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Biodegradability and methane productivity during anaerobic co-digestion of refractory leachate

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ABSTRACT

Mature landfill leachate was anaerobically co-digested with synthetic wastewater to evaluate the degradability and methane productivity in various mixing ratios. The proportion of leachate was increased in three equal steps from 0% to 100%, and then decreased again through the same steps back to 0%. Both COD removal efficiency and methane production decreased as the leachate proportion in the influent was increased. When the influent contained 100% leachate, and when 33% synthetic wastewater was reintroduced, methane production was suppressed relative to COD removal. During the same phases, NH_4^+ accumulated, suggesting an excess of NH_4^+ mineralization versus uptake. After 100% leachate was supplied, methane yield decreased to near zero, and the production of methane remained suppressed relative to COD reduction even as more synthetic wastewater was reintroduced, until 100% synthetic wastewater was resupplied. This decline in methane yield might be caused by deterioration of methanogenic bacterial activity following treatment of 100% leachate.

1. Introduction

Anaerobic co-digestion is the simultaneous treatment of more than one organic waste stream. The potential benefits of co-digestion include the dilution of toxic compounds, an improved balance of nutrients, the development of synergistic microbial consortia, and the increased loading of biodegradable organic matter, which can lead to higher biogas yields and digestion rates (Ahring et al., 1992; Cecchi et al., 1996; Sosnowski et al., 2003; Hartmann and Ahring, 2005). Bouallagui et al. (2009) co-digested fruit and vegetable waste with fish waste, abattoir wastewater or activated sludge and observed that abattoir wastewater and activated sludge additions enhanced biogas yields by 51.5% and 43.8%, respectively. The higher biogas yields were attributed to the lowered carbon to nitrogen (C/N) ratio that resulted from the wastewater or waste addition. Lo et al. (2009) co-digested municipal solid waste (MSW) with MSW fly ash. They suggested that the enhanced methane gas production was results of optimal alkali, trace metals concentrations and near-neutral pH created by the fly ash amendment.

Landfill leachate, a byproduct of landfill waste degradation contains high concentrations of heavy metals and organic materials should be treated before being discharged to natural waters. Aged, or mature leachate, which is produced by older landfills, is very refractory, with high humic and fulvic fractions within its organic matter (Huo et al., 2008; Kulikowska and Klimiuk, 2008). For this reason mature leachate is difficult to treat alone (Renou et al., 2008). However, leachate can be co-digested with sewage, septage, and domestic wastewater. During co-digestion of 1 part landfill leachate with 3 parts septage, Lin et al. (1999) reported that up to 86% of chemical oxygen demand (COD) was removed at an organic loading of 315 g-COD m^{-3} day^{-1}. During co-digestion of intermediately-aged landfill leachate with sewage sludge at a ratio of 1:20, both biogas and methane yield increased by 13% and 16.9%, respectively, in comparison with sludge treatment alone (Montusiewicz and Lebiocka, 2011). Unfortunately, since the primary purpose of co-digestion is to improve the digestibility of refractory substrates it was not possible to distinguish the decomposition rate or methane productivity of each substrate separately (Mata-Alvarez et al., 2000; Hartmann and Ahring, 2005). Therefore, it is as yet unclear whether co-digestion improves the decomposition of leachate or of the co-digested substrate.

It is likely, in an environment where both microbially favorable and unfavorable substrates are available, that the favorable

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substances are preferentially decomposed. For instance, Loomis and Magasanik (1967) examined the differential consumption of monosaccharides by *Esherichia coli*. Although the bacteria could metabolize lactose in the absence of glucose (the more favorable substrate), the presence of glucose suppressed lactose degradation. Although anaerobic co-digestion is more complex than this example, as microbial reactions are mediated by a sequential series of reactions driven by diverse microbes in the wastewater treatment (Pavlostathis and Giraldo-Gomez, 1991; Speece, 1996), the decomposition of refractory material in leachate may be similarly suppressed if a more labile substrate is available. Therefore, it is important to evaluate the effect of co-digestion on the degradability and methane productivity of refractory and labile carbon pools.

Landfill leachate contains a complex mixture of both refractory and labile organic compounds (Alkalay et al., 1998). The refractory substances accumulate with increasing landfill age (Huo et al., 2008; Renou et al., 2008). Variability in the ratio of refractory to labile organic matter in leachate may lead to variable treatment performance. Therefore, it is important to evaluate the biodegradability and methane productivity of mixture with differing ratios of refractory to labile substrates for effective landfill leachate management.

