In-situ biogas upgrading with H_2 addition in an anaerobic membrane bioreactor (AnMBR) digesting waste activated sludge

Akira Hafuka,[a,*] Sota Fujino,[a] Katsuki Kimura,[a] Kazuyuki Oshita,[b] Naoya Konakahara,[c] Shigetoshi Takahashi[c]

[a] *Division of Environmental Engineering, Graduate School of Engineering, Hokkaido University, North-13, West-8, Kita-ku, Sapporo 060-8628, Japan*

[b] *Department of Environmental Engineering, Graduate School of Engineering, Kyoto University, Katsura C1-3, Nishikyo-ku, Kyoto, 615-8540, Japan*

[c] *Technology Center, Takuma Co., Ltd., 2-2-33 Kinrakuji-cho, Amagasaki, 660-0806, Japan*

*Corresponding author (A. Hafuka): E-mail address: ahafuka@eng.hokudai.ac.jp; Tel: +81-11-706-6273.

Abstract

Biological in-situ biogas upgrading is a promising approach for sustainable energy-powered technologies. This method increases the CH_4 content in biogas via hydrogenotrophic methanogenesis with an external H_2 supply. In this study, an anaerobic membrane bioreactor (AnMBR) was employed for in-situ biogas upgrading. The AnMBR was operated in semi-batch mode using waste activated sludge as the substrate. Pulsed H_2 addition into the reactor and biogas recirculation effectively increased the CH_4 content in the biogas. The addition of 4 equivalents of H_2 relative to CO_2 did not lead to appreciable biogas upgrading, although the acetate concentration increased significantly. When 11 equivalents of H_2 were introduced, the biogas was successfully upgraded, and the CH_4 content increased to 92%. The CH_4 yield and CH_4 production rate were 0.31 L/g-VS_{input} and 0.086 L/L/d, respectively. In this phase of the process, H_2 addition increased the acetate concentration and the pH because of CO_2 depletion. Compared with a continuously-stirred tank reactor, the AnMBR system attained higher CH_4 content, even without the addition of H_2. The longer solid retention time (100 d) in the AnMBR led to greater degradation of volatile solids. Severe membrane fouling was not observed, and the transmembrane pressure remained stable under 10 kPa for 117 d of continuous filtration without cleaning of

the membrane. The AnMBR could be a promising reactor configuration to achieve in-situ biogas upgrading during sludge digestion.

Keywords: Biomethanation; power to gas; anaerobic digestion; mesophilic; sewage sludge; membrane fouling

1. Introduction

Wastewater treatment processes generate significant amounts of sludge, which places a heavy burden on the environment (Ahmad et al., 2016). Dewatering followed by incineration is widely used to treat sludge. Anaerobic digestion (AD) is another option to treat sludge and it generates biogas. Biogas, which represents a potential energy source, generally consists of methane (CH_4) and carbon dioxide (CO_2). The CH_4 and CO_2 contents of biogas range from 40%–75% and from 25%–60%, respectively (Ryckebosch et al., 2011). This insufficient CH_4 content makes it difficult to use the biogas directly as a bio-natural gas; therefore, biogas upgrading, i.e., increasing the CH_4 content in biogas, is often conducted (Nguyen et al., 2021; Sun et al., 2015). The obtained biogas with higher CH_4 content (>90%) can be used as vehicle fuel or natural gas (Deng and Hagg, 2010). Biogas

upgrading methods can be categorized as physical, chemical, or biological technologies (Angelidaki et al., 2018), and some physical and chemical technologies are now commercially available. Physical strategies, such as water scrubbing, pressure swing adsorption, and membrane separation remove CO_2 from the biogas to increase its relative CH_4 content; therefore, these physical techniques release CO_2 into the atmosphere (Fu et al., 2021). Chemical absorption using amines has also been applied to remove CO_2 from biogas; however, this method requires energy-intensive regeneration of the amine solution (Ardolino et al., 2021). In contrast to absorption technologies, chemical reactions can convert CO_2 into CH_4 using catalysts and an external H_2 source via the Sabatier reaction ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$). However, chemical reactions have disadvantages, including the high reaction temperature (~300 °C) (Xia et al., 2016). Biological technologies that convert CO_2 to CH_4 via hydrogenotrophic methanogenesis ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) have attracted significant attention in recent years owing to their mild operational conditions (i.e., low pressure and temperature) (Angelidaki et al., 2018; Fu et al., 2021; Lai et al., 2021). Biological technologies can be further categorized as in-situ, ex-situ, or hybrid-type biogas upgrading (Angelidaki et al., 2018; Zhao et al., 2021). In contrast to the ex-situ and hybrid methods, the in-situ approach can achieve biogas generation and

67 upgrading in a single reactor by suppling H_2 directly to the anaerobic digester.

