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Ammonia-methane two-stage anaerobic digestion of dehydrated waste-activated sludge

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Abstract In repeated batch-wise thermophilic anaerobic digestion of dehydrated waste-activated sludge with 80% (w/w) water content (DWAS), although methane production reached 30 % of total organic carbon in DWAS in the first run of 15d, it gradually decreased and finally stopped in the subsequent runs together with an increase in ammonia concentration. When the loading of DWAS on anaerobic digestion was investigated, methane production at 30d significantly decreased with the increase in the amount of organic matter per kg wet sludge while enormous proportion of organic nitrogen in the DWAS was converted to ammonia within 5d. The addition of ammonia to sludge significantly decreased methane production rate. When ammonia was removed from the DWAS using a physicochemical method after 10d of ammonia production and it was applied to semi-continuous anaerobic digestion, stable methane production was observed.

Keywords: Dehydrated waste-activated sludge, Ammonia stripping, Anaerobic digestion,

1 **Introduction**

2

3 The activated sludge process has been widely used throughout the world to treat BOD or
4 COD in municipal sewage and low-strength industrial wastewaters. It transforms dissolved
5 organic pollutants in wastewater into carbon dioxide, water and biomass (Held et al., 2002;
6 Rocher et al., 1999). Although some of the biomass is returned to the aerating tank, huge
7 quantities of biomass are exhausted as waste-activated sludge (WAS) in this process. In
8 Japan, for example, 428 million m³ of WAS, corresponding to 2 million tons as the dry
9 weight criterion, is exhausted per year, even though the distribution of the sewage line was
10 66.7%. Although a large proportion of WAS (39%) is treated as landfill after being
11 concentrated by gravity, dehydrated by a filter process, dried by heating and finally
12 incinerated by combustion, this is a costly process, and still requires too much landfill space.

13 Conventional anaerobic digestion using concentrated WAS whose total solid
14 concentration is only 1.5 to 3% (w/v) is also used to reduce the amount of WAS by
15 gasification into methane and CO₂. This treatment is very useful because of the recovery of
16 energy as methane; however, this process requires a very big anaerobic digester due to the
17 slow degradation of sludge, which generally takes 20 to 30 days of hydraulic retention time
18 (HRT). This process, therefore, has been mainly used in large-scale sewage treatment
19 plants. On the other hand, it has not been employed in small-scale plants because the use of
20 an anaerobic digester is considered to be not always profitable in small-scale sewage
21 treatment plants from an economic point of view. In small-scale sewage treatment plants,
22 the sewage sludge is usually collected by waste disposers after dehydration to ca. 80% water
23 content. Only some of such excess sludge is recycled into, for example, compost and
24 cement ingredients, since the demands for such products are too small for all excess sludge to
25 be treated. If anaerobic digestion of dehydrated WAS (DWAS; moisture content < 80%) can

1 be done with the same efficiency as conventional anaerobic digestion of sewage sludge, the
2 reactor size can be decreased to 1/10 that in conventional plants, which would reduce the
3 initial costs of plant construction. Moreover, if anaerobic digestion of the DWAS is
4 possible, the need for a treatment process of the wastewater discharged after anaerobic
5 digestion is obviated or minimized, which also decreases both the initial and running costs of
6 the treatment. Although a report on the effect of water contents up to 89 % on anaerobic
7 digestion was previously carried out (Fujishima et al., 2000; Lay et al., 1997), there is no
8 report on direct anaerobic digestion of the DWAS.

9 Sewage sludge usually contains a high amount of proteins, generally a 50% content on a
10 dry basis, derived from biomass that is obtained as the result of aerobic treatment of organic
11 matter from wastewater. The amino groups bonded with the carboxyl group in proteins are
12 released as ammonia in anaerobic digestion. Inhibition by ammonia has been reported in
13 swine manure, sewage sludge and methanogenic bacteria (Hansen et al., 1999; Hendriksen
14 & Ahring, 1991; Sosnowski et al., 2003; Sprott & Patel, 1986; Sung & Liu, 2003), and
15 methane production has been shown to rapidly decrease if the concentration exceeds a
16 threshold level (Hashimoto, 1986; Koster & Lettinga, 1984; Koster & Lettinga, 1988). The
17 acclimation of methanogenic bacteria to high ammonia concentration is effective for
18 maintaining stable methane production from an organic solution containing a high
19 concentration of ammonia (Koster & Lettinga, 1988; Parkin et al., 1983; Robbins et al., 1989;
20 Sung & Liu, 2003; van Velsen, 1979). However, the ammonia concentration will easily
21 exceed the level at which methane production is inhibited if only DWAS is applied to
22 anaerobic digestion.

23 To treat organic wastes that contain a high amount of nitrogen compound using anaerobic
24 digestion, other wastes such as garbage and paper wastes that have a relatively low nitrogen
25 content were frequently added to decrease the ammonia concentration to less than the

1 threshold level (Kayhanian, 1999; Sosnowski et al., 2003). However, the amount of sewage
2 sludge is too great for a sufficient amount of garbage and paper wastes to be collected to
3 maintain the ammonia concentration at less than the threshold level. Furthermore, since the
4 amount of waste that can be collected fluctuates, it is difficult to operate the treatment
5 steadily.

6 In this study, methane production from DWAS under thermophilic conditions was
7 investigated by using methanogenic sludge unacclimated to high concentrations of ammonia
8 with repeated batch anaerobic digestion. The ammonia production increased up to 7,500
9 mg-N/kg-total wet solid of total ammonia concentration even though the methane production
10 was stopped when an accumulation of ammonia of *ca.* 3000 mg-N/kg- total wet solid was
11 reached. However, gas stripping of the ammonia produced by DWAS brought about a
12 significant recovery of methane production in both repeated batch and lab-scale anaerobic
13 digestion.

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15

16 **Materials and Methods**

17

18 **Sludges**

19

20 The dehydrated waste activated sludge (DWAS) was collected at a sewage treatment facility
21 in Hiroshima, Japan. The characteristics of DWAS are described in Table 1. The original
22 methanogenic sludge was collected at a sewage treatment facility in Hiroshima where a
23 concentrated WAS (*ca.* 2.5%, v/w) was treated with thermophilic methane fermentation and
24 then dehydrated. The digested excess sludge was anaerobically incubated at 55 °C for 60
25 days in our laboratory in order to consume the substrate completely, and it was then used as

1 the acclimated methanogenic sludge to initiate methane fermentation by mixing with DWAS
2 at an adequate ratio. The characteristics of the acclimated methanogenic seed sludge are
3 also described in Table 1.

4

5 Culture conditions

6

7 The acclimated methanogenic sludge was mixed with DWAS in several different ratios
8 ranging from 4:1 to 1:4 (20 g as the total wet solid). After the mixed sludge was transferred
9 to serum bottles (approximately 125ml), the headspace in the serum bottle was purged with
10 N₂ gas for 1 min. All cultivation was carried out under thermophilic (55 ± 1 °C) and static
11 conditions. To test the effect of ammonia concentration on methane production, the sludge
12 was supplemented with ammonium chloride (Hansen et al., 1998; Sung & Liu, 2003).
13 Sodium acetate, sodium propionate and sodium butyrate were added into the sludge to test the
14 effect of (volatile fatty acid) VFA concentration on methane production.

