Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 19(2007) 416–420
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Start-up performances of dry anaerobic mesophilic and thermophilic digestions of organic solid wastes

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Received 19 May 2006; revised 16 August 2006; accepted 22 September 2006

Abstract

Two dry anaerobic digestions of organic solid wastes were conducted for 6 weeks in a lab-scale batch experiment for investigating the start-up performances under mesophilic and thermophilic conditions. The enzymatic activities, i.e., β-glucosidase, N-α-benzoyl-L-argininamide (BAA)-hydrolysing protease, urease and phosphatase activities were analysed. The BAA-hydrolysing protease activity during the first 2–3 weeks was low with low pH, but was enhanced later with the pH increase. β-Glucosidase activity showed the lowest values in weeks 1–2, and recovered with the increase of BAA-hydrolysing protease activity. Acetic acid dominated most of the total VFAs in thermophilic digestion, while propionate and butyrate dominated in mesophilic digestion. Thermophilic digestion was confirmed more feasible for achieving better performance against misbalance, especially during the start-up period in a dry anaerobic digestion process.

Key words: anaerobic digestion; enzymatic activity; mesophilic; solid wastes; thermophilic

Introduction

Biological treatment has been demonstrated as one of the most advantageous methods for maximizing recycle and recovering its components. Anaerobic digestion of sorted organic fraction of municipal solid wastes, especially food wastes, is the utmost attractive alternative and the most cost-effective technology (Baere, 2000; Edelmann *et al*., 2000). Generally, the overall anaerobic organic solid digestion can be roughly classified into hydrolysis, acidogenesis, acetogenesis and methanogenisis (Veeken *et al*., 2000), each metabolic stage is functioned by a series of microorganisms. In these four stages, hydrolysis, which includes various enzyme functions involved carbon, nitrogen and phosphorus cycles, is reported as the most rate limiting stage (Vavilin *et al*., 1996; Christ *et al*., 2000). In addition, it is usually difficult to maintain an appropriate circumstance to each microorganism and the problems caused by misbalance of production and consumption of intermediary products often occur in practical anaerobic digestion process, especially during the start-up period. Therefore, whether or not the anaerobic digestion process can restore its efficiency from the misbalance circumstance, such as the lower pH condition owning to the accumulation of volatile fatty acids (VFAs) and the consumption of alkalinity, and progress smoothly and quickly toward a better performance in a completely mixed one phase digester is very important. Accordingly, investigating enzymatic activities related to carbon, nitrogen, and phosphorus cycles will give some important indices for anaerobic digestion performance (Benitez *et al*., 1999).

One of the most important factors affecting anaerobic digestion of organic solid wastes is temperature (Ahring, 1994; Cheunbarn and Pagilla, 2000). Generally, anaerobic digestion process is operated under mesophilic or thermophilic condition, in which thermophilic digestion is reported more efficient method (Griffin *et al*., 1998; Ahring *et al*., 2001). On the other hand, compared with wet anaerobic digestion, dry anaerobic digestion is beneficial to its compact digester with high organic loading rate and its energetically effective performance (Pavan *et al*., 2000; Kuroshima *et al*., 2001). This process also results in a lower outcome of leachate and easy handle of digested residues that can be further treated by composting process or be used as fertilizer (Brummeler, 2000). So far, few reports can be found on the study of dry anaerobic digestion of food wastes, and the explanation of solid wastes anaerobic digestion performance in the start-up period from the viewpoint of enzymatic activity is not extensively investigated yet. Hence, the aims of this study were to investigate the start-up performance of dry anaerobic digestion with the emphasis on the enzyme activities of microorganisms by co-digestion of food wastes and sewage sludge under meso- and thermo-philic conditions in two completely mixed one phase anaerobic digesters in

Project supported by the Grant-in-Aid for Science Research of Japan Society for the Promotion of Science (JSPS), Japan. *Corresponding author. E-mail: lvshuguang@ecust.edu.cn.

a lab-scale batch experimental process.

