collection process of KG, a large amount of plastic used as boxes and bags are mingled in KG. If these boxes or bags are made from non-biodegradable plastic, it is necessary to remove them from KG before anaerobic digestion to avoid negative effect on microorganisms' activity or mechanical troubles in digesters. The separation process brings extra cost for the whole treatment process and hence weakens the economic advantage of anaerobic digestion. An option for solving this problem is to replace the non-biodegradable plastic with polylactide (PLA), which is one of the well-known biodegradable plastics, and to treat KG and PLA together in an anaerobic digestion system to avoid the extra separation cost with an increase of biogas production. Therefore, an effective and economical anaerobic digestion system for KG and PLA is important to be developed on the bases of biodegradation characteristics of PLA.

For last decade, many researches have focused on the PLA biodegradation. Ho and Pometto [3] studied the biodegradation of PLA in soil under different temperatures of 28, 40 and 55°C for 182 days and found that increase in soil temperature drastically enhanced the biodegradation of PLA. Onyari et al. [4] investigated the biodegradation of PLA films using thermophilic organisms and found that the PLA films undergo fast biodegradation at the temperature of 45°C than at the temperature of 37°C. Other researches also showed that the PLA degradation rate increased with the incubation temperature [5,6]. However, the biodegradation period took more than 2 or 3 months, and generally applicable and effective anaerobic digestion methods for PLA have not been proposed yet. Aqueous solutions of ammonia are used as a chemical reaction medium for degradation of polymer materials such as poly-ethylene naphthalate, poly-ethylene terephthalate, poly carbonate and poly-hexamethylene carbonate, but they are accomplished under hydrothermal or supercritical conditions of the temperature of over 120°C and the high pressure of over 10 MPa [7-9]. If PLA is degraded by biological and enzymatic reaction with existence of ammonia under the temperature and the pressure ranges suitable for biological reaction, PLA is transformed to methane easily in an anaerobic digestion process, as some amount of ammonia is produced during anaerobic digestion of KG as the product of protein hydrolysis. However, researches focused on biological degradation of PLA with ammonia under an anaerobic condition are scarce.

Compared with mesophilic (37°C) and thermophilic (55°C) anaerobic digestion, hyperthermophilic (over 55°C) digestion has the advantage on effective solubilization of organic particles [10)]. In our laboratory, a two-phased system of hyperthermophilic/thermophilic (70–80°C/55°C) anaerobic digestion has been developed for KG and sewage sludge treatment [11,12)]. Osumi and Tsubota [13)] showed that the hyperthermophilic treatment enhanced the energy recovery of the anaerobic digestion system and no additional energy is required. For PLA biodegradation under an anaerobic digestion condition, hyperthermophilic treatment is expected to promote the biodegradation extent. However, few researches of the biodegradation of PLA under a hyperthermophilic anaerobic digestion conditions have been published to date.

The objective of this study was to evaluate the biodegradability of PLA under thermophilic (55°C) and hyperthermophilic (80°C) anaerobic digestion conditions and possibility of enhancement by existence of ammonia. For this purpose, batch experiments were performed in order to evaluate possibility of PLA degradation in terms of methane conversion ratio.

2. EXPERIMENTS

(1) Substrate and seed sludge

In the batch experiments, a kind of plastic bag and artificial KG were used as organic substrates. The plastic bag was made of PLA wholly with a thickness of 0.1 mm. It was cut by a shredder (M-450Cs, Fellowes, Japan) into small pieces with the size of 2×2 mm. The composition of the KG was prepared by simulating the actual KG based on a survey in Japan [14)] and consisted of 14 kinds of foods [11)]. The characteristics of the PLA and KG were summarized in Table 1.

Table 1 Characteristic of PLA and KG

KG		PLA	
TS (g/L)	191	TCOD (g/g)	1.38
VS (g/L)	184	TS (g/g)	1.00
SS (g/L)	96.4	VS (g/g)	0.99
VSS (g/L)	94.4		
TCOD (g/L)	230		
SCOD (g/L)	136		
Total nitrogen (mg N/L)	5030		
NH_4^+-N (mg/L)	89.5		

