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Temperature-phased anaerobic co-digestion of sewage sludge and micro algae for enhanced biomethane production

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ABSTRACT

Temperature-phased anaerobic digestion (TPAD) has recently gained attention for its capability to improve the hydrolysis stage, considered to be the rate-liming step of anaerobic digestion (AD). The TPAD system involves thermophilic pretreatment with a short retention time (1–3 d) prior to mesophilic AD with its longer retention time (10–20 d). In this study, effects of varying pretreatment conditions affecting the degree of solubilization during the thermophilic stage on methane yields of the mesophilic stage were investigated to improve TPAD performance. Three different conditions of the thermophilic stage were simulated in laboratory scale using sewage sludge (mixed primary and secondary sludge) and microalgae (*Chlorella vulgaris*) as substrates: 2 d hydraulic retention time (HRT) at 65°C, 2 d HRT at 75°C and 3 d HRT at 75°C. Biochemical methane potential (BMP) tests during the mesophilic stage were performed after the thermophilic stages under each pretreatment condition. Cumulative methane yield ranked by decreasing yield: 215.73 mL CH_4/g VS_{added} after pretreatment at 75°C with 2 d HRT; 147.55 mL CH_4/g VS_{added} after pretreatment at 65°C; 139.82 mL CH_4/g VS_{added} after pretreatment at 75°C with 3 d HRT. Increase in solubilization under thermophilic treatment resulted in enhanced performance of co-digestion.

Keywords: Temperature-phased anaerobic digestion; Thermophilic pretreatment; Co-digestion; Sewage sludge; Microalgae

1. Introduction

Large volumes of sludge are generated by municipal wastewater treatment plants (WWTPs) after undergoing physical, chemical, and biological processes. Average annual production of excess sludge in the US, Europe and China amounts to 240 million wet tons [1]. Treatment and disposal of excess sludge comprises 25–65% of the total operational cost of WWTPs [2]. Anaerobic digestion (AD) is a biological degradation process to stabilize and reduce organic waste prior to disposal or reuse. Microorganisms reduce the amount of sludge by breaking down organic matter in the absence of oxygen. Methane, a renewable energy source, is produced during the process. AD consists of hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis is known to be the rate-limiting step during AD. During this stage, cell wall rupture and degradation of extracellular polymeric substances take place to release readily available organic material to undergo acidogenesis. However, speeding up AD requires some sort of pretreatment of cells, a major constituent of organic fraction of sludge and an unfavorable substrate for microbial degradation.

Several pretreatment methods have been studied to improve the rate of hydrolysis and enhance biogas produc-

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tion during AD. Among these methods, temperature-phased anaerobic digestion (TPAD) has recently gained attention for its capability to shorten hydrolysis via disrupting chemical bonds of the cell wall, thus solubilizing the cell components. The TPAD system involves thermophilic pretreatment with a short retention time (1–3 d) prior to mesophilic anaerobic digestion with a longer retention time (10–20 d). The thermophilic stage allows optimization of hydrolytic and acidogenic conditions. In the mesophilic stage, longer retention time and neutral pH enhance conversion of organic components to methane [3]. Each stage of the TPAD system allows different groups of microorganisms to operate under their own optimum environmental conditions [4]. Kinetic separation of the faster acidogenesis stage from the slower methanogenesis enhances the individual steps. The reduction of volatile solids by the TPAD system is 7.5–14.5% higher than by control single-stage mesophilic AD [5].

The main objective of this study is to evaluate TPAD performance under batch conditions for co-digestion of sewage sludge and *Chlorella vulgaris* by varying operating temperature and HRT in the thermophilic stage. To improve the TPAD system, the study aims (1) to determine if increasing the temperature has a substantial effect on solubilization during the thermophilic stage of TPAD, and (2) to compare the effects of varying operating temperature and HRT in the thermophilic stage on the methane yields of the mesophilic stage.