The co-digestion experiment using mature leachate as the refractory substrate and synthetic wastewater as a labile substrate was performed. During the experiment, the ratio of these two materials was varied to evaluate the degradability and methane productivity of both the leachate and synthetic wastewater.

2. Materials and methods

2.1. Co-digestion substrates

The co-digestion experiment was conducted in an upflow anaerobic sludge blanket (UASB) reactor using varying mixtures of leachate and synthetic wastewater as influent.

The leachate was from the Benowo landfill, a controlled dump 3.4 km southeast of the coast in northwest Surabaya, East Java, Indonesia, which has been in operation since 2001. The landfill covers an area of 34 ha and receives 6064 t per day of municipal solid waste (MSW) from Surabaya City (Ferita, 2006). Leachate from the landfill flows through ditches and gathers in an artificial pond. The chemical oxygen demand (COD) of the leachate from an artificial pond was varied from 2000 to 17000 mg L^{-1} annually (Kawai et al., accepted for publication).

The leachate used in this experiment was obtained from the artificial pond in June 2009.The average total COD and salinity of the leachate were 3058 mg L^{-1} and 1.08%, respectively (Table 1). For our experiment the leachate was standardized to a COD of 2000 mg L^{-1} and a salinity of 0.5% by dilution with distilled water and the addition of sodium chloride. Synthetic wastewater was made as described by Sekiguchi et al. (1998) and contained a buffered mixture of labile carbohydrates, protein, and macro- and micronutrients.

For our experiment, the influent substrate was created by mixing the leachate and synthetic wastewater (Table 2) in varying ratios (see Section 2.2 below). The reactor was inoculated with seed sludge collected from an anaerobic treatment plant of municipal sewage sludge in the Hokubu Sludge Treatment Center, Yokohama, Japan. Upon arrival at our laboratory, the sludge was allowed to settle for 3 days to increase the solids content. The total solid (TS) content of the final product was 30 g L^{-1}, of which 25 g $L^{-1}$was volatile solids (VS).

Table 1
Characteristics of the landfill leachate and synthetic wastewater used in the co-digestion experiments. Leachate was from the Benowo landfill, Surabaya, Indonesia.

Chemicals	Unit	Leachate	Synthetic wastewater
COD	mg L^{-1}	3058	2000
$NH_4^+ - N$	mg L^{-1}	254	86
TN	mg L^{-1}	414	131
DOC	mg L^{-1}	311	607
Salinity	%	1.08	0.3
BOD/COD	–	0.15	0.95
C/N	–	0.75	4.6

2.2. Experimental protocol

Anaerobic co-digestion of our influent mixtures took place in a 5-L upflow anaerobic sludge blanket (UASB) reactor. The reactor was kept at constant-temperature (37 ± 1 °C), while the influent mixture was kept at 4 °C. The influent supplied to the reactor by a peristaltic pump. Initially, the reactor was fed with 100% synthetic wastewater (Table 2). The proportion of the leachate in the influent was increased in three steps (Phase 2–4) to 100% leachate (Phase 4). After 75 days, the proportion of leachate was again decreased in three steps (Phase 5–7) until the reactor was being fed with 100% synthetic wastewater (Phase 7). At each mix ratio condition, the reactor was operated for over 14 times the hydraulic retention time (HRT) to allow stabilized condition development. Stabilized conditions were established when the variation in the product effluent COD was constant with ±3% during three times the HRT of operation. The HRT and organic loading rate (OLR) were kept constant at 1 day and 2 g-COD L^{-1} day^{-1}, respectively.

Samples of biogas and influent were collected once every three days and sample of effluent was collected every day to measure biogas production and influent and effluent composition. The influent and effluent were analyzed for soluble COD, pH, nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) concentration; in addition, the total nitrogen (TN) content of the influent was also determined (see Section 2.3 below).