The seed sludge used in the batch experiments was taken from a thermophilic methane fermentation reactor in a hyperthermophilic/thermophilic (80/55°C) anaerobic digestion system operated continuously for one year with the hydraulic retention time (HRT) of 25 d and organic loading rate (OLR) of 6 g COD/(L·d). The substrate of the reactor was a mixture of the cut PLA and the artificial KG mentioned above with a composition of 1:4 (based on TCOD value). The ratio of COD/N of the mixture was 57, which was in a suitable range for anaerobic digestion. The concentrations of SS and VSS of the seed sludge were 19.6±1.2 g/L and 18.1±1.2 g/L, respectively. The sludge used in the batch experiments was prepared by the following procedure: a given volume of sludge taken from the continuous reactor was centrifuged under 3,000 rpm (1,207g) for 10 minutes, and then the solid phase was separated from the supernatant and mixed with hot water at 55°C to the original volume. After fully mixed, the mixture was centrifuged under the same condition and the solid phase was separated again from the supernatant. This operation was repeated once more and the obtained sludge was inoculated as the washed sludge.

(2) Experimental condition

a) PLA dissolution experiments

Transformation of PLA to lactic acid was firstly examined as it is the first step of PLA degradation. Three batch experiments, Experiments S-A, S-B and S-C, were performed to evaluate the dissolution of PLA under hyperthermophilic and thermophilic conditions. The experimental condition is summarized in Table 2. In Experiment S-A, PLA hydrolysis under a thermophilic (55°C) condition was evaluated. The PLA concentration was set at 36 g/L, and the washed sludge was mixed at 17.1 g/L. pH was controlled at 8 by combination of a controller and automatic titration of 10N KOH. NH_4Cl was added to evaluate the effect of ammonia on PLA degradation, and four experiments under different ammonia concentrations of 0, 1, 2 and 4 g N/L were compared. In Experiment S-B, pure water was used to treat the PLA under a hyperthermophilic (80°C) condition, and the ammonia addition and the pH control were same as those of Experiment S-A. The experimental operation of Experiment S-C was same as that of Experiment S-B except that the washed sludge was

Table 2 Experimental operations of PLA dissolution experiments

Experiment No.		Reaction medium	Temperature (°C)	Ammonia addition (g N/L)	pH (-)	Experimental period (d)
S-A	S-A-1	Washed sludge	55	0	8	8
	S-A-2			1		
	S-A-3			2		
	S-A-4			4		
S-B	S-B-1	Water	80	0	8	3
	S-B-2			1		
	S-B-3			2		
	S-B-4			4		
S-C	S-C-1	Washed sludge	80	0	8	3
	S-C-2			1		
	S-C-3			2		
	S-C-4			4		

used to treat the PLA instead of the pure water.

The cylindrical glass reactors with a maximum working volume of 1 L were used. Nutrients necessary for microbe metabolization [15, 16] were added into the solution at the beginning of each batch experiment. The concentrations of the nutrients were as follows: Yeast Extract, 4,500 mg/L; $(NH_4)_2 \cdot HPO_4$, 450 mg/L; $MgSO_4 \cdot 7H_2O$, 90 mg/L; $MnSO_4 \cdot 4H_2O$, 18 mg/L; $FeSO_4 \cdot 4H_2O$, 18 mg/L; $NiCl_2 \cdot 6H_2O$, 0.5 mg/L; $CoCl_2 \cdot 6H_2O$, 10 mg/L. The reactor was closed with a silicon plug after purged with N_2 gas for 5 minutes. Sampling ports for gas and sludge were also equipped in the reactor. The mixing rate was set at 200 rpm by a stirrer.

b) Methane fermentation experiments

Three methanogenesis batch experiments were conducted. The substrates and the washed sludge were mixed, and cultivated under a thermophilic condition. The experimental condition is summarized in Table 3.

In Experiment M-A, 10 g COD/L of PLA was used as an organic substrate. In Experiment M-B, a mixture of PLA (5 g COD/L) and artificial KG (5 g COD/L) was used as organic substrates. In Experiment M-C, the PLA was pretreated under the same condition as the PLA dissolution experiment of Experiment S-C-4 with ammonia addition of 4 g N/L for two days, and then set under a thermophilic methanogenesis condition. The pretreated mixture of PLA and the washed sludge (200 ml) was used as organic substrates. It was mixed with another washed sludge (800 ml) as seed and cultivated under a thermophilic condition. Blank experiments with the washed sludge but without substrate addition were performed in the three experiments.