2. Materials and methods

2.1. Experimental set-up

Sludge samples were collected from the municipal WWTP in Gwangju, South Korea. The sludge samples were mixed with primary sludge and waste activated sludge. The collected sludge was stored immediately at below 4°C prior to use. Microalgae biomass was used with *Chlorella vulgaris*. The seed for the thermophilic stage of the TPAD was inoculated with sludge harvested from a laboratory-scale anaerobic digestion reactor operating at 55°C. The inoculum for the mesophilic stage of the TPAD was used with sludge digested from a full-scale AD process located in the same WWTP. The primary and secondary sludge was mixed at a ratio of 1:1 based on the concentration of volatile solids (VS). Microalgae were added to the sludge mixture at a ratio of 10% VS-based. The substrate to inoculum ratio for both the thermophilic and mesophilic stage of the TPAD was mixed at 1:2 VS/VS.

2.2. TPAD tests

Thermophilic AD as the first stage of the TPAD system was performed in a 5 L reactor with a working volume of 3 L. A mechanically-operated mixer ensured continuous stirring at 200 rpm. The reactor was flushed with high purity N_2 gas for approximately 2 min and sealed with rubber stoppers to create anaerobic conditions. Heating tape was used to achieve operating temperatures. Three different operating conditions were applied during the thermophilic AD. Temperature and HRT were altered every period. For period I, the operating temperature was maintained at 65°C and 2 d of HRT. After 372 h (more than 3*HRT), period I of the thermophilic AD was considered to be stabilized. The temperature and HRT were then increased to 75°C and 3 d, respectively, during period II. After another 372 h, the temperature was maintained at 75°C, while the HRT was decreased to 2 d for period III. Samples were collected every 12 h and identical amounts were replenished with fresh substrate to ensure the HRT.

Sludge collected at the end period of the thermophilic stage was fed as substrate to the mesophilic stage, the second stage of the two-stage test. The mesophilic stage was carried out as biochemical methane potential (BMP) tests for 30 d in 250 mL serum bottles with a working volume of 150 mL. The bottles were flushed with high purity N_2, sealed with a butyl rubber stopper retained with an aluminum crimp-cap and then stirred in a temperature-controlled incubator at 150 rpm and 37°C. Biogas production and composition were measured daily using a gas chromatograph (GC).

2.3. Analytical methods

Total solids (TS), VS, chemical oxygen demand (COD) and soluble oxygen demand (sCOD), were analyzed according to standard methods [6]. Protein concentration was measured using a Pierce BCA Protein Assay Kit, and carbohydrate analysis was performed using the phenol-sulfuric acid method. The pH of the samples was analyzed using a pH meter (Orion star A221, Thermo Fisher Scientific). Biogas composition (CH_4, N_2, CO_2) was determined using GC (Model YL6500 GC, Young Lin Instrument Co., Korea) equipped with a thermal conductivity detector with a Carboxen-1000 column. High purified helium gas was used as a carrier gas. The inlet, oven, and detector temperature were maintained at 150°C. Methane yield was calculated as the net amount of methane produced per unit VS added. All chemical measurements were carried out in duplicate.

2.4. Statistical analysis

To predict methane production of the combinations of substrates with sewage sludge and microalgae in the TPAD, the values of cumulative methane production were analyzed by a modified Gompertz model using the following equation:

$$G(t) = G_0 \cdot \exp\left\{-\exp\left[\frac{Rmax \cdot e}{G_0}(\lambda - t) + 1\right]\right\} \qquad (1)$$

where $G(t)$ = cumulative methane production (mL/gVS$_{added}$), G_0 = methane production potential (mL), R_{max} = the maximum methane production rate (mL/day), λ = lag phase (d), t = digestion time (d), e = 2.7183. Experimental kinetic data were evaluated for their fit to the modified Gompertz model by the Generalized Reduced Gradient Nonlinear Solving method using Microsoft Excel Solver.

3. Results and discussion

3.1. Thermophilic pretreatment

Initial pH during the initial 5 days of period I decreased significantly from 9.2 to 5.5, and then the pH value stabilized at around 5.5 (Fig. 1a). The initial decrease in pH might result from formation of acidic compounds such as volatile acids through enzymatic breakdown of organic matter [7]. The pH values regardless of change in temperature and HRT in both periods II and III were almost constant within a range of 5.0–6.0. A notable increase in initial sCOD concentrations can be observed in period I (Fig. 1b). This result might explain the initial decrease in pH via an increase in organic acids under the same conditions. Increase in sCOD could result from breakdown of cells of sludge and microalgae, thus releasing the contents into the digestion broth [7].