2.3. Analytical procedures

COD, TN, NH_4^+, NO_2^-, NO_3^-, were measured by standard methods (APHA, 1998). COD was determined colorimetrically (Model DR/2400 Spectrophotometer, Hach) following dichromate digestion. pH was measured electrochemically (Model F-22, Horiba). NH_4^+, NO_2^-, and NO_3^- were measured on filtered samples (GC-50 0.45-μm glass-fiber filter, Advantec) by ion chromatograph (Model SSC-600, Senshu Kagaku). The ion chromatograph was calibrated both before and after, resulting in a measurement error of ±1%. Total nitrogen (TN) was analyzed by ultraviolet spectrophotometry (Model V-530, JASCO) after peroxydisulfate digestion.

Microbial cell density was determined on sludge samples fixed with formalin (2% final concentration) immediately after sampling. The fixed samples were stored at 4 °C and stained with SYBR Gold solution (10% final concentration) for 10 min in the dark (Chen et al., 2001; Shibata et al., 2006) after which cell numbers were immediately counted by microscopy Axioskop 2 plus, Carl Zeiss. The methane content of biogas samples was analyzed by gas chromatography (Model GC-2014AT, Shimadzu) and was expressed as the methane production (L-methane L^{-1}-reactor volume day^{-1}).

2.4. Calculations

In each phase of the experiment (Table 2), the steady-state values of COD removal efficiency and methane yield were

Table 2
Operational parameters for the co-digestion experiments using an upflow anaerobic sludge blanket reactor. SW: synthetic wastewater; OLR: organic loading rate; HR: hydraulic retention time.

Operation period (days)	Phase	Leachate fraction (%)	Feed COD concentration (mg L^{-1})		Feed COD (mg-COD day^{-1})	
			Leachate	SW	Leachate	SW
0–14	1	0	0	2000	0	10000
15–35	2	33	2000	2000	3333	6667
36–54	3	67	2000	2000	6667	3333
55–75	4	100	2000	0	10000	0
76–91	5	67	2000	2000	6667	3333
92–115	6	33	2000	2000	3333	6667
116–142	7	0	0	2000	0	10000

determined. COD removal efficiencies while the proportion of leachate was increasing (Phase 1–4) were determined by curve-fitting using the following equation:

$$y_t = a \times \exp(-bt) + C \quad (1)$$

where y_t is the COD removal efficiency (%) at time t (days following experiment initiation), and a, b and C are constants. The value of C represents the steady-state COD removal efficiency (%) of each phase. Steady-state COD removal efficiencies while the proportion of leachate was decreasing (Phase 5–7) were determined by curve-fitting using the following equation:

$$y_t = a \times (1 - \exp(-bt))/(1 + \exp(-bt)) + C \quad (2)$$

The steady-state COD removal efficiency of each phase was determined as the sum of a and C.

Methane yield was determined as the volume of methane produced per mass influent COD. The steady-state methane yield of each phase was determined as the mean methane yield after three HRTs since methane yield fluctuated throughout each phase. The specific methane yield was calculated as the volume of methane produced per mass COD removed.

3. Results and discussion

3.1. pH, COD removal efficiency and microbial cell density

The pH of both influent and effluent ranged between 7.1 and 8.8, within the range of the optimum pH levels for anaerobic digestion (Speece, 1996) (Fig. 1). COD removal efficiency decreased from 93% to 24% as the proportion of the leachate in the influent was increased from 0% to 100% (Phase 1–4) (Fig. 2a). When synthetic wastewater was again introduced into the influent (Phase 5) the COD removal efficiency rapidly increased, attaining a steady-state value of 68%, 1.8 times higher than that attained during Phase 3, during which the same influent leachate proportion (33%) was used (Fig. 2a). When the leachate proportion was subsequently increased to 67% (Phase 6), COD removal efficiency remained constant at ca. 66% but recovered to 94% when 100% synthetic wastewater was reintroduced (Phase 7) (Fig. 2a). The microbial cell density of

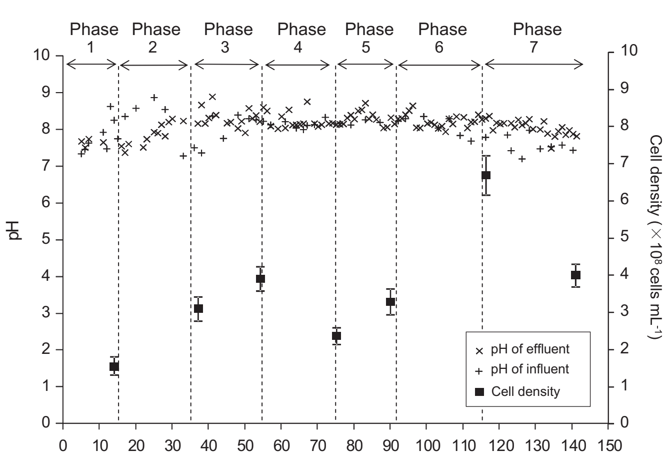

Fig. 1. pH and microbial cell density in the co-digestion experiment using the upflow anaerobic sludge bed reactor. Operational phases described in Table 2. Errors are standard deviations.