Table 3 Experimental operations of methane fermentation experiments

Experiment	Substrate	Tempera-true (°C)	Experiment period (d)
M-A	PLA	55	60
M-B	PLA + KG	55	60
M-C	Pretreated PLA	55	22

The reactors used in the methane fermentation experiments and nutrients addition were same as those of the PLA dissolution experiment. The periods of Experiments M-A and M-B, and M-C were 60 days and 22 days, respectively.

(3) Chemical analysis

Lactic acid and other six kinds of organic acids of acetic acid, propionic acid, i-butyric acid, n-butyric acid, i-valerianic acid and n-valerianic acid were measured using HPLC (CDD-10Avp, Shimadzu, Japan). COD, SS, VSS, T-N, NH_4^+-N were measured according the standard method [17]. Dissolved samples were prepared by filtration through a glass filter with a pore size of 1 μm (ADVENTEC). Gas produced was collected by gasbag and analyzed for gas composition by gas chromatography (GC-14B, Shimadzu, Japan) and gas volume was analyzed by syringe.

In Experiments S-A, S-B and S-C, the degree of PLA hydrolysis was evaluated by the ratio of lactic acid generated from PLA to the total PLA added to the reactor. This ratio was defined as the PLA transformation ratio as shown by the following equation under the assumption that the amount of smaller molecule organic acid and gas produced from lactic acid was negligible compared with the produced lactic acid:

$$\text{PLA transformation ratio}\,(\%) = (C_{LCA} / C_{PLA\,added}) \times 100 \qquad (1)$$

Where C_{LCA} (g/L) is the lactic acid concentration at a given time and $C_{PLA\,added}$ (g/L) is the concentration of PLA at the initial time.

The net methane conversion ratio of the substrates in the three methane fermentation experiments was calculated as follows:

$$\text{Net methane conversion ratio}\,(\%) = \frac{COD_{Total\,methane} - COD_{Methane\,from\,sludge}}{COD_{Substrate}} \times 100 \qquad (2)$$

Where $COD_{Total\ methane}$ is the COD of totally generated methane, $COD_{Methane\,from\,sludge}$ is the COD of the methane generated from the blank experiment, and $COD_{substrate}$ is the initial COD of the substrate.

3. RESULTS AND DISCUSSION

(1) PLA hydrolysis under different anaerobic digestion conditions

Fig.1 shows the change in lactic acid concentration in the three PLA dissolution experiments. As shown in the figure, the PLA showed obviously different hydrolysis characteristics under the thermophilic and the hyperthermophilic conditions. In Experiment S-A, the lactic acid was not produced under all the ammonia addition conditions. Methane or other gas production was not also observed during the whole experiment period of 192 h, longer than Experiments S-B, and S-C conducted with the same substrate of the PLA. The addition of ammonia from 1 to 4 g N/L did not promote PLA transformation. Under the thermophilic condition, it was difficult for the PLA to be hydrolyzed to lactic acid, which consequently means the difficult methane fermentation.

Under the hyperthermophilic condition, the PLA was obviously transformed to lactic acid. In Experiment S-B, PLA transformation occurred even in hot water (80°C), without ammonia addition but at a very low lactic acid concentration of 2.3 g/L at the end of experiment. The PLA transformation was promoted by addition of ammonia, and the lactic acid concentration was increased to 7.89 g/L by the addition level of 4g N/L.

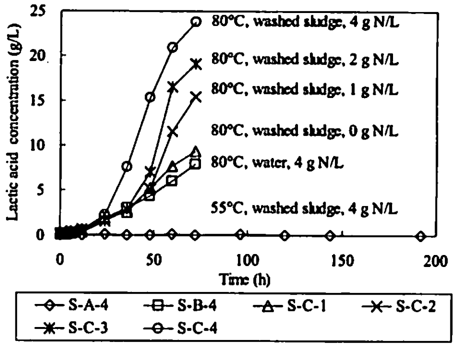

Fig.1 Lactic acid concentration in Experiment S-A, S-B and S-C.

In Experiment S-C in which the washed sludge was used to treat the PLA, the lactic acid concentration was much higher than that in Experiment S-B, and lactic acid production was enhanced much by addition of ammonia. During the process of the four experiments, similar changing trends were observed as follows: the lactic acid concentration increased slowly in the initial 12 hours and then sharply increased while the other organic acids showed no obvious increasing trend. During the whole experimental period of Experiments S-B and S-C, no methane or hydrogen production was observed.