15 For continuous ammonia production of sludge, 20 g sludge prepared at the mixing ratio of
16 1:1 for seed sludge acclimated at 55 for 60 days and DWAS was transferred to a serum
17 bottle (approximately 125ml). After headspace in the serum bottle was purged with N₂ gas
18 for 1 min, treated sludge was culture at 55°C under the static condition. After culture for 10
19 days, semi-continuous operation was started. The half amount of all sludge (10 g) was
20 drawn out and the same amount of the fresh DWAS (10 g) was added. At that time, pH was
21 adjusted between 7.0 and 8.0 using sodium hydrocarbon because pH adjustment is very
22 difficult in dry ammonia production. The repetition described above was performed in half a
23 day of SRT (d). In the case of SRT 1.33 d and 1.5 d, the mixing ratio of seed sludge and
24 DWAS was set to 1 : 3 and 1 : 2, respectively. For, semi-continuous culture, the three

1 quarter and two thirds amount of all sludge (15 and 13.3 g) was drawn out and the three
2 quarter and two thirds amount addition of the sludge (15 and 13.3 g) at SRT 1.33 and 1.5d,
3 respectively, every 24hours. All experiments were carried out in triplicate.

5 Stripping of ammonia from sludge

7 To remove ammonia from the DWAS, a novel apparatus for stripping ammonia from solid
8 waste was constructed in this experiment as illustrated in Fig. 1. The apparatus consisted of
9 a reactor for ammonia stripping and an absorbing tower for ammonia recovery from the gas
10 containing ammonia.

11 The reactor was equipped with an inner rotating vessel with a volume of 11 l (5 l of
12 working volume) and an agitator with twelve windingly disposed blades to achieve efficient
13 kneading of the DWAS with high viscosity. The ammonia- accumulated DWAS for the
14 stripping experiment was prepared by culturing DWAS mixed with sludge acclimated for
15 ammonia formation for 3d at a ratio of 3:1 and transferring it into the reactor. To prepare the
16 sludge acclimated for ammonia formation, repeated batch culture was carried out at 55°C by
17 mixing the methanogenic sludge with DWAS at a ratio of 3:1. Then 75% (w/w) of the mixed
18 sludge was withdrawn and the mixture was supplemented with the same amount of fresh
19 DWAS through sufficient mixing every week. By using the acclimated sludge, $7500 \pm$
20 $600 \text{ mg-N/kg-total wet solid}$ of ammonia was accumulated in the sludge at 3d of culture.
21 After the pH of the sludge was raised to approximately 11 with 4N Ca(OH)_2 solution, the
22 reactor temperature was raised to 85 °C (Liao et al., 1995). Then, N_2 gas was continuously
23 circulated between the vessel and absorbing towers containing 800ml of 4N sulfuric acid at
24 ca. 5 l/min for 24 h under rotating speeds of 3 rpm for the inner vessel and 16 rpm for the

1 agitator. After the ammonia stripping, the pH of the sludge was adjusted to 7.0 with 10 N
2 HCl. The ammonia-removed DWAS was used for repeated batch-wise and continuous
3 methane production.

4

5 Semi-continuous labo-scale methane production

6

7 The schematic drawing of laboratory scale reactor used in this study was illustrated in Fig. 2.
8 The reactor has 10l of working volume (19l of total volume) equipped with a horizontal blade
9 operated at 16 rpm of rotational speed and was maintained at 55°C. A cumulative
10 production of biogas was measured using a wet gas meter after desulfurized with iron oxide
11 pellets.

12 The digestion was started by adding 125g of ammonia stripped DWAS into 5kg of the
13 acclimated methanogenic sludge corresponding to 40d of SRT (3.28kg -VS/m³/d) every day.
14 Then, SRT increased to 20d (6.51kg-VS/m³/d) at 18d from the start of the culture and 17d
15 (7.49kg-VS/m³/d) at 108d. The treated sludge in the reactor was withdrawn every two or
16 three days as the total volume in the reactor became 5 kg. SRT means that the bed volume
17 per DWAS volume added per day.

18

19 Analyses

20

21 The pH of any dehydrated sludge was measured after 0.1g wet weights of the sludge were
22 withdrawn to 1.5 ml plastic tubes and suspended with 0.9 ml deionized water. The
23 suspension was centrifuged at 12,000 × g for 10 min at 4 °C, and the clear supernatant was
24 used for measurements of the amounts of VFAs and ammonia.

25 Gas production was measured periodically by displacement of saturated aqueous NaCl in a

1 graduated cylinder. The composition of CO₂ and CH₄ was determined by a gas
2 chromatograph (GC 8A, Shimadzu, Kyoto, Japan) with a thermal conductivity detector
3 equipped with a glass column (2 m × 3 mm) packed with Unibeads C 60/80 (Shinwakakou,
4 Kyoto, Japan) at 140 °C. Argon was used for carrier gas at a pressure of 100 kPa. Acetate,
5 propionate, and butyrate were measured by HPLC (Shimadzu, Kyoto, Japan) equipped with
6 Aminex HPX-87H column, 300 mm × 7.8 mm (Bio-rad, Tokyo, Japan). The column
7 temperature was 65 °C. The flow rate was 0.8 ml/min with 0.005 mM H₂SO₄ solution. The
8 total ammonia-nitrogen was determined with a commercial available ammonia test kit (Wako,
9 Osaka, Japan). Total organic carbon (TOC) was determined by a TOC analyzer (TOC-5000,
10 Shimadzu, Kyoto, Japan).

11

12

13 **Results**

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15 Methane fermentation from untreated dehydrated waste-activated sludge

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17 To test the availability of the dehydrated waste-activated sludge for methane fermentation,
18 repeated batch culture was carried out at 55°C by mixing the acclimated methanogenic sludge
19 with DWAS at the ratio of 3:1, which corresponded to 21 g TOC/kg-total wet solid (Fig. 3A).

20 Every 15d, one-third of the mixed sludge was withdrawn, and the remainder was
21 supplemented with the same amount of fresh DWAS through sufficient mixing. Fifteen days
22 after the initiation of the culture, 550 mmol/kg-total wet solid of methane was produced
23 where 57 % of TOC was removed (methane 33 % and CO₂ 24%). In a second run (15-30d),
24 however, the methane production decreased to 200 mmol/kg-total wet solid. TOC removal

1 was only 27% (methane 12 % and CO₂ 15 %). Methane was hardly produced in the third
2 run (30-45d) and not produced at all in fourth run (45-60d). With regard to volatile fatty
3 acids in this repeated batch culture, acetate accumulated significantly after 30d (the third and
4 fourth runs) and reached 250 mmol/kg total wet solid at the end of the culture, at which the
5 propionate and butyrate accumulations were 75 and 70 mmol/kg total wet solid, respectively.
6 Ammonia concentration increased in proportion to the addition of fresh DWAS and reached
7 7,600 mg-N/kg-total wet solid at the end of the culture.