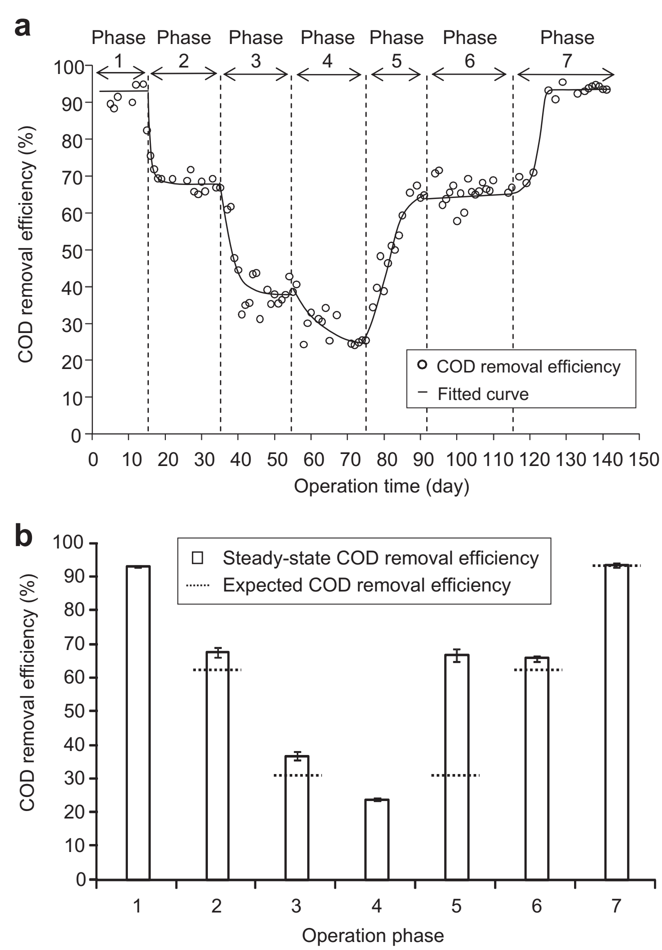

Fig. 2. (a) COD removal efficiency in the co-digestion experiment using the upflow anaerobic sludge bed reactor. (b) Steady-state COD removal efficiency (white bars) and the expected COD removal efficiency from synthetic wastewater based on the Phase 1 removal efficiency (dotted lines). Phases are described in Table 2. Errors are standard deviations.

reactor sludge increased from 1.56×10^8 to 3.94×10^8 cells mL^{-1} as the proportion of leachate was increased (Phase 1–3) but decreased to 2.39×10^8 cells mL^{-1} at the end of Phase 4 when 100% leachate was supplied (Fig. 1). As the proportion of the leachate in the influent was gradually decreased again, cell density increased again to 6.8×10^8 cells mL^{-1} (Phase 6) and thereafter decreased to 4.03×10^8 cells mL^{-1} at the end of the experiment. It is possible that washout of sludge was caused by going up with produced biogas during Phase 7.