Comparing the final PLA transformation ratio in Experiments S-A, S-B and S-C, which was summarized in Table 4, the hyperthermophilic biological treatment promoted the transformation of the PLA to the final hydrolysis product, lactic acid. Fig. 2 shows the measured ammonia concentration in Experiments S-B and S-C. It was lower than the added value. This could be because ammonia is vaporized under hyperthermophilic conditions, but it was stable during the experiment. The previous studies demonstrated that ammonia solution was effective for the degradation of various poly materials [7-9)], and ammonia attacked at the electron deficient center C–O of polyethylene terephthalate during the depolymerization process at the room temperature [18)]. The mechanisms of PLA hydrolysis with ammonia aqueous were assumed to be same as that of other polymers. But most of the previous hydrolysis processes were accomplished under hydrothermal and supercritical conditions. In our study, the ammonia solution was found to be effective for PLA degradation in anaerobic digestion under the microbial active range of temperature and pressure.

Table 4 Final PLA transformation ratios in Experiments S-A, S-B and S-C

	Period (d)	Ammonia addition (g N/L)	Final PLA transformation ratio (%)
S-A-1	8	0	0
S-A-2		1	0
S-A-3		2	0
S-A-4		4	0.1
S-B-1	3	0	6.4
S-B-2		1	9.6
S-B-3		2	14.6
S-B-4		4	21.9
S-C-1	3	0	25.7
S-C-2		1	42.4
S-C-3		2	52.5
S-C-4		4	65.2

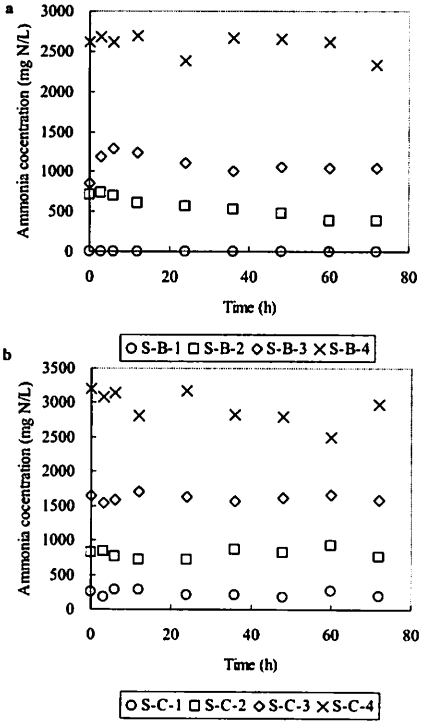

Fig. 2 Measured ammonia concentration in Experiments S-B and S-C, a: Experiment S-B; b: Experiment S-C.

(2) Biodegradation of PLA as the only organic substrate

Fig.3 shows the changes in net methane conversion ratio, organic acids and SCOD in Experiment M-A. The total organic acids were the sum of the seven kinds of organic acids measured. Other SCOD (O-SCOD) was calculated by the difference between SCOD and the total organic acids measured. The net methane conversion ratio of the PLA increased quickly during days 8–20. After that, the methane production got stable with a slight increase. It increased slowly from 7.5 % at day 20 to the final value of 11.7 % at day 60. The low net methane conversion ratio of PLA indicated that it was difficult for microbes to utilize the PLA to generate methane gas under the thermophilic condition when the PLA was used as the only organic substrate.

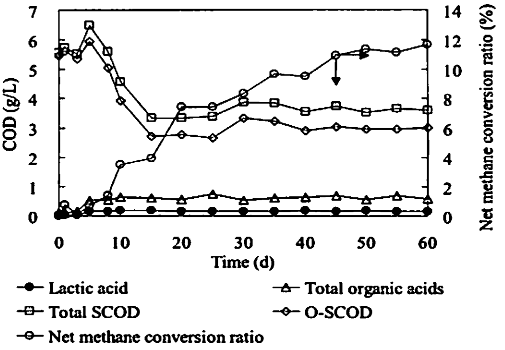

Fig.3 Changes in net methane conversion ratio, organic acids and SCOD during Experiment M-A.