8

9 Characteristics of ammonia release from DWAS

10

11 In order to examine the effect of the loading dose of DWAS on methane production, batch
12 cultures were carried out using sludge in which the acclimated methanogenic sludge was
13 mixed with DWAS at various ratios. The methane yield at 5d was quite low even at the
14 lowest DWAS loading ratio (Fig.4C), while the ammonia and VFA concentrations linearly
15 increased with the increase in DWAS loading ratio after 5d (Fig.4B, 4D). The Weak pH
16 elevations such as 0.1 to 0.2 were observed during for 25d against starting pH, of which
17 distributed 8.1 to 7.6 according to decrease of DWAS loading ratio, as shown Fig.3A. The
18 methane was observed only at DWAS loading ratio lower than 0.33 (= 28g-TOC/kg total wet
19 solid) after 5d, while the ammonia and VFA concentrations get to almost plateau irrespective
20 of DWAS loading ratio at 5d, as shown Fig. 3B –D. The ammonia concentration reached
21 8,000 to 9,000 (mg/kg-total wet solid) on higher DWAS loading ratio at 25d, typically 60% or
22 more of the nitrogen in organic matter was converted to ammonia with in 5d, at every DWAS
23 loading ratio (Fig. 3E). Although likewise the ammonia release, the VFA concentrations at
24 25d were similar to that at 5d of culture, the explanation of this result is not the same as that

1 for ammonia production because VFA concentration changes due to the production of
2 methane and the conversion of butyric and propionic acid to acetate and hydrogen. It is well
3 known that ammonia is a significant inhibitor of methane production (Hansen et al., 1999;
4 Hendriksen & Ahring, 1991; Sosnowski et al., 2003; Sprott & Patel, 1986; Sung & Liu,
5 2003). Since a high concentration of VFA also inhibits methane production, VFA
6 accumulation could affect methane production. However, VFA appears to be consumed if
7 the ammonia concentration is low, because a higher consumption of VFA by methanogenesis
8 is expected at low concentrations of ammonia.

9

10 Continuous ammonia production from DWAS

11

12 Since most of ammonia was released at least during 5 d, it was considered that acceleration of
13 ammonia production might be possible by using a semi-continuous operation. Fig. 5 shows
14 ammonia production in sludge at the stable condition as the function of SRT at 37°C and
15 55°C. At 37 °C, ammonia production was increased up to SRT 4 d, and then decreased at
16 loading rate after that. At 55 °C, ammonia production was increased up to SRT 1.33 d more
17 rapidly than that at 37°C, and then became stable after that. Ammonia production was very
18 close after SRT 4 d at 37°C and 55°C where the amount of ammonia per DWAS was 5.75
19 (50.1% of ammonia conversion) and 5.41 g-N/kg-t.w.s (52.8% ammonia conversion),
20 respectively.

21 VFA production showed similar tendency, the ammonia production from dehydrated
22 excess sludge was possible at mesophilic and thermophilic conditions using which digested
23 sludge. In batch culture, cultivation after 10 days, ammonia conversion rate was shown 44.2
24 % at 37 °C, and 55.6 % at 55 °C. Unlike batch culture, by repeating continuous culture of
25 ammonia, the ammonia conversion rate in excess sludge reached to 50.1 and 52.8 % in the

1 short period (SRT 4 day at 37 °C and 1.33 day at 55 °C). Even if mesophilic and
2 thermophilic condition, ammonia production was possible. However, remaining 50 % was
3 not converted into ammonia, and the limit of accumulation of ammonia was 8 g TAN/kg
4 t.w.s. (data not shown).

5

6 Effect of ammonia stripping from sludge on methane fermentation

7

8 Ammonia removal from sludge that was treated for ammonia production for 5d as described
9 in the Methods section was carried out, as was methane fermentation using the treated sludge.
10 A typical time course for ammonia removal from ammonia-fermented DWAS is shown in
11 Fig. 6. When the initial pH of the sludge was set to 11.7 using $\text{Ca}(\text{OH})_2$, and the temperature
12 was increased to 85°C, the ammonia concentration dramatically decreased from 7,700 to
13 1,900 mg-N/kg-total wet solid after 1h. After that, the ammonia concentration slowly
14 decreased and reached 490 mg-N/ kg-t.w.s. at 4 h. In this condition, VFA concentrations
15 such as acetate, propionate and butyrate concentrations increased slightly, suggesting that few
16 VFAs were released during ammonia removal.

17 After ammonia production for 5 d, the repeated batch culture using DWAS from which
18 ammonia had been removed was performed by the same method as that used for untreated
19 sludge as shown in Fig. 3A. The ammonia concentration was kept at around 2,000 mg-N/
20 kg-t.w.s. during the experiment (Fig. 3B). At each withdrawal of treated sludge and addition
21 of fresh sludge, a constant amount of methane (560 ± 47 mmol/kg-t.w.s.) was produced,
22 and the VFA concentration was kept below 80mmol/kg-t.w.s.. This result clearly indicates
23 the elimination of ammonia from the sludge after the conversion of nitrogen in the organic
24 fraction.

25

1 Semi-continuous production of methane from ammonia-stripped DWAS

2

3 The digestion of ammonia-stripped DWAS was performed using a labo-scale reactor shown
4 in Fig. 2 over 150d. In Fig. 7, the profile of the digestion was illustrated. Just after the
5 time when the digestion was started at SRT 40d ($3.28\text{kg-VS/m}^3/\text{d}$), VFA accumulation was
6 observed, but it faded away by 14d. Biogas production reach plateau after 5d and then
7 stabilized at $0.62\text{Nm}^3/\text{kg-VS}$. After SRT was increased to 20d ($6.51\text{kg-VS/m}^3/\text{d}$) at 18d of
8 the culture, although VFA accumulated for 2 weeks, it disappeared after 32d, and then no
9 VFA accumulation and stable biogas production was observed for 60days. The biogas
10 composition was CH_4 63%, CO_2 35% H_2 2%. Biogas yield was $0.58\text{Nm}^3/\text{kg-VS}$. When
11 SRT changed to 17d at 108d, slight VFA accumulation was observed for a few days but
12 disappeared then stable biogas production was found in which biogas yield was
13 $0.51\text{Nm}^3/\text{kg-VS}$. The accumulation of VFA, mainly propionate was started after 140d
14 maybe due to over loading of organic matter. During the operation pH was not controlled
15 but was quite stable around 8. Average ammonia concentration was lowered with shorter
16 SRT, that is, 2700, 2,100, and 1,800mg-N/kg-ww at SRT 40, 20 and 17d, respectively.