68

69 The power-to-gas strategy has also contributed to the increased attention regarding
70 biological biogas upgrading (Thema et al., 2019). This approach focuses on converting
71 surplus electricity from renewable energy sources (e.g., wind and solar energy) to gas fuel,
72 which is relatively easier to store and transport. Dihydrogen gas can be generated from the
73 electrolysis of water using surplus electricity. In general, further conversion of H_2 to CH_4 is
74 desirable because the volumetric energy density of H_2 is relatively low, and current
75 infrastructure can be used to store, transport, and use CH_4 (Götz et al., 2016). Therefore, in-
76 situ biogas upgrading with H_2 addition has been studied using batch tests (Luo et al., 2012;
77 Wang et al., 2016), continuously-stirred tank reactors (CSTRs) (Bassani et al., 2015; Jensen
78 et al., 2018; Zhu et al., 2019), and up-flow anaerobic sludge blankets (Bassani et al., 2016;
79 Park et al., 2021; Xu et al., 2020).

80

81 Anaerobic digestion of sludge enables biogas generation, while also reducing the volume of
82 sludge and destroying pathogens (Appels et al., 2008). However, the quality of effluent is
83 generally poor because of the low growth rates of anaerobic microorganisms and washout

of these microorganisms from the digester. To solve these problems, anaerobic membrane bioreactors (AnMBRs) have been applied for sludge digestion (Abdelrahman et al., 2020). An AnMBR comprises a system that couples membrane filtration with anaerobic treatment. The distinguishing feature of AnMBRs involves the decoupling of hydraulic retention time and solid retention time (Skouteris et al., 2012), which can prolong the SRT without simultaneously extending the HRT following solid-liquid separation with membranes. Therefore, AnMBRs generate higher-quality effluent than conventional CSTRs because the membrane removes suspended solids (SS). Additionally, anaerobic microorganisms can be retained inside the digester. Deschamps et al. (2021) have recently reported in-situ biogas upgrading with H_2 addition in an AnMBR treating industrial wastewater. However, in-situ biogas upgrading in an AnMBR configuration has not yet been applied for sewage sludge. Therefore, the objective of the current study was to achieve in-situ biogas upgrading in an AnMBR digesting sewage sludge. Waste activated sludge (WAS) was used as the substrate, and the WAS digestion and biogas upgrading performance of the AnMBR system were investigated. We tested the hypothesis that the amount of H_2 added would be a key factor for in-situ biogas upgrading in the AnMBR.

2. Materials and Methods

2.1 Reactor setup

Figure 1 shows a schematic diagram of the AnMBR experimental apparatus, which comprised a jar fermenter (BMS-03NP4, Biott Corporation, Tokyo, Japan) and an external membrane unit. The external cross-flow configuration was selected to mitigate membrane fouling (Hafuka et al., 2019). A vessel with a working volume of 2.37 L was continuously mixed with a stirrer at 40 rpm. The temperature was held constant at 37 °C (i.e., mesophilic conditions) with a heating jacket. The external membrane unit had a working volume of 0.1 L, and the unit contained a polyvinylidene fluoride hollow fiber microfiltration (MF) membrane (Microza®, Asahi Kasei Corp., Tokyo, Japan). The total effective area of the membrane was 0.0016 m^2, the inner diameter of each membrane fiber was 2.6 mm, and the pore size of the membrane was 0.1 μm. The vessel was inoculated with seed sludge (2.16 L) obtained from a full-scale mesophilic digester in a sewage treatment plant. The concentrations of total solids and volatile solids of the seed sludge were 13.1 g/L and, 8.9 g/L, respectively. Before starting the reactor operation, air in the reactor was purged with N_2 gas. The WAS fed into the AnMBR was obtained from another full-scale sewage treatment plant using a conventional activated sludge process. Raw WAS was screened