1 Materials and methods

1.1 Experimental layout

The experiments were conducted in two 8.0 L lab-scale anaerobic digesters each with a working volume of 4.5 L. Instead of food residue, dog food, crashed homogeneously, was used as feedstock to simulate food wastes to some extent. The dog food used in the experiment consisted of 25% protein, 28% carbohydrate, 8% lipid and some essential trace nutrients, respectively. Woodchip as bulking material and excess sludge from municipal wastewater treatment plant (Ube, Yamaguchi Prefecture, Japan) with 93% of water content as co-digestion substrate were mixed thoroughly with dog food. The total wet weight of completely mixed mixtures which was similar to the dry organic fraction of municipal solid wastes in each reactor was 1310 g with the moisture of 57%, in which dog food 540 g, woodchip 50 g, and excess sludge 720 g. Two digesters were separately put into 35 and 55°C water bath for maintaining mesophilic and thermophilic conditions. The experiments lasted 6 weeks. The mixtures were completely mixed once a day manually and sampling was taken on 0, 2, 5, 9, 14, 21, 28, 35, and 42 d. Subsequently, the aqueous extraction was made by mechanically shaking the samples (10 g) at 150 r/min with 100 ml deionized water for 2 h at room temperature. The suspension was centrifuged at 12000 r/min for 10 min. Supernatant was filtered through 0.45 μm filter paper and then analyzed further.

1.2 Analytical methods

Samples were oven dried for 24 h at 105°C for moisture determination, and for 8 h at 550°C after oven drying for ash measurement. Total wet weight loss was calculated from weighing of the filled digester and the amounts of taken sampling. The pH was measured immediately after the sample was extracted by deionized water. Filtered samples were analyzed for total organic carbon (TOC), volatile fatty acid (VFA), alkalinity, total nitrogen (T-N), ammonium and nitrite or nitrate nitrogens. TOC was determined by Shimadzu TOC-5000 analyzer. GC-8APF/FID was used for VFA analysis. T-N, NH_4-N, NO_2-N, NO_3-N and alkalinity were analyzed according to the Standard Method (APHA, 1995).

1.3 Enzymatic activity analytical procedure

All samples for enzymatic activity analyses were air dried at least one day under 30°C, then ground and sieved through 1 mm pore size mesh. Assays of hydrolases activities, including β-glucosidase, N-α-benzoyl-L-argininamide (BAA)-hydrolysing protease, urease and phosphatase activities, were as described by Garcia-Gil *et al*. (2000).

Urease and BAA-hydrolysing protease activities were determined in 0.1 mol/L phosphate buffer at pH 7, and 1 mol/L urea and 0.03 mol/L BAA were used as substrates, respectively. Two millimeter of buffer and 0.5 ml of substrate were added to 0.5 g of the prepared sample and then incubated at 30°C for urease or 37°C for protease for 90 min. Both activities were determined by NH_4-N released.

Phosphatase and β-glucosidase activities were determined using *p*-nitrophenyl phosphate disodium (*p*-NPP, 0.115 mol/L) or *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 mol/L) as substrates, respectively. These assays are based on the release and detection of *p*-nitrophenol (PNP). Two milliliters of 0.1 mol/L maleate buffer (pH 6.5 for both phosphatase and β-glucosidase activities) and 0.5 ml of substrate were added to 0.5 g of sample and incubated at 37°C for 90 min. The reaction was stopped by cooling rapidly to 2°C for 15 min, 0.5 ml of 0.5 mol/L $CaCl_2$ and 2 ml of 0.5 mol/L NaOH were then added and the mixture was centrifuged at 4000 g for 10 min. To stop the β-glucosidase assay, trashy-droxymethyl aminomethane instead of NaOH was used. The amount of PNP was determined using a spectrophotometer at 398 nm.

The control for the enzymatic assays was conducted under the same procedure by adding the substrate to the sample after incubation and immediately prior to stopping the reaction.