17

18

19 **Discussion**

20

21 In this study, when DWAS with water content of 80% was applied to anaerobic digestion in
22 repeated batch culture, methane production was significantly inhibited by the increase in
23 ammonia concentration (Fig. 3A). In batch cultures at a TOC loading that was altered by
24 changes in the ratio of seed methanogenic sludge to DWAS, ca. 85% of organic nitrogen in
25 DWAS was converted to ammonia after 25d (Fig. 4). This percentage conversion may be

1 reasonable because it was demonstrated by Krylova et al. (1997) that the anaerobic digestion
2 of poultry manure for 25d at 7% of total solids and at a 55°C temperature resulted in the
3 transformation to ammonia nitrogen of 85% organic nitrogen. A point to note in the present
4 study is that of the organic nitrogen in DWAS that was released as ammonia within 25 d, 81%
5 of it was released within 5d. It was also observed that the acclimation of
6 ammonia-producing sludge with repeated batch cultures shortened the solid retention time of
7 DWAS for the conversion of ammonia from organic nitrogen without a decrease in
8 conversion efficiency by up to 3d (unpublished data). During ammonia production, pH was
9 kept at 7.1 ± 0.8 and the amount of ammonia produced was strongly correlated to the
10 production of VFA at the correlation factor of 0.93 indicating simultaneous production of
11 ammonia and VFA from amino acids derived from easy degradable VS.

12 This is a very important finding if ammonia inhibition is to overcome, because if the
13 removal process of ammonia is introduced prior to methane fermentation, anaerobic digestion
14 of DWAS can be allowed to occur without considering the ammonia concentration in the
15 methane fermentation tank. In this study, since the addition of ammonia of more than
16 2,000mg-N/kg-total wet weight significantly inhibited methane formation (data not shown),
17 ammonia removal was essential for sustainable methane production from DWAS.

18 Technology for the stripping of ammonia, especially from aqueous solution, is very
19 common. In terms of anaerobic treatment, air stripping has been investigated for the
20 recovery of ammonia from materials to be digested (Bonmatí & Flotats, 2003). On the other
21 hand, ammonia stripping from organic matter with high TS contents exceeding 15% (w/w)
22 has not yet been investigated. This may be due to the difficulty of handling highly solid
23 matter that has poor fluidity and the fact that equipment design for the stripping of ammonia
24 from such solid matter had not been considered. To overcome this problem, a novel
25 apparatus for stripping ammonia from solid waste was constructed in this experiment as

1 illustrated in Fig. 1. Treatment for 4 h decreased the total ammonia concentration in DWAS
2 by less than 500 mg-N/kg total wet solid under initial conditions of pH 11.7 at 85°C (Fig. 6).
3 Using the sludge after the removal of ammonia, sustainable methane production was achieved
4 with the repeated batch culture, and the ammonia concentration was kept at around
5 2000mg-N/kg total wet solid as shown in Fig. 3B. This clearly suggests that if the ammonia
6 was efficiently removed, DWAS can be treated with an anaerobic digester without the
7 addition of water and/or a C/N adjuster as proposed previously (Kayhanian, 1999).

8 The semi-continuous digestion for prolonged periods was demonstrated the effectiveness
9 of anaerobic ammonia release and its stripping in DWAS for methane production. During
10 the operation, ammonia concentration was kept below 3,000mg-N/kg-ww, leading to stable
11 biogas production. The higher TOC loading was, the lower ammonia concentration and
12 biogas yield were. Since easy degradable organic solids in DWAS already are degraded in
13 the ammonia release, organic solids in ammonia-stripped DWAS are degraded very slowly
14 during the digestion and maybe the decrease of biogas yield and ammonia concentration is
15 due to the decrease of degradation of organic solid.

16 Yield of ammonia-nitrogen was calculated based on the results for 120 to 133d at SRT of
17 20d in the semi-continuous culture. Although 10% of TKN was ammonia originally,
18 ammonia was increased to 59% of TKN during the phase for ammonia production. During
19 ammonia stripping, 57% of TKN was removed as ammonia gas, which was 97% of ammonia
20 in DWAS. Ammonia was increased to 16% from 2% in the stage of methane production
21 together with degradation of VS. This indicates that 73% of TKN is converted to ammonia
22 during the whole process and 57% of TN was stripped as ammonia gas. This means if
23 DWAS is treated for methane production without ammonia stripping, ammonia concentration
24 in DWAS reaches more than 10,000mg-N/kg-t.w.s.. Ammonia stripping of DWAS after the
25 conversion to ammonia is effective for sustainable methane production from DWAS.

1 Based on the results for 120 to 133d at SRT of 20d in the semi-continuous culture, 26% of
2 VS was degraded at the phase of ammonia production in which the composition of CO₂ and
3 H₂ was 89% and 11%, respectively. Although no degradation of VS was not found at the
4 ammonia stripping, 33% of VS was recovered as biogas in which the composition of CH₄ was
5 62%. This means that VS degradation was 59% during the whole process.

6 The dry methane fermentation of DWAS is beneficial because the use of DWAS
7 decreases the size of the anaerobic digester, increases the methane productivity per digester
8 volume, and minimizes the process for wastewater treatment in comparison to conventional
9 anaerobic digestion. Furthermore, the use of organic wastes with lower water content means
10 that a low energy input is required to increase the temperature of the waste for the effective
11 stripping of ammonia. Thus, stripping the ammonia prior to the anaerobic digestion of
12 DWAS can be competitive with anaerobic digestion of organic wastes diluted with water
13 and/or C/N adjuster for assuring that the ammonia concentration does not exceed the
14 threshold value of inhibition. However, the stripping efficiency in this study is still lower
15 than that of stripping ammonia from filtered pig slurry from filtered pig slurry at an initial pH
16 of 11.5 and 80°C using a stripping tower (Bonmatí & Flotats, 2003), and the reactor
17 configuration seems to be too complex to scale up for practical use. Therefore, an effective
18 and practical process for ammonia removal from organic matter that has a low moisture
19 content should be intensively investigated.

20 The stripping of ammonia from DWAS is also useful for the production of nitrogen
21 fertilizers such as ammonia sulfate and urea, which are produced from ammonia by
22 Haber-Bosch synthesis out of its constituent elements. The total annual production of
23 ammonia was 121 million tons in 2005
24 (<http://minerals.usgs.gov/minerals/pubs/commodity/nitrogen/>). The amount of ammonia
25 sulfate and urea used for fertilizers in Japan was 0.66 million tons in 2004, and 0.18 million

1 tons of ammonia were used to produce them. Since 0.26 million tons appeared to be
2 recovered from DWAS, we concluded that if all sewage treatment centers used DWAS for
3 ammonia production, the amount of ammonia recovered would be enough to meet the demand
4 for chemical fertilizers in Japan. Furthermore, since this ammonia production could be
5 applied to garbage and animal feces such as cow and chicken manure as previously reported
6 (Bonmatí & Flotats, 2003), a major part of the ammonia supply could be collected from these
7 biomass wastes. In addition, ammonia recovered from nitrogen-rich biomass could also be
8 connected to ammonia fuel cells to produce (Maffei et al., 2005; McFarlan et al., 2004;
9 Metkemeijer & Achard, 1994). At present, the nitrogen in organic wastes is still treated as
10 something to be disposed by means of nitrification and denitrification in wastewater treatment
11 plants.