118 through a 1 mm mesh screen, and then the supernatant was removed to thicken the sludge.
119 The AnMBR was operated in semi-batch mode. Sludge feeding (0.21–0.57 L) and sludge
120 withdrawal (0.16–0.57 L) were performed manually once per week. The digested sludge
121 was circulated continuously using a peristaltic pump, and membrane filtration of the
122 digested sludge was performed at a constant cross-flow velocity of 0.5 m/s. This cross-flow
123 velocity is within the range of reported values (Abdelrahman et al., 2020). The digested
124 sludge was continuously filtered through the hollow fiber membrane, and the filtrate was
125 collected (i.e., inside-out filtration). The membrane flux was held constant by controlling
126 the filtrate flow rate with a peristaltic pump. The pressure inside and outside of the
127 membrane was measured daily using manometers, and the transmembrane pressure (TMP)
128 values were calculated from these data. The pH and oxidation-reduction potential (ORP) of
129 the digested sludge inside the fermenter were measured using an attached pH/ORP meter.
130 The biogas produced from the fermenter was stored in an aluminum gas bag, and the
131 volume of the biogas was measured using a wet gas meter (W-NK-0.5A, Shinagawa Co.,
132 Tokyo, Japan). The HRT and SRT of the reactor were controlled by changing the filtrate
133 flow rate and the sludge withdrawal volume according to Equations 1 and 2, respectively:

134 $$\mathrm{HRT\ (d)} = \frac{\text{Working volume of the reacotor (L)}}{\text{Input of WAS (L/d)}} \qquad (1)$$

$$\text{SRT (d)} = \frac{\text{Working volume of the reactor (L)}}{\text{Withdrawal of digested sludge (L/d)}} \quad (2)$$

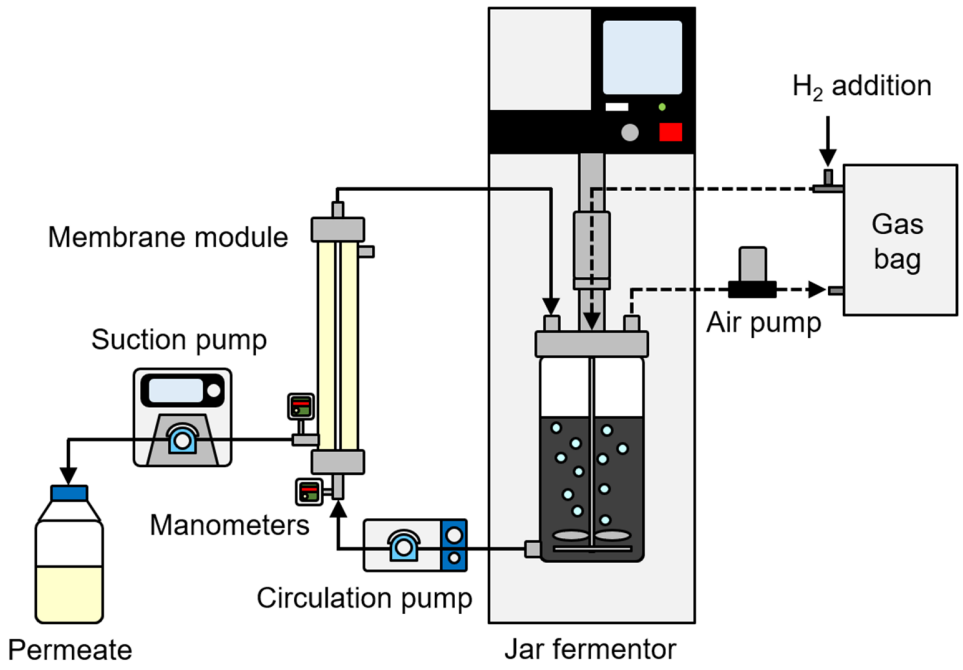


Figure 1. Schematic diagram of the AnMBR.