In order to evaluate the removal of leachate COD separate from that of synthetic wastewater COD, the COD removal efficiency attributable to synthetic wastewater alone (expected COD removal efficiency) was determined at each phase, under the assumption that synthetic wastewater continued to be decomposed at the same efficiency (93%) as it was during Phases 1 and 7 (Fig. 2b). As the proportion of the leachate in the influent was increased during Phase 2 and 3, the COD removal efficiency exceeded that expected due to synthetic wastewater alone by ca. 6%, suggesting that some leachate COD was being removed by co-digestion. The excess COD removal attributable to leachate is indicated in Fig. 2b by the difference between the observed COD removal (white bars) and that expected due to wastewater alone (dotted lines). When the reactor was supplied with 100% leachate, all of the COD removal (24%) must necessarily have been leachate COD. This significant removal of leachate COD increased further to 37% during Phase 5, when 33% synthetic wastewater was reintroduced into the influent (Fig. 2b). This observation implies that microorganisms were degrading a significant amount of refractory organic materials from leachate during Phase 5. As the proportion of synthetic wastewater was increased to 67% (Phase 6), the COD removal attributable to leachate decreased again to 4% (Fig. 2b), suggesting that the microbial community, which had developed the ability to decompose organic material of leachate in the previous phase, subsequently lost that ability, owing to the increased availability of labile synthetic wastewater in the influent. By Phase 7, when 100% synthetic wastewater was supplied, COD removal efficiency returned to the level seen at the beginning of the experiment.

3.2. *Methane yield*

The methane production (L-methane L^{-1}-reactor volume day^{-1}) decreased as the proportion of leachate in the influent was increased (Fig. 3b). When only synthetic wastewater was supplied to the reactor (Phase 1), the methane production was 0.34 L L^{-1} day^{-1}. In contrast, when only the leachate was supplied (Phase 4), very little methane was produced. When synthetic wastewater was reintroduced, the methane production gradually recovered, eventually attaining a production of 0.46 L L^{-1} day^{-1} during the last phase of the experiment with 100% synthetic wastewater.

The steady-state methane yields when each synthetic wastewater and the leachate was supplied (Phases 1 and 4) were 0.17 L g-COD^{-1} and 0.0058 L g-COD^{-1}, respectively (Fig. 3a, white bar). These results were similar to the results of the biological methane potential (BMP) tests of synthetic wastewater and the leachate, which were <0.25 L g-COD^{-1} and <0.01 L g-COD^{-1}, respectively (unpublished data). The steady-state methane yield decreased from Phase 1 to Phase 4. As the proportion of synthetic wastewater was again increased in the influent, the methane yield increased to 0.24 L g-COD^{-1} with 100% synthetic wastewater (Phase 7).

In order to evaluate methane yield from leachate COD, separate from that of synthetic wastewater COD, the methane yield attributable to synthetic wastewater alone (expected methane yield) was determined at each phase, under the assumption that methane yield from synthetic wastewater continued at the same efficiency

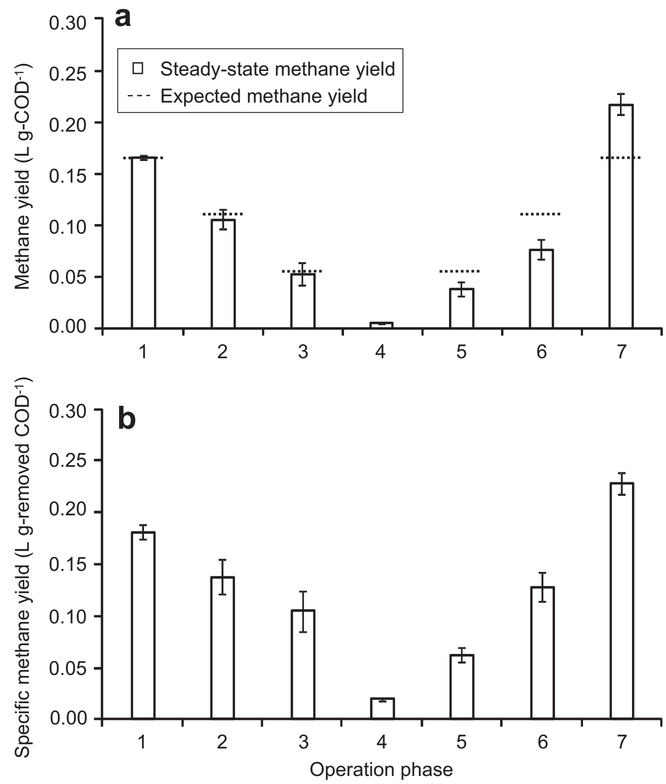

Fig. 3. (a) Steady-state methane yield (white bars) and expected methane yield from synthetic wastewater based on the Phase 1 yield (dotted lines). (b) Specific methane yield of each phase in the co-digestion experiment using the upflow anaerobic sludge bed reactor. Phases are described in Table 2. Errors are standard deviations.