12

13

14 **Acknowledgements**

15

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17 and Technology.

18

(Figure legends)

Fig. 1. Reactor system for ammonia-stripping from dehydrated waste activated sludge. 1. heater. 2. outer vessel. 3. inner rotating vessel. 4. stirring device. 5. stirring motor. 6. thermo couple. 7. water bath. 8. absorbing tower containing 4N sulfuric acid. 9. trap bottle 10. Pump. 11. Drain. 12. check valve closure.

Fig.2 Schematic drawing of apparatus for semi-continuous methanogenic fermentor. 1. Fermentation chamber with a volume of 19 l (10 l of working volume), 2. Water bath kept at 55 °C. 3. Desulfurizer by iron-oxide. 4. Gas flow meter. 5. Inlet port. 6. Outlet port. 7 Gas sampling port.

Fig. 3 Typical time course of semi-continuous anaerobic digestion of dehydrated waste activated sludge (DWAS) (A) and DWAS with ammonia stripping for 6 h after ammonia production (B). The culture was initiated with mixed sludge that was prepared by mixing acclimated dehydrated methanogenic sludge (ca. 80% water content) with DWAS at a ratio of 3:1. After 15 d of cultivation at 55°C, 25% of the weight of the mixed sludge was withdrawn, and the same amount of DWAS was added. This procedure was repeated 3 times. Symbols : open square, acetate; closed diamond, propionate; open circle, butyrate. kg-t.w.s.: kg of total wet solid, VFA: volatile fatty acids.

Fig. 4 Effect of the loading ratio of dehydrated waste activated sludge (DWAS) on anaerobic digestion. Sludge was prepared by mixing the acclimated methanogenic sludge with DWAS at various ratios and cultured for 25d.

1 **Fig. 5** Production of ammonia and VFA at various SRT condition on continuous ammonia
2 production. Closed square shows incubation temperature at 55 °C and open
3 diamond shows incubation temperature at 35°C.

4

5 **Fig. 6** Typical time course of ammonia stripping from sludge containing ammonia that was
6 prepared by mixing acclimated dehydrated methanogenic sludge with dehydrated
7 sewage sludge at a ratio of 3:1 and cultured for 10 d. Ammonia stripping was
8 performed at temperature 85°C and starting pH at 10.5. Symbols: closed square, pH;
9 open square, ammonia concentration. Lower graph: closed square, acetate; open
10 circle, butyrate; and closed triangle, propionate.

11

12 **Fig. 7** Semi-continuous production of methane from ammonia stripped DWAS using the
13 laboratory scale reactor. Symbols: solid line , SRT; dashed line , VS loading rate;
14 open diamond, gas production rate; close diamond, gas yield; open circle, pH; close
15 circle, ammonia as mg-N per kg-t.w.s.: kg of total wet solid. VFA, volatile fatty
16 acids.

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Table 1. Characteristics of dehydrated waste activated sludges (DWAS) and seed methanogenic sludges

Parameter	Unit	DWAS	Seed sludge
Water content	%w/w	83	80
TS	%w/w	17	20
VS	%TS	90	53
TOC	g-C/kg-TS	421	268
TKN	g-N/kg-TS	77	32
AN	g-N/kg-TS	19	3

TS total solids, VS volatile solids, TOC total organic carbon, TKN total Kjeldahl nitrogen, AN ammonia nitrogen

Fig. 1 Nakashimada *et al.*

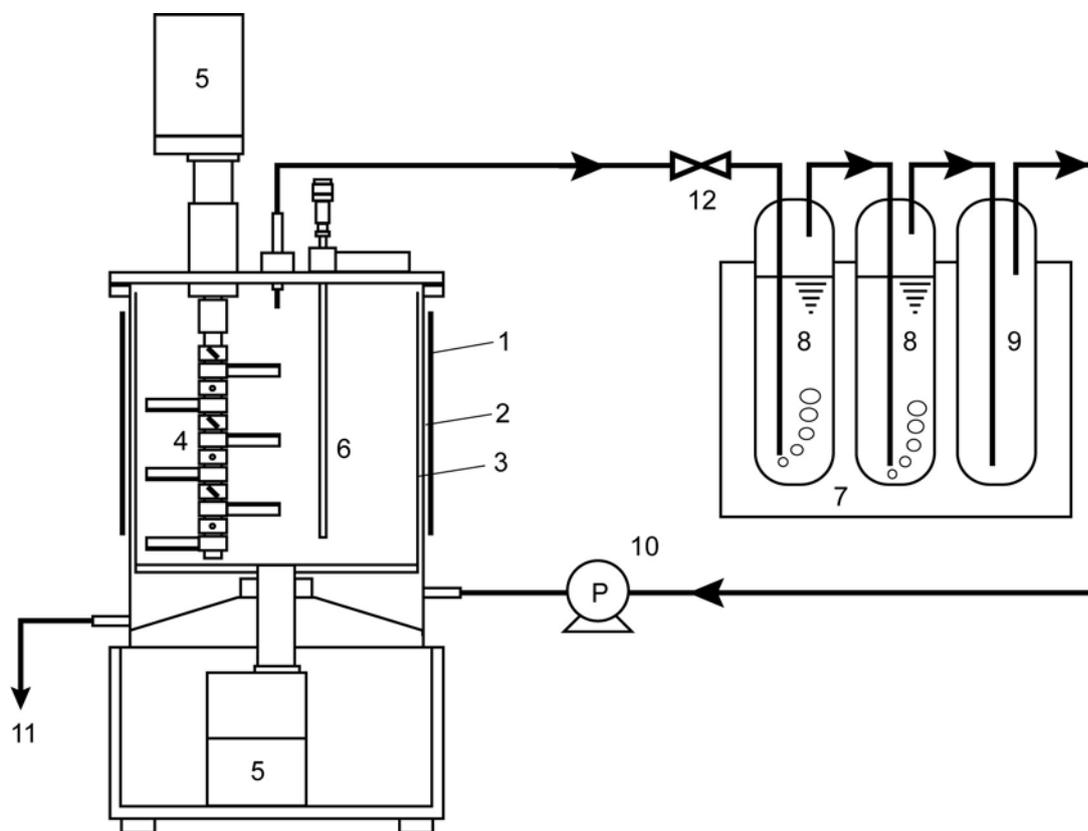


Fig. 1. Reactor system for ammonia-stripping from dehydrated waste activated sludge. 1. heater. 2. outer vessel. 3. inner rotating vessel. 4. stirring device. 5. stirring motor. 6. thermo couple. 7. water bath. 8. absorbing tower containing 4N sulfuric acid. 9. trap bottle 10. Pump. 11. Drain. 12. check valve closure.