The substrates' solubilization calculated by variations in sCOD concentrations in influent and effluent was approximately 20% for period I (at 65°C and 2 d of HRT). This is in line with the results of a study by Nges and Liu [7] reporting that 2 d pretreatment of dewatered sludge at 50°C yielded 18 % COD solubilization. After increasing both temperature and HRT during period II, solubilization was not improved. However, %COD solubilization was enhanced to approximately 36% after decreasing HRT to 2 d at 75°C during period III. Ge et al. [8] achieved 27% COD solubilization from thermal pretreatment of waste activated sludge at 60–70°C and 2 d HRT. These results suggest that both an increased thermophilic temperature and a shorter retention time might enhance solubilization of substrate materials via increased production of extracellular hydrolytic enzymes.

In AD, destruction of proteins and carbohydrates is important to for reduce organic solid wastes. Proteins are chains of amino acids linked by peptide bonds. These peptide bonds are hydrolyzed by exoenzymes such as proteases and peptidases releasing amino acids. Degradation of amino acids produces volatile fatty acids (VFAs) which are precursors for methane formation [9]. Carbohydrates are macromolecules containing numerous monomers of sugars. Exoenzymes degrade sugars producing organic acids and alcohols which are then degraded to VFAs. As seen in Fig.1c and 1d, removal of protein and carbohydrate was improved during periods II and III. This suggests that increase in temperature might improve fermentation of proteins and carbohydrates via lysis of the chemical bonds of the cell walls and membranes of the substrates, allowing the proteins and carbohydrates to be more accessible for degradation [10].

3.2. *Kinetic modeling using a modified Gompertz equation*

As shown in Fig. 2, the highest methane yield (215.73 mL CH_4/g VS_{added}) was produced from period III thermophilic pretreatment (75°C, 2d HRT). This was followed by period I (65°C, 2 d HRT) showing production of methane of 147.56 mL CH_4/g VS_{added}. The lowest methane yield (139.82 mL CH_4/g VS_{added}) resulted from period II pretreatment (75°C, 3d HRT). These results are consistent with previous studies