(0.17 L g-COD^{-1}) as it was during Phase 1 (Fig. 3a). As leachate was introduced to the reactor, the observed steady-state methane yield (white bar) decreased relative to the expected methane yield due to wastewater alone (dotted line), suggesting that the introduction of leachate inhibited the production of methane from wastewater, and that little or no leachate COD was converted to methane by co-digestion. During Phases 5 and 6, when the proportion of synthetic wastewater reintroduced into the influent was increased, the steady-state methane yield remained lower (by 0.017 L g-COD^{-1} and 0.035 L g-COD^{-1}, respectively) than that expected based on the methane yield of 100% synthetic wastewater. This inhibition of methane yield was even greater than that observed during Phases 2 and 3, which had the same proportion of leachate, suggesting that methanogenic activity may have deteriorated during Phase 4, when the reactor was fed with the highly refractory, 100% leachate (Fig. 3a). In order to maintain methanogenesis, a minimum proportion of labile organic material may have to be supplied to reactors co-digesting such refractory leachate. In contrast, the steady-state methane yield in the final phase (Phase 7) of 100% synthetic wastewater was higher than that observed during the initial 100% wastewater phase (Phase1), possibly a result of methanogenesis from labile organics accumulated during the preceding two phases when methanogenesis was inhibited.

In order to evaluate the contribution of methanogenesis to COD reduction, methane production was normalized to COD reduction (specific methane yield) (Fig. 3b). The specific methane yield remained near and above 0.14 L g-removed COD^{-1} during the initial phases of increasing leachate proportion (Phase 1–3). However, the specific methane yield dropped precipitously to 0.024 L g-removed COD^{-1} when 100% leachate was supplied (Phase 4). When synthetic wastewater was reintroduced, the specific methane yield increased proportionally, finally reaching a high of 0.23 L g-removed COD^{-1} by the final 100% synthetic wastewater Phase 7. This pattern reflects our earlier observation that after the reactor was supplied with 100% leachate, methanogenesis was suppressed, even when synthetic wastewater was reintroduced.

3.3. COD consumption and ammonium ion concentration

The carbon to nitrogen ratio (C/N) of the leachate used in this study was low (0.75) relative to that of synthetic wastewater (4.6) (Table 1). Mature landfill leachate typically has a low C/N ratio (Trabelsi et al., 2000; Calli et al., 2006; Renou et al., 2008), because organic carbon is converted to gaseous forms, (Trabelsi et al., 2000), while ammonium accumulates as a result of hydrolysis and fermentation of nitrogenous organic material such as protein (Lema et al., 1988; Carley and Mavinic, 1991; Kjeldsen et al., 2002; Salminen and Rintala, 2002). In the present study, leachate DOC was lower than, and TN higher than, that of synthetic wastewater (Table 1). The organic material within the leachate was highly refractory, as can be seen from the low BOD/COD ratio (Table 1) and methane yield (Fig. 3). Previous studies also reported that mature landfill leachate generally contained high concentrations of refractory organic matter (Kang et al., 2002; Kjeldsen et al., 2002; Huo et al., 2008). However, although 24% of influent COD was removed when 100% leachate was supplied to our co-digestion reactor (Phase 4), methane yield was near zero. And although the steady-state COD removal efficiency during Phase 5 and 6 was higher than the expected COD removal attributable to the synthetic wastewater fraction (Fig. 2b), methane yields remained lower than the expected methane yield from synthetic wastewater (Fig. 3a). These results indicate that all of the additional removed organic matter (COD) was, however, not converted to methane, and methane conversion was inhibited after the 100% leachate phase when synthetic wastewater was reintroduced (Phase 4, 5 and 6). It is possible that organic matter was instead converted to CO_2 when 33% and 67% leachate were supplied to the reactor (Phase 4 and 5). The apparent low CO_2 production during these phases can be attributed to the dissolution of CO_2 into alkaline water (ca. pH 8 as effluent) in the reactor.