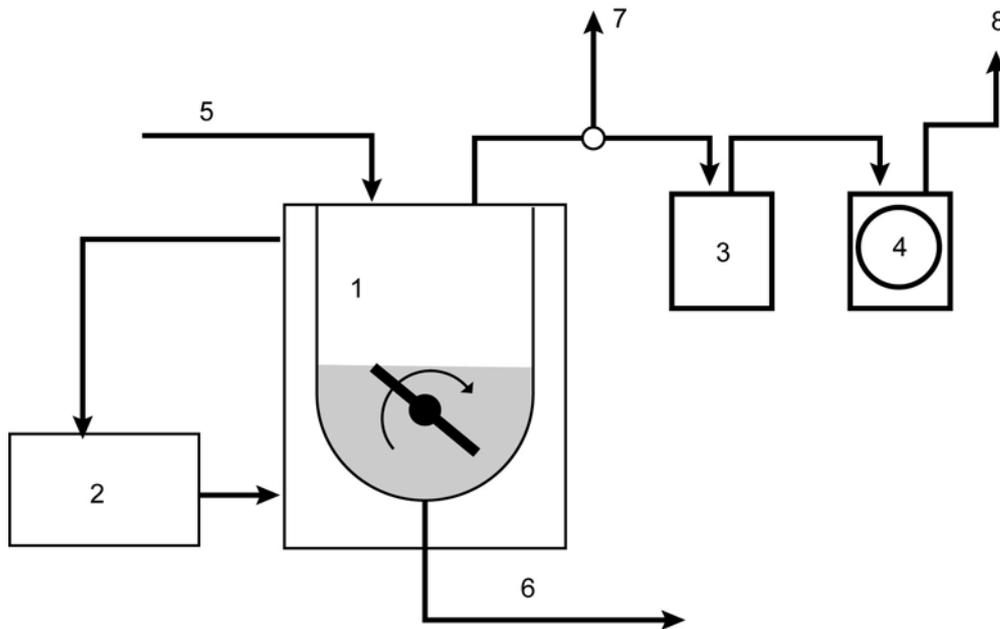


Fig.2 Schematic drawing of apparatus for semi-continuous methanogenic fermentor. 1. Fermentation chamber with a volume of 19 l (10 l of working volume), 2. Water bath kept at 55 °C. 3. Desulfurizer by iron-oxide. 4. Gas flow meter. 5. Inlet port. 6. Outlet port. 7 Gas sampling port.

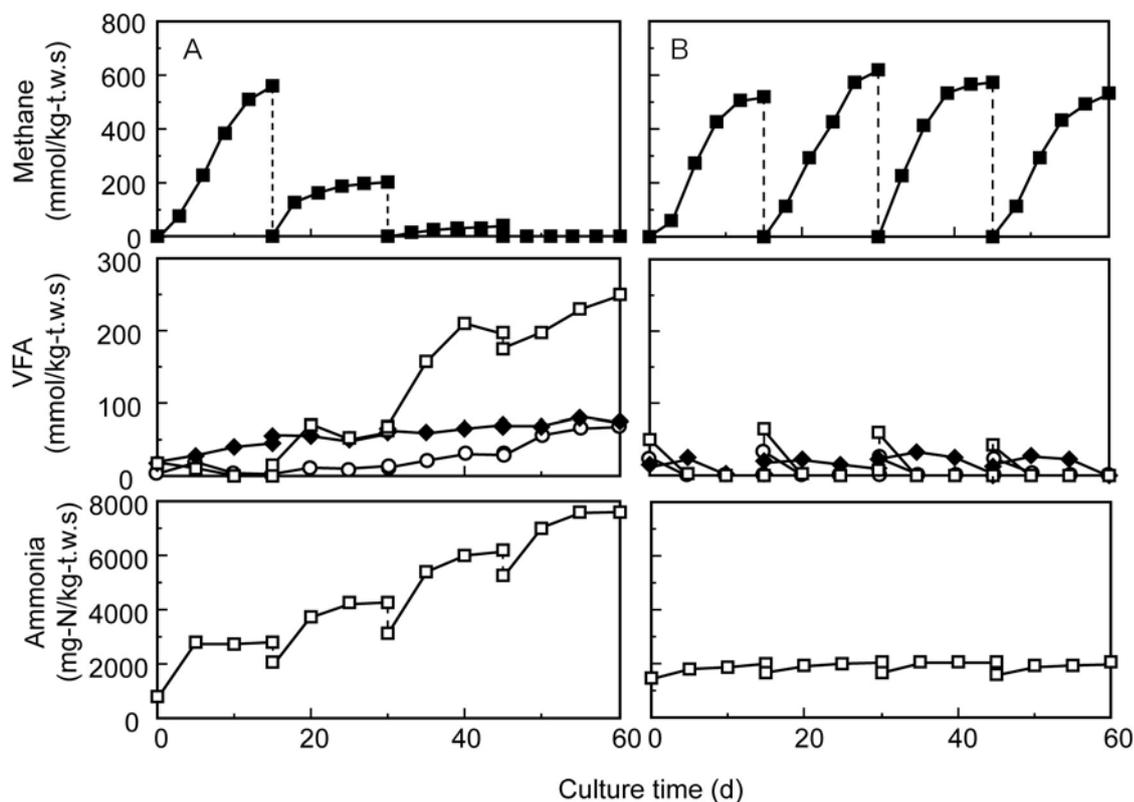


Fig. 3 Typical time course of semi-continuous anaerobic digestion of dehydrated waste activated sludge (DWAS) (A) and DWAS with ammonia stripping for 6 h after ammonia production (B). The culture was initiated with mixed sludge that was prepared by mixing acclimated dehydrated methanogenic sludge (ca. 80% water content) with DWAS at a ratio of 3:1. After 15 d of cultivation at 55°C, 25% of the weight of the mixed sludge was withdrawn, and the same amount of DWAS was added. This procedure was repeated 3 times. Symbols : open square (), acetate; closed diamond, () propionate; open circle (), butyrate. kg-t.w.s.: kg of total wet solid, VFA: volatile fatty acids.