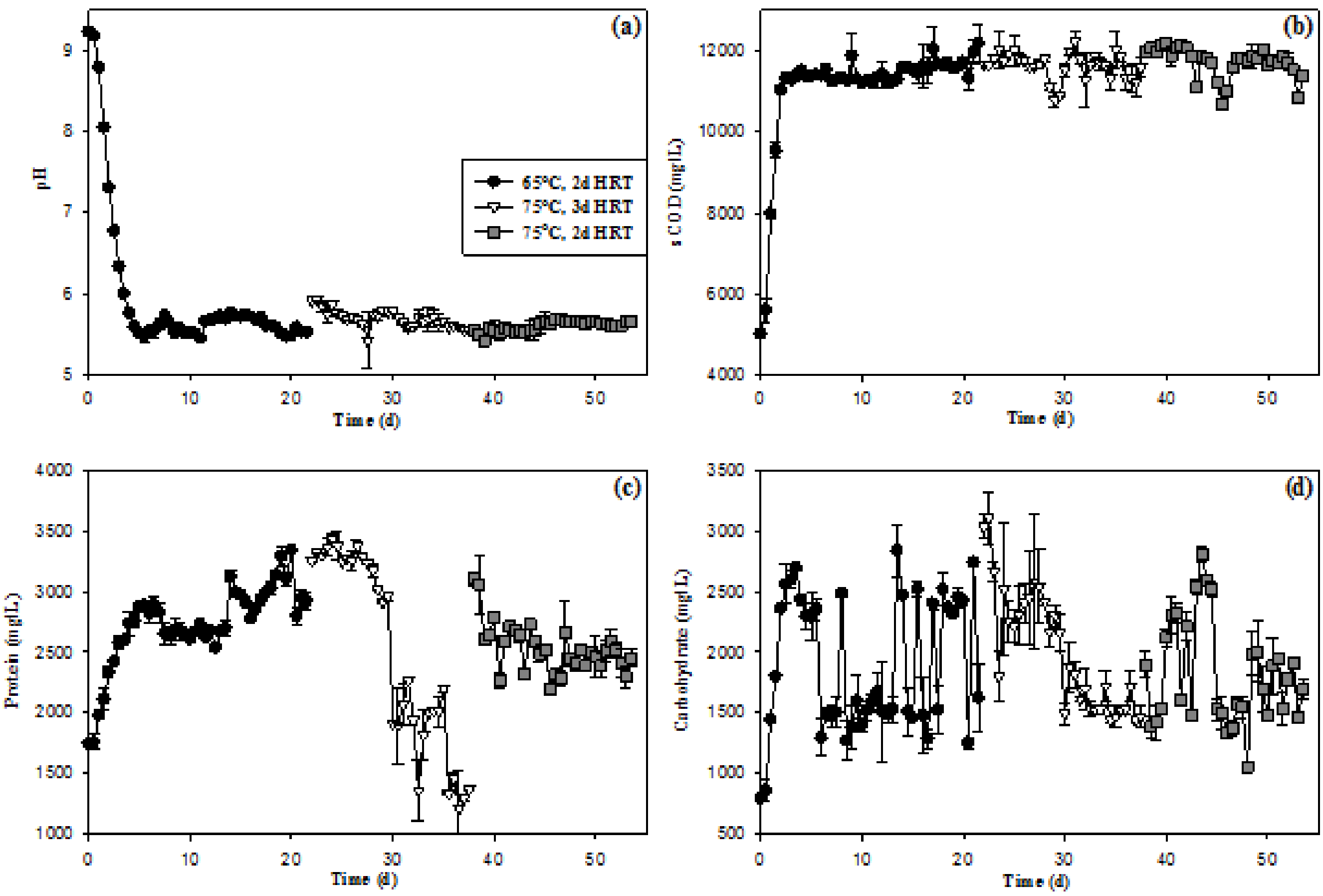

Fig. 1. Effect of varying pretreatment temperatures and HRT on (a) pH (b) sCOD (c) protein and (d) carbohydrates.

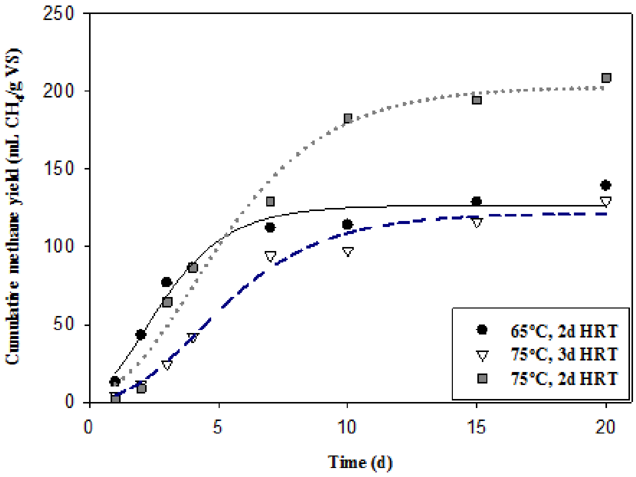

Fig. 2. Actual cumulative methane yield vs predicted cumulative methane yield using the Gompertz Model.

by Ge et al. [3,11] who found that an increase in pretreatment temperature improved methane yield (Table 1). This can be attributed to increased destruction of complex organics at higher temperatures, generating a higher proportion of methane. The increase in temperature may have stimulated growth of the microbial population or production of extracellular hydrolytic enzymes, which were then passed downstream to the methanogenic pathways. The elevated temperature might also enhance disintegration of the sludge and microalgae, resulting in reduced particle size thereby increasing the surface area available to the microbial community. Increasing hydraulic retention time from 2 to 3 d did not increase methane yield. The extent of solubilization may not have been influenced by extending the retention time. With increasing retention times, more available substrates might be consumed by the microbial community in the first stage, as a result of increased energy requirements of the cells to maintain cellular activity. This might limit substrate availability of methanogenesis in the second stage, precluding microbes from producing methane.