2 Results and discussion

2.1 Start-up performances of meso- and thermo-philic anaerobic digestions

The start-up performances of two batch dry anaerobic digestions under meso- and thermo-philic conditions are shown in Fig.1. The pH decreased from the beginning of 6.7 to 4.2 under mesophilic condition and to 5.8 under thermophilic condition in two weeks, then gradually restored to 7.6 after 4 weeks in 35°C digester and 8.0 after 3 weeks in 55°C digester. The initial decrease of pH was due to the lower original alkalinity (3000 mg/L as $CaCO_3$) in the mixtures for pH buffering. Biodegradable organic solid wastes were gradually hydrolysed and degraded, resulting in the final dry weight loss of 48% and 50% in correspondence with the increase of ash from 8% to 15% and 17% of dry weight for mesophilic and thermophilic digestions, respectively.

The organic solid components were generally hydrolysed and decomposed into water-soluble matters by secreted microbial enzymes, and subsequently degraded or absorbed into the cells by microbial activation. Therefore, the analyses after water extraction from mixtures are useful indices for monitoring the progress of dry anaerobic digestion. During the first 5 d, the soluble TOC increased rapidly and reached 79 mg TOC/g dw or 122 mg TOC/g dw in meso- or thermo-philic digester, particularly more organic solid wastes were hydrolysed in thermophilic condition owing to the high temperature. Thereafter soluble TOC concentration maintained steady until week 4 (35°C) or week 3 (55°C), and began to increase slightly again, finally both reached the same value of 142 mg TOC/g dw. Correlated to soluble TOC, the same trend was also found in deionized water extracted T-N results owing to the soluble

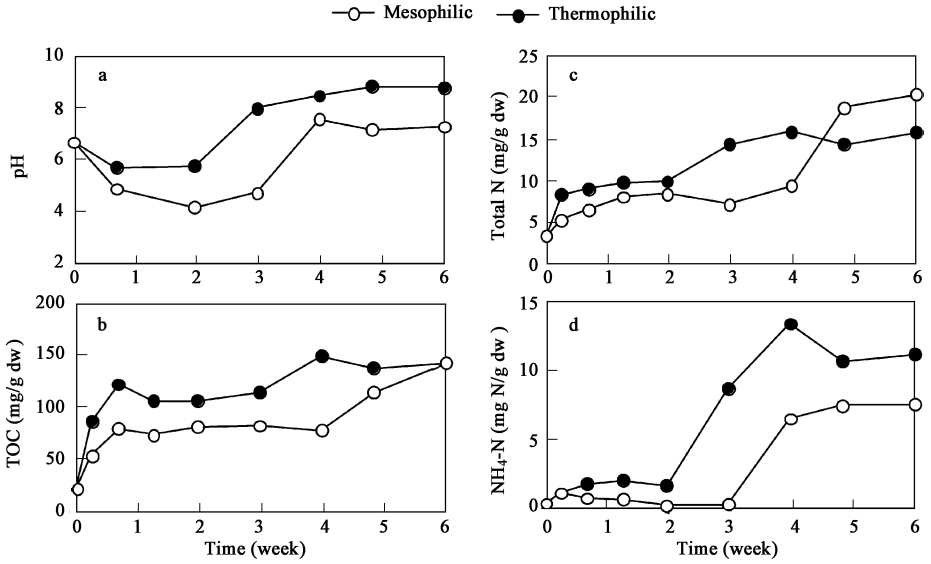

Fig. 1 Changes of chemical parameters in meso- and thermo-philic anaerobic digestions. (a) pH; (b) TOC; (c) T-N; (d) NH_4-N.

organic nitrogen in company with the hydrolysis of solid organic matters. Nitrite or nitrate nitrogen was negligible in all samples. No large amount of ammonium nitrogen was extracted from the beginning of both digesters. However, rapid increase of ammonium nitrogen occurred after 4 weeks or 3 weeks for meso- or thermo-philic digestion, and maintained high until the end of the experiment.