The concentration of lactic acid, which is the final product of PLA hydrolysis, was 0.03 g COD/L at the beginning of the experiment. It slowly increased to the highest value of 0.19 g COD/L at day 15 and then decreased slightly to the final value of 0.17 g COD/L at day 60. The total organic acids concentration increased from 0.12 g COD/L at the beginning of the experiment to the highest value of 0.75 g COD/L at day 25. After that, the total organic acids concentration decreased slightly to the final value of 0.58 g COD/L. The SCOD at the beginning of the experiment was 5.57 g/L, and it increased to the highest value of 6.49 g/L at day 5 and then decreased gradually to the value of 3.34 g/L at day 15. After that, the SCOD concentration got stable in the range of 3.3–3.9 g/L. The O-SCOD concentration variation trend was in accordance with the SCOD variation trend. The value increased from 5.5 g COD/L at the beginning of experiment to the highest value of 6.0 g COD/L at day 5. Then it decreased gradually to the value of 2.7 g COD/L at day 15 and then got stable. The variation of the O-SCOD and the total organic acids indicated that a part of dissolved matters from PLA was difficult to be transformed to organic acids during the experiment process. The acids concentration and the SCOD variation indicated that there was no acids accumulation in the reactor during the whole experiment. These results showed that the low methane conversion ratio was caused by the low transformation of PLA to organic acids under the thermophilic condition.

(3) Co-digestion of PLA and kitchen garbage under thermophilic condition

Experiment M-B was performed to evaluate the biodegradability of PLA under the co-digestion condition with KG. The changes in the net methane conversion ratio, organic acids and SCOD are shown in Fig.4.

The net methane conversion ratio increased to 61.0 % before day 20, and then increased slowly to the final value of 64.9 % at day 60. The lactic acid concentration was 0.04 g COD/L at the beginning of the experiment. It increased to the highest value of 0.31 g COD/L at day 40 and then decreased gradually to the final value of 0.10 g COD/L. The total organic acids concentration increased from 1.38 g COD/L at the beginning of the experiment to the highest value of 3.05 g COD/L at day 8. After that, the total organic acids concentration decreased gradually to the final value of 1.27 g COD/L. The SCOD was 5.95 g/L at the beginning of the experiment, and it increased to the highest value of 8.67 g/L at day 3 and then decreased gradually to the value of 3.57 g/L at day 25. After that, the SCOD concentration got stable. The O-SCOD variation trend was in accordance with that of the total SCOD. The value increased from 4.57 g COD/L at the beginning to the highest value of 6.37 g COD/L at day 3. Then it decreased gradually to the value of 1.02 g COD/L at day 25 and then got stable.

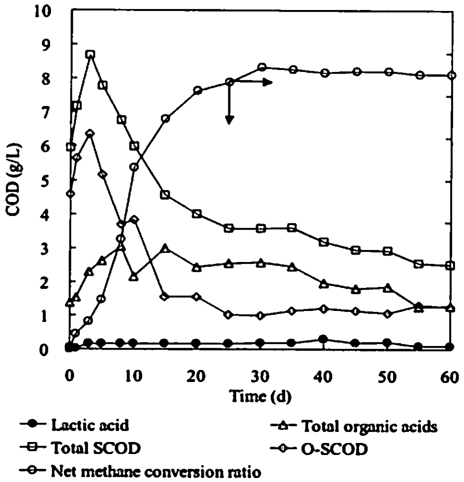

Fig.4 Changes in net methane conversion ratio, organic acids and SCOD during Experiment M-B

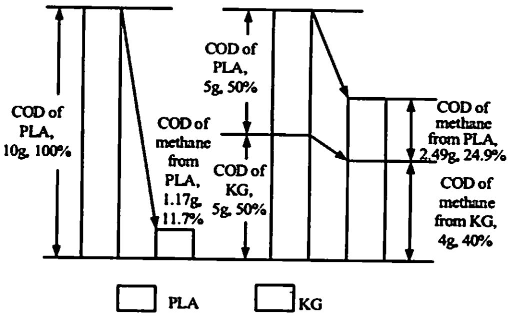

Fig.5 COD balance in Experiment M-A and M-B

Fig.5 shows the net methane conversion ratio of PLA in Experiments M-A and M-B. Park et al. [19] studied the methane conversion ratio of the KG with the same composition using single/two-phase anaerobic digestion systems and achieved the average methane conversion ratio of about 80%. In Experiment M-B, the COD of KG took half of the total substrate COD. Assuming that the methane conversion ratio of the KG was also 80%, the methane generated from the KG was 40% of the total substrate COD and the methane from the PLA was 24.9% of the total substrate COD. This means that the net methane conversion ratio of the PLA was 49.8%, which was much higher than the value of 11.7 % in Experiment M-A. This indicated that co-digestion improved the biodegradability of the PLA.