The experimental results of methane production from TPAD systems for co-digestion of sewage sludge and microalgae were fitted to a modified Gompertz model (Fig. 2). The results of kinetic equation analysis using the Gompertz model, are presented in Table 2. The R^2 values obtained ranged from 0.97 to 0.98, indicating that the methane production is a good fit to the model. Mesophilic digestion with substrate from the thermophilic stage under period III conditions displayed both the highest potential (G_0 = 203.06093 mL) and rate of methane production (R_{max} = 26.2057). Considering production rates of methane from all the BMP tests lasting between from 15 to 20 days, HRT of mesophilic stage in TPAD seemed optimal if operated for 15 days. The results of kinetic parameters could be used to predict methane production potential, maximum methane production rate, and the minimum time needed to produce biogas under specific conditions of operating temperature and HRT during TPAD with combination substrates of sewage sludge and *Chlorella vulgaris*.

Experimentally obtained cumulative methane yields were higher at 2 d HRT during the thermophilic pretreat-

Table 1
Comparison of methane yields from various TPAD studies

Pre-treatment conditions	Substrate	Inoculum	Operation	Main results	Reference
50°C 2 d HRT	Pctivated sludge	Digested sludge	Continuous	Pretreatment temperature from 35 to 50°C increased methane yield (25%)	[3]
55°C 3 d SRT	Primary and secondary sludge	Mixed anaerobic culture	Continuous	Single stage mesophilic AD to TPAD increased methane production from 420 to 480 mL/g VS destroyed in the mesophilic stage (14%)	[5]
65°C 2 d HRT	Activated sludge	Digested sludge	Batch	Pretreatment temperature from 50 to 65°C increased methane yield from 160 to 300 mL/g VS added (86%)	[8]
50, 60, 65, 70°C 2 d HRT	Activated sludge	Digested sludge	Batch	Methane production increased from pretreatment of 50°C to 60°C but decreased with further increase in temperature to 65°C and 75°C	[11]
55°C 6 d HRT	Food waste	Digested sludge	Continuous	Almost similar methane yield (440 and 450 mL/g VS added) for single stage mesophilic AD and TPAD	[12]
55°C 10 d HRT	Sewage sludge and sugar beet pulp lixiviation	Digested sludge	Continuous	Single stage mesophilic AcD produced higher methane yield (630 mL/TVS removed) than TPAcD (211 mL/TVS removed)	[13]
55°C 3, 4, 5, 6 d HRT	Organic fraction of municipal solid waste	Digested sludge	Batch	Pretreatment HRT of 3 d and 6 d were found to be the most viable for methane production (600 mL/g VS removed)	[14]

Table 2
Kinetic coefficients of the Gompertz Model under different pretreatment conditions

Period	G_0 (mL)	R_{max} (mL/d)	λ (d)	R^2
I	126.4968	26.4513	0.3787	0.9653
II	121.7287	16.7730	1.4808	0.9842
III	203.0603	26.2057	1.1474	0.9762

ment than those obtained at 3 d HRT. Increasing the thermophilic temperature from 65 to 75°C while maintaining HRT at 2 d improved methane yield, but negative effects were observed when HRT was increased to 3 d. The parameters obtained from the modified Gompertz model indicated that an HRT of 2 d during thermophilic pretreatment is effective for methane production in TPAD. Thus, it would be better to maintain 2 d of HRT in thermophilic pretreatment for the TPAD of sewage sludge and *Chlorella vulgaris*. Compared to typical anaerobic digestion systems, our revised TPAD could decrease total HRT in addition to improving the efficiency of co-digestion.

4. Conclusions

Pretreatment conditions were varied for the temperature-phased, anaerobic co-digestion of sewage sludge and microalgae to compare their operation performance. Increasing the temperature in the thermophilic digester improved solubilization during the initial stage of TPAD, facilitating methane production during the mesophilic stage. However, extending retention time during the thermophilic stage did not further increase methane production. The highest cumulative methane of 215.73 mL CH_4/g VS_{added} in the TPAD was achieved after pretreatment under conditions of 75°C and 2 d HRT; a total HRT of 17 d was desirable in the TPAD.

Acknowledgments

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