2.2 VFA variation pattern

Being the intermediary product of anaerobic digestion, VFAs in the mixtures were analysed, and the results are shown in Fig.2. Acetic acid dominated the most of total VFAs throughout the entire start-up period in thermophilic digestion. The amount of VFA climbed to its maximum value (40 mg COD/g dw) after 3 weeks, and at the end of the experiment all VFAs seemed to be consumed completely. In contrast to thermophilic digestion, VFA in the mesophilic reactor climbed to its maximum (72 mg COD/g dw) on week 5 though it increased slowly, especially during the first 3 weeks, which correlated to a relatively lower soluble TOC. Propionic acid constituted the majority of total VFAs in weeks 2–4 and then butyric acid dominated for the rest experiment. At the end of the experiment, there were still large quantity of VFAs remaining in the mixture and not consumed yet in mesophilic digestion, indicating the slowly degrading rate of VFA in mesophilic digester. By comparison, thermophilic digester maintained a much more balance, without significant accumulation of VFAs and decrease in pH during the start-up period.

2.3 Enzymatic activity assessment

Being the function of various enzymes excreted from micro-organisms in hydrolysis and biodegradation of organic solid waste in anaerobic digestion procedure, enzymatic activities might be the most direct biomarkers that could give some indications on the phenomenon occurred in the digestion proceed. In this study, the changes in hydrolases enzyme activities throughout the start-up anaerobic digestion in both meso- and thermo-philic digesters are investigated and illustrated in Fig.3. β-Glucosidase is hydrolytic enzyme involved in the carbon cycle, while BAA-hydrolysing protease involved the nitrogen cycle. At the beginning the activity of β-glucosidase showed a slight increase trend, but dropped rapidly in one week and increased again from week 3. This drop of β-glucosidase activity during the second week was caused by the lower pH condition (Veeken *et al.*, 2000). The activity of BAA-hydrolysing protease remained lower during the first 2 weeks in both digesters, but rapid increase was observed in week 3 reaching the maximum of 75 μmol NH_4-N/(g·h) dw in thermophilic digester. While in mesophilic digester the activity reached the maximum of 25 μmol NH_4-N/(g·h) dw

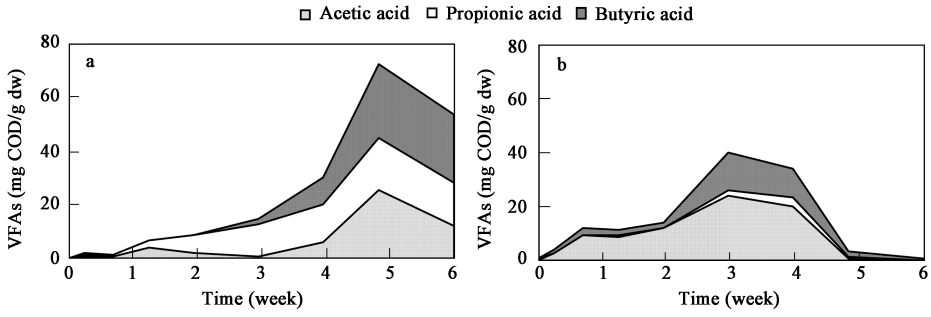

Fig. 2 Variations of VFAs in anaerobic digestions. (a) mesophilic condition; (b) thermophilic condition.