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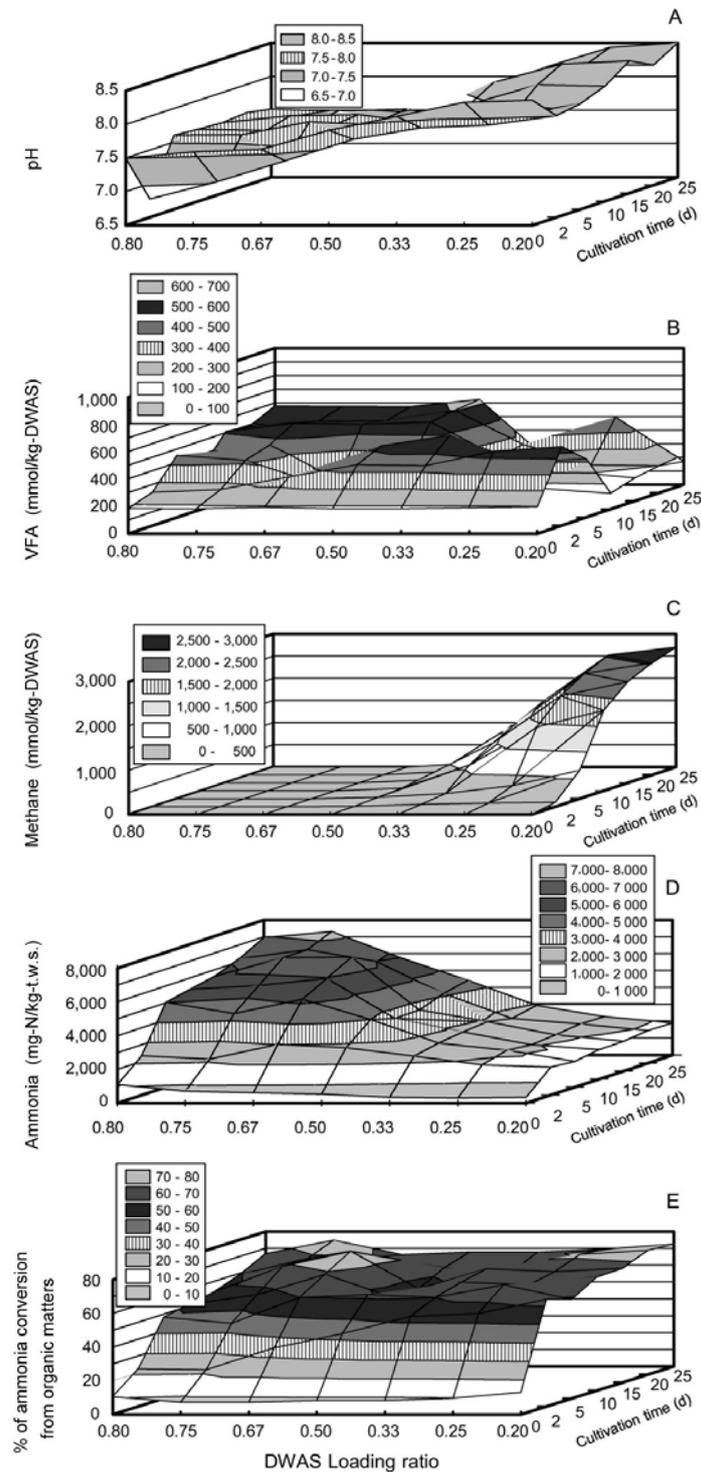


Fig. 4 Effect of the loading ratio of dehydrated waste activated sludge (DWAS) on anaerobic digestion. The sludges were prepared by mixing the acclimated methanogenic sludge with DWAS at various ratios and cultured for 25d.

Fig. 5 Nakashimada *et al.*

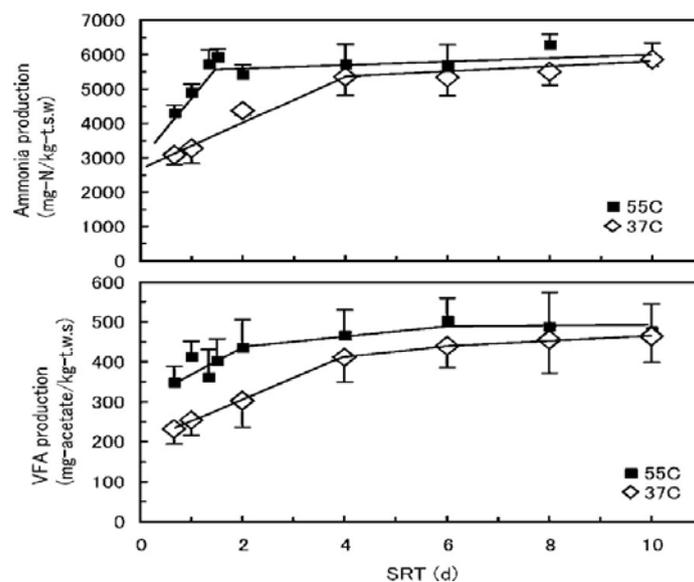


Fig.5 Production of ammonia and VFA at various SRT condition on continuous ammonia production. Closed square () show incubation temperature at 55 °C and open diamond() show incubation temperature at 35°C.

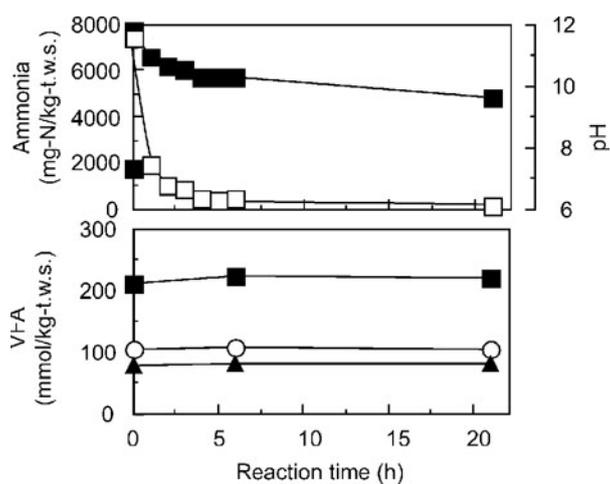


Fig. 6 Typical time course of ammonia stripping from sludge containing ammonia that was prepared by mixing acclimated dehydrated methanogenic sludge with dehydrated sewage sludge at a ratio of 3:1 and cultured for 10 d. Ammonia stripping was performed at temperature 85°C and starting pH at 10.5. Symbols: square () pH; open square () ammonia concentration. Lower graph: closed square () acetate; open circle () butyrate; and closed triangle () propionate.