Changes in ammonium concentration between the influent and the effluent during treatment in the reactor result from the balance of NH_4^+ mineralization from organic matter, and NH_4^+ uptake through microbial biomass production. Significant nitrification (conversion of NH_4^+ to NO_2^- and NO_3^-) was unlikely in the anoxic environment of the reactor. Removal of NH_4^+ indicates that NH_4^+ uptake exceeds mineralization. Ammonium removal efficiency decreased as the proportion of leachate in the influent increased from Phases 1and 4 (Fig. 4). In Phase 1 through 3, NH_4^+ was removed by assimilation into microbial biomass, which accumulated in the reactor (Fig. 1). As increasing proportions of leachate were added, the mineralization of leachate organic matter may have increased the NH_4^+ loading, reducing the removal efficiency. The negative NH_4^+ removal efficiency in Phases 4 and 5 (when the reactor was fed with 100% leachate, and when 33% synthetic wastewater was reintroduced) indicated that NH_4^+ mineralization exceeded uptake during these phases. Ammonium was likely generated from leachate organic matter, as well as from microbial biomass, which decreased when the substrate was changed from 67% to 100% leachate (Fig. 1). During Phase 6, when the proportion of synthetic wastewater was increased to 67%, NH_4^+ once again underwent a net removal. Microbial cell density increased from 3.3×10^8 to 6.8×10^8 cells mL^{-1} from Phase 5 to 6 (Fig. 1), suggesting that mineralized NH_4^+ may have been taken up into microbial biomass.

In contrast to the present study, Montusiewicz and Lebiocka (2011) reported an increase in methane yield with adding

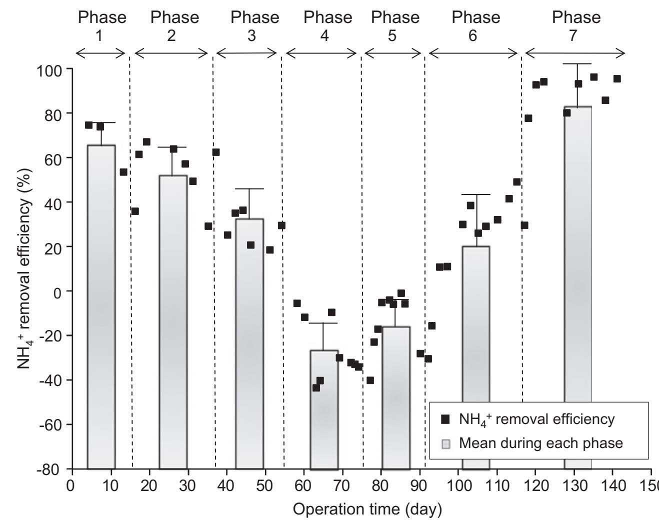

Fig. 4. Ammonium ion removal efficiency in the co-digestion experiment using the upflow anaerobic sludge bed reactor. Phases are described in Table 2. Means and standard deviations for each Phase are indicated by the vertical bars.

leachate during co-digestion of excess sludge with refractory leachate at sludge:leachate ratios of 1:0, 20:1 and 10:1. Although they hypothesized that this increase resulted from the promotion of organic material solubilization by leachate. It could also be a result of increased methane production rather than of refractory leachate. Lin et al. (1999) also conducted co-digestion of septage and leachate with mixed ratios of 1:0, 1:1, 2:1, and 3:1 by changing the organic loading rate (OLR). Although methane production was highest at a ratio of 1:1, the methane yield varied little between treatments (from 305 to 336 L kg-COD^{-1}). However, they used a relatively labile leachate. In contrast, the aged or mature leachate from older landfills, such as in the present study, is known to be very refractory, and difficult to treat. Our study revealed that when labile organic matter was resupplied after the reactor treated 100% leachate, both refractory and labile materials were decomposed (i.e. COD was removed).

4. Conclusions

We investigated the anaerobic co-digestion of refractory leachate with labile synthetic wastewater with respect to COD removal efficiency and methane yield. As the proportion of leachate in the influent was increased, the efficiency of COD removal from leachate increased until 67% wastewater was again reintroduced. Once the influent contained 100% leachate and even when 33% synthetic wastewater was reintroduced into the influent, the specific yield of methane relative to the COD removed became low, even though the COD of both leachate and synthetic wastewater continued to be decomposed. During the same phases, NH_4^+ underwent net production. This decline in the ability of methanogens to metabolize labile organics may have been an after effect of the deterioration of methanogenic activity induced during the 100% leachate treatment phase. We concluded that leachate may be treated continuously in UASB reactor without degrading the microbial activity of digestion, by alternately conducting mono-digestion of refractory leachate and co-digestion with labile materials.

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