The results of Experiments M-A and M-B show that co-digestion of PLA with KG is a promising option to improve the performance of anaerobic digestion of PLA. The benefits of co-digestion include dilution of potential toxic compounds, improved balance of nutrients, synergistic effects of

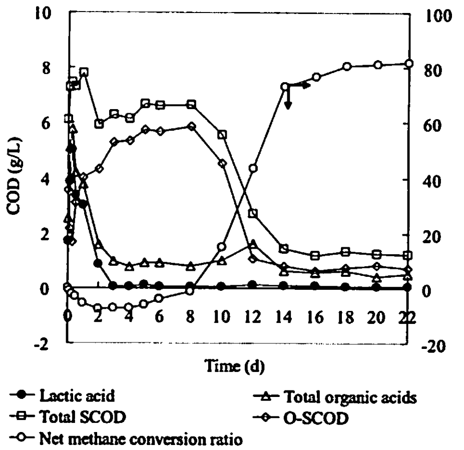

Fig.6 Changes in net methane conversion ratio, organic acids and SCOD during Experiment M-C

microorganisms, increased load of biodegradable organic matter and better biogas production [20]. The difference of methane conversion ratios of the PLA under those two experiments can be explained by the mechanisms of microbial co-metabolism, i.e. microbial transformation of a non-growth substrate in the obligate presence of a growth substrate. When the PLA was used as the only substrate, the PLA was more stable than the KG due to the thermal and mechanical property and the activity of microbes was low because of lack of bioavailable components for metabolisms. Under the co-digestion condition, the addition of KG can maintain the activity of microbes and supply the necessary nutrient for metabolisms.

(4) Promotion effect of hyperthermophilic pretreatment on PLA biodegradation

In Experiment M-C, the hyperthermophilic pretreatment with ammonia addition was performed to promote the PLA biodegradation. The results are shown in Fig.6. After the pretreatment, the biodegradation extent of the PLA was much higher than those in Experiments M-A and M-B. In the initial 8 days, the net methane conversion ratio of the PLA was minus because the total methane production was lower than that in the blank experiment. This indicated that some inhibition effect possibly caused the low methane production when the PLA pretreated under the hyperthermophilic condition was used as the substrate for methane fermentation.

At the beginning of the experiment, the total organic acids concentration was 2.53 g COD/L and pH value was 7.83. The organic acids concentration increased to the highest value of 5.77 g COD/L at hour 6. The pH value decreased to the lowest value of 6.91 at hour 6, and then pH gradually recovered to the value of 7.81 until day 8. The decrease of pH was possibly caused by the high organic acids concentration and it would take several days for microbes to get acclimatized to the high organic acids concentration. The net methane conversion ratio of the PLA increased sharply to the value of 73.0% at day 14 and then increased gradually to the final value of 81.8% at day 22. The lactic acid concentration was 1.72 g COD/L at the beginning of the experiment. It increased to the highest value of 5.03 g COD/L at hour 6 and then decreased to the value of 3.03 g COD/L at hour 24. The lactic acid continued to decrease to 0.89 g COD/L at day 2 and to the final value of 0.07 g COD/L at day 22. The total organic acids concentration increased from 2.53 g COD/L at the beginning of the experiment to the highest value of 5.77 g COD/L at hour 6. After that, the total organic acids concentration decreased gradually to the final value of 0.51 g COD/L at day 22. The SCOD was 6.10 g/L at the beginning of the experiment, and then increased to the highest value of 7.78 g/L at day 1 and then decreased gradually to the value of 2.71 g/L at day 12. After that, the SCOD concentration got stable. At the beginning of the experiment, the O-SCOD was 3.57 g COD/L. It decreased to the value of 1.68 g COD/L at hour 6 then increased gradually to the value of 5.85 g COD/L at day 8. After day 8, O-SCOD decreased sharply to the value of 1.07 g COD/L at day 12 and then the value got stable. The increase of O-SCOD from hour 6 to day 8 was possibly caused by the quick release of the hydrolysis products of the PLA. After day 8, the SCOD decreased sharply while the total organic acids were stable which indicated the rate of methane production was equal to the rate of organic acids production from the PLA hydrolysis products.