Fig. 7 Nakashimada *et al.*

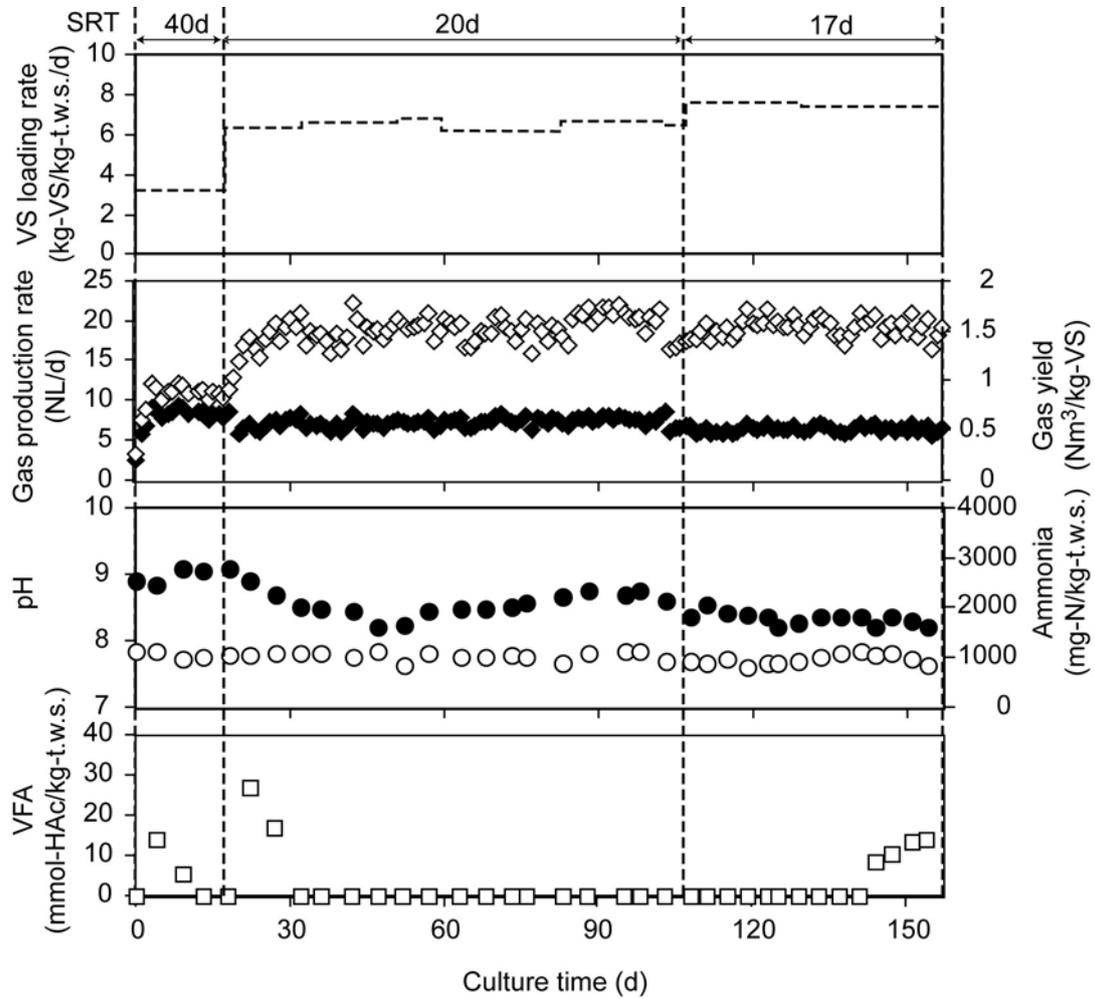


Fig. 7 Semi-continuous production of methane from ammonia stripped DWAS using the laboratory scale reactor. Symbols: solid line , SRT; dashed line , VS loading rate; open diamond (), gas production rate; close diamond (), gas yield; open circle (), pH; close circle (), ammonia. kg-t.w.s.: kg of total wet solid, VFA volatile fatty acids.

References

- Bonmatí A, Flotats X (2003) Air stripping of ammonia from pig slurry: characterisation and feasibility as a pre- or post-treatment to mesophilic anaerobic digestion. *Waste Manage (Oxford)* 23: 261-272
- Fujishima S, Miyahara T, Noike T (2000) Effect of moisture content on anaerobic digestion of dewatered sludge: ammonia inhibition to carbohydrate removal and methane production. *Water Sci Technol* 41: 119-127
- Hansen KH, Angelidaki I, Ahring BK (1998) Anaerobic digestion of swine manure: inhibition by ammonia. *Water Res* 32: 5-12
- Hansen KH, Angelidaki I, Ahring BK (1999) Improving thermophilic anaerobic digestion of swine manure. *Water Res* 33: 1805-1810
- Hashimoto AG (1986) Ammonia inhibition of methanogenesis from cattle wastes. *Agricultural Wastes* 17: 241-261
- Held C, Wellacher M, Robra KH, Giibitz GM (2002) Two-stage anaerobic fermentation of organic waste in CSTR and UFAF-reactors. *Biores Technol* 81: 19-24
- Hendriksen HV, Ahring BK (1991) Effects of ammonia on growth and morphology of thermophilic hydrogen-oxidizing methanogenic bacteria. *FEMS Microbiol Ecol* 85: 241-246
- Kayhanian M (1999) Ammonia inhibition in high-solids biogasification: An overview and practical solutions. *Environ Technol* 20: 355-365
- Koster IW, Lettinga G (1984) The influence of ammonium-nitrogen on the specific activity of pelletized methanogenic sludge. *Agricultural Wastes* 9: 205-216
- Koster IW, Lettinga G (1988) Anaerobic digestion at extreme ammonia concentrations. *Biological Wastes* 25: 51-59

- Krylova NI, Khabiboulline RE, Naumova RP, Nagel MA (1997) The influence of ammonium and methods for removal during the anaerobic treatment of poultry manure. *J Chem Technol Biotechnol* 70: 99-105
- Lay J-J, Li Y, Noike T (1997) Influences of pH and moisture content on the methane production in high-solids sludge digestion. *Water Res* 31: 1518-1152
- Liao PH, Chen A, Lo KV (1995) Removal of nitrogen swine manure wastewaters by ammonia stripping. *Biores Technol* 54: 17-20
- Maffei N, Pelletier L, Charland JP, McFarlan A (2005) An intermediate temperature direct ammonia fuel cell using a proton conducting electrolyte. *J Power Sources* 140: 264-267
- McFarlan A, Pelletier L, Maffei N (2004) An intermediate-temperature ammonia fuel cell using Gd-doped barium cerate electrolyte. *J Electrochem Soc* 151: A930-A932
- Metkemeijer R, Achard P (1994) Ammonia as a feedstock for a hydrogen fuel-cell - reformer and fuel-cell behavior. *J Power Sources* 49: 271-282
- Parkin GF, Speece RE, Yang CHJ, Kocher WM (1983) Response of methane fermentation systems to industrial toxicants. *J Water Pollut Control Fed* 55: 44-53
- Robbins JE, Gerhardt SA, Kappel TJ (1989) Effects of total ammonia on anaerobic digestion and an example of digester performance from cattle manure-protein mixtures. *Biological Wastes* 27: 1-14
- Rocher M, Goma G, Begue AP, Louvel L, Rols JL (1999) Toward a reduction in excess sludge production in activated sludge process. *Appl Microbiol Biotechnol* 51: 883-890
- Sosnowski P, Wieczorek A, Ledakowicz S (2003) Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes. *Adv Environ Res* 7: 609-616
- Sprott GD, Patel GB (1986) Ammonia toxicity in pure cultures of methanogenic bacteria. *Sys Appl Microbiol* 7: 358-363

Sung SW, Liu T (2003) Ammonia inhibition on thermophilic anaerobic digestion.
Chemosphere 53: 43-52

van Velsen AFM (1979) Adaptation of methanogenic sludge to high ammonia-nitrogen
concentrations. Water Res 13: 995-999