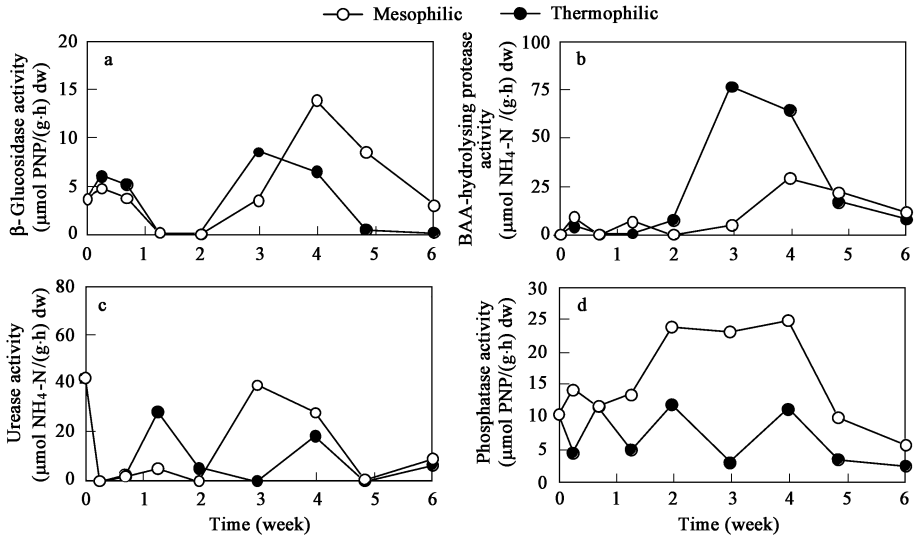

Fig. 3 Variations of enzymatic activities in meso- and thermo-philic anaerobic digestions. (a) β-glucosidase; (b) BAA-hydrolysing protease; (c) urease; (d) phosphatase.

in week 4. Thereafter the activities of BAA-hydrolysing protease in both digesters decreased.

The activity of urease, which catalyses the hydrolysis of urea to CO_2 and NH_4-N, varied considerably throughout the entire start-up digestion period in both digesters. Being of fewer available urea component in the mixtures, there was no significant positive effectiveness on digestion for urease enzyme. On the other hand, phosphatase is an enzyme with relatively broad specificity, capable of hydrolysing various organic phosphate esters. A significant fluctuation of phosphatase activity was observed and it is not clear yet what caused these fluctuations. Organic phosphate compounds, presented in the dog food and sewage sludge, might act as inducers of enzyme synthesis. Orthophosphate, as necessary nutrient for micro-organisms, may partly be transformed into microbial cells with the micro-organism growth, and inversely released from cell decay.

Carbohydrate is generally easily hydrolysed and gradually difficult for lipid and protein. Whereas, lignin is thought to be refractory throughout anaerobic digestion. Most readily biodegradable organic carbohydrates were hydrolysed quite well during the first one week, but β-glucosidase activities showed the lowest values in weeks 1–2 due to the lower pH condition, but recovered later with the increase of BAA-hydrolysing activity. On the other hand, BAA-hydrolysing activities began to increase after 2 weeks, especially in thermophilic digestion. These results suggested that protein hydrolysis began to occur after 2 weeks and reached its maximum on week 4 or week 3 for 35°C or 55°C digestion, respectively. One week later these phenomena were observed in mesophilic digestion and less than half of the maximum BAA-hydrolysing activity was determined compared with thermophilic digestion. Protein degradation is suitable in neutral pH range, and the lower pH condition would inhibit protein degradation. This can be confirmed from the analytical results of ammonium nitrogen and pH in this study. Even though adding $NaHCO_3$ into digester could restore the pH quickly and provide buffering capacity (Griffin *et al*., 1998), ammonia, which released and solubilised later as protein was hydrolysed and amino acid produced, could finally cause pH increase. In addition, hydrolysed products of protein also could enhance β-glucosidase activity. However, it should be noted that more ammonia released from protein hydrolysis might result in toxicity to methanogenesis (Sung and Liu, 2001), therefore, more attention should be paid on the dry anaerobic digestion of organic solid wastes with high protein content.

3 Conclusions

It is concluded from this study that enzymatic activities can provide important explaination for anaerobic digestion performance. Dry anaerobic digestion under mesophilic condition exhibited a poor start-up performance. Thermophilic digestion maintained a much more balanced system, without a significant accumulation of VFAs or a lower decrease in pH. Compared with mesophilic digestion, thermophilic digestion is more feasible process for achieving a better performance against misbalance, especially during the start-up period of a dry anaerobic digestion system.

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