The promotion effect of the hyperthermophilic pretreatment can be explained as follows: The matrix of PLA is firstly broken by the hydrolytic degradation and oligomers are released to the solution as the hydrolysis products [3]. When the PLA without hyperthermophilic pretreatment was used as the only organic substrate (Experiment M-A), the released hydrolysis products were difficult to be transformed to organic acids. This was proved by the fact that the O-SCOD concentration was stable after day 15 and it was the dominant part of the total SCOD (Fig.2). Furthermore, there was no obvious organic acids accumulation during the whole experiment period. Methanogenesis is inhibited when the ammonia concentration exceeds a certain threshold value [21]. Fig. 7 shows the measured ammonia concentration in the three methane fermentation experiments. The ammonia concentrations were lower than 300 mg N/L; these low concentrations were not associated with any obvious inhibition to methane production [22].

After the hyperthermophilic pretreatment, the biodegradability of the PLA was promoted and this agreed with the fact that there was an obvious increase of O-SCOD in the following thermophilic anaerobic digestion caused by the quick release of the PLA hydrolysis products. These products can be further transformed to organic acids and then the final product of methane gas. This well agrees with the fact that lactic acid and O-SCOD showed increase followed by decrease and the net methane conversion ratio showed rapid increase with the decrease of O-SCOD. In practical anaerobic digestion of KG, pretreatment of boxes and bags made of PLA is required, because they may get tangled in mixers or pumps. The developed method

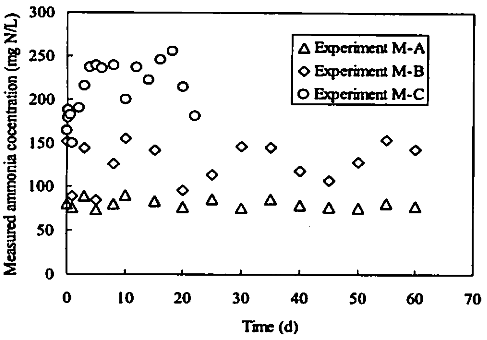

Fig.7 Measured ammonia concentration in Experiments M-A, M-B and M-C

can make anaerobic digestion processes of KG and other organic waste more economical by co-digestion of PLA and other organic waste without the separation of PLA mingled in collection.

4. CONCLUSION

In this research, biodegradation of the PLA under different anaerobic digestion condition was studied by the batch experiments. Hyperthermophilic condition can promote hydrolysis of PLA to lactic acid and more existence of ammonia can enhance the hydrolysis dramatically. The methane fermentation experimental results demonstrated that under the thermophilic anaerobic digestion, the PLA was transformed to methane gas, but that the net methane conversion ratio was only 11.7% for 60 days treatment. Co-digestion of the PLA with the KG increased the net methane conversion ratio of the PLA to 49.8% for 60 days treatment. Hyperthermophilic biological pretreatment can promote the transformation of PLA to the hydrolysis product, lactic acid, greatly. After two days pretreatment with ammonia addition of 4 g N/L, the PLA was easily utilized by the microbes to produce methane gas in the following thermophilic anaerobic digestion. In this situation, the net methane conversion ratio of the PLA was 81.8% after 22 days fermentation. The anaerobic treatment of PLA can make the treatment process of KG more economical by co-digestion of PLA without the separation of PLA mingled in collection process.

5. ACKNOWLEDGEMENTS

The authors would like to express the deepest respect and appreciation to Mr. Takumi OISHI, for his contributions in experiment.

REFERENCES

1) Kim, H.W., Han, S.K., Shin, H.S.: The optimisation of food waste addition as a co-substrate in anaerobic digestion of sewage sludge. *Waste Manage.*, Vol.21, pp. 515-526, 2003.

2) Hong, F., Tsuno, H., Hidaka, T. and Cheon, J.: Study on applicability and operation factor of thermophilic methane fermentation to garbage treatment in high concentration under once-a-day feeding condition, *Environ. Eng. Res.*, Vol. 40, pp. 333-342, 2003.

3) Ho, K.L.G., Pometto, A.L.: Temperature effects on soil mineralization of polylactic acid plastic in laboratory respirometers, *J. Polym. Environ.*, Vol.7, pp. 101-108, 1999.

4) Onyari, J.M., Mulaa, F., Muia, J., and Shiundu., P. : Biodegradability of Poly (lactic acid), Preparation and Characterization of PLA/Gum Arabic Blends. *J. Polym. Environ.*, Vol.16, pp. 205-212, 2008.

5) Copinet, A., Bertrand, C., Govindin, S., Coma, V., and Couturier, Y. : Effects of ultraviolet light (315 nm), temperature and relative humidity on the degradation of polylactic acid plastic films. *Chemosphere*, Vol. 55, pp. 763-773, 2004.

6) Dunne, M., Corrigan, OI., and Ramtoola, Z.: Influence of particle size and dissolution conditions on the degradation properties of polylactide-co-glycolide particles. *Biomaterials*, Vol. 21, pp. 1659-1668, 2000.

7) Zenda, K. and Funazukuri, T.: Depolymerization of poly (ethylene terephthalate) in dilute aqueous ammonia solution under hydrothermal conditions. *J. Chem. Technol. Biotechnol.*, Vol. 83, No. 10, pp. 1391-1396, 2008.

8) Arai, R., Zenda, K., Hatakeyama, K., Yui, K., and Funazukuri,T.: Reaction kinetics of hydrothermal depolymerization of poly (ethylene naphthalate), poly (ethylene terephthalate), and polycarbonate with aqueous ammonia solution. *Chem. Eng. Sci.*, Vol. 65, No. 1, pp. 36-41, 2010.

9) Mormann, W. and Spitzer, D., Fractionation and ammonolyticdegradation of poly (hexamethylene carbonate) in (supercritical) ammonia. *E-Polymers*, Vol. 12, 2004. URL: http://www.e-polymers.org/journal/download.cfm?abstract_Id=574&pdf_name=mormann_100204.pdf

10) Scherer, P.A., Vollmer, G.R., Fakhouri, T., and Martensen, S.: Development of a methanogenic hyperthermophilic conditions. *Water Sci. Technol.*, Vol. 41, No. 3, pp. 83-91, 2000.

11) Lee, M., Hidaka, T. and Tsuno, H.: Effect of temperature on performance and microbial diversity in hyperthermophilic digester system fed with kitchen garbage. *Bioresour. Technol.*, Vol. 99, No. 15, pp. 6852-6860,2008.

12) Lee, M., Hidaka, T., Hagiwara, W. and Tsuno, H.: Comparative performance and microbial diversity of hyperthermophilic and thermophilic co-digestion of kitchen garbage and excess sludge. *Bioresour. Technol.*, Vol. 100, No. 2, pp. 578-585, 2009.

13) Osumi, S. and Tsubota, J.: Estimation of improved energy balance of anaerobic digestion plant by hyper thermal hydrolysis. *J. Jpn. Institute Energy.*, Vol. 89, No.2, pp.

167-171, 2010.

14) Tanigawa, N., Takemoto, T., Ohki, H., and Kawasaki, T.: Detailed component of garbage. *J. Jpn. Waste Manage. Assoc.*, Vol. 50, pp. 116-119, 1997.

15) Hidaka, T., Horie, T., Akao, S., and Tsuno, H.: Kinetic model of thermophilic L-lactate fermentation by Bacillus coagulans combined with real-time PCR quantification. *Water Res.*, Vol. 44, pp. 2554-2562, 2010.

16) Leighton, I.R. And Forster, C.F.: Effect of heavy metals on acidogenesis and volatile fatty acid degradation in thermophilic anaerobic digestion. *Process Saf. Environ.*, Vol. 73, pp. 285-289, 1995.

17) APHA, AWWA, WEF. Standard Methods for the examination for Water and Wastewater, APHA: MD, 1998.

18) Mittal, A., Soni, R.K., Dutt, K., Singh, S.: Scanning electron microscopic study of hazardous waste flakes of polyethylene terephthalate (PET) by aminolysis and ammonolysis. *J. Hazard. Mater.* Vol. 178, 390-396, 2010.

19) Park, Y.J., Hong, F., Hidaka, T. and Tsuno, H.: Comparison of thermophilic anaerobic digestion characteristics between single-phase and two-phase systems for kitchen garbage treatment. *J. Biosci. Bioeng.*, Vol. 105, pp. 48-54, 2008.

20) Sosnowski, P., Wieczorek, A. and Ledakowicz, S.: Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes. *Adv. Env. Res.*, Vol. 7, pp. 609-616, 2003.

21) Siles, J.A., Brekelmans, J., Martin, M.A., Chica, A.F., Martin, A.: Impact of ammonia and sulphate concentration on thermophilic anaerobic digestion. *Bioresour. Technol.* Vol. 101, 9040-9048, 2010.

22) Angelidaki, I. And Ahring, B.K.: Thermophilic anaerobic digestion of livestock waste: the effect of ammonia. *Appl. Microbiol. Biotechnol.* Vol. 38, 560-564, 1993.

(Received May 30, 2011)