2.2 Reactor operating conditions

The whole operating period of the reactor was 221 days, and it was divided into seven separate phases, each involving distinct operational conditions (Table 1). The HRT and the SRT values were selected based on a previous study (Abdelrahman et al., 2020). During phase 1 (operation days 1–42), both the HRT and the SRT were set to 80 d (i.e., CSTR

146 mode without using the membrane module). Phase 1 was considered the acclimatization
147 period. The operation of the AnMBR began in phase 2 (operation days 42–102), during
148 which the HRT and SRT were set to 30 and 100 d, respectively. During phase 3 (operation
149 days 102–123), biogas was continuously re-circulated at 0.52 L/min between the fermenter
150 and gasbag using a diaphragm-type air pump (APN-10D3-W, IWAKI CO., LTD., Tokyo,
151 Japan). Biogas produced in the fermenter was passed through the gasbag and then re-
152 introduced into the fermenter through a diffuser. The diffuser, which had 12 pinholes (ϕ =
153 0.6 mm), was located at the bottom of the vessel. Phase 4 (operation days 123–137)
154 involved in-situ biogas upgrading with added H_2 and biogas recirculation. Hydrogen gas
155 (99.99%) was collected from canned standard hydrogen gas (1020-11201, GL Sciences
156 Inc., Tokyo, Japan) and added to the gasbag five times per week (totally 0.54 L/week; see
157 supplementary material). During phase 5 (operation days 137–158), the H_2 addition rate
158 was increased to 1.50 L/week. To confirm the effect of AnMBR operation on CH_4 content
159 in biogas, CSTR operation without H_2 addition was continued during phase 6 (operation
160 days 158–179) and phase 7 (operation days 179–221).
161

Table 1. Operational conditions of each phase of the reactor.

Phase No.	Operational mode	HRT (d)	SRT (d)	Filtration flux (LMH)	H_2 addition (L/week)	Biogas recirculation rate (L/min)
1	CSTR	80	80	-	-	-
2	AnMBR	33	107	1.29	-	-
3	AnMBR	30	102	1.46	-	0.52
4	AnMBR	30	98	1.38	0.54	0.52
5	AnMBR	29	98	1.46	1.50	0.52
6	CSTR	54	54	-	-	0.52
7	CSTR	29	29	-	-	0.52

164 *2.3 Analytical methods*

165 Selected physical and chemical properties of the WAS, membrane permeate, and digested
166 sludge were analyzed weekly. The concentrations of total solids (TS), volatile solids (VS),
167 chemical oxygen demand with potassium dichromate (COD_{Cr}), ammonium nitrogen (NH_4^+-
168 N), and alkalinity were determined as previously described (Hafuka et al., 2019). The
169 concentrations of volatile fatty acids (VFAs; e.g., acetate, propionate, *i*-butyrate, *n*-butyrate,
170 *i*-valerate, and *n*-valerate) in the digested sludge were measured for samples after
171 centrifugation and filtration (0.20 μm; 25HP020AN, Toyo Roshi Kaisya, Ltd., Tokyo,
172 Japan) using a high-performance liquid chromatography system (LC-10AD, Shimadzu
173 Corporation, Kyoto, Japan) equipped with an electrical conductivity detector and a
174 stainless-steel-packed column with dimensions 0.3 m × 8.0 mm (Shim-pack SCR-102H;
175 Shimadzu Corporation, Kyoto, Japan). The CH_4, CO_2, and H_2 contents of the biogas were
176 determined using a gas chromatograph (GC-14B, Shimadzu Corporation, Kyoto, Japan)
177 equipped with a thermal conductivity detector and a 6.0 m × 3.0 mm stainless-steel-packed
178 column (Shincarbon St; Shinwa Chemical Industries, Ltd., Kyoto, Japan).

179

180 VS degradation efficiency was calculated based on the VS mass balance in each phase.

Biogas production rate and biogas yield were calculated by using cumulative biogas volume obtained in each phase. The CH_4 production rate and CH_4 yield were calculated by using average CH_4 content in biogas obtained in each phase. The efficiency of the COD rejection by the membrane was calculated according to Equation 3,

$$\text{COD rejection efficiency of the membrane (\%)} = \frac{\text{COD}_{\text{digested sludge}} - \text{COD}_{\text{permeate}}}{\text{COD}_{\text{digested sludge}}} \times 100 \qquad (3)$$

where $COD_{\text{digested sludge}}$ (i.e., total COD) and COD_{permeate} (i.e., soluble COD) are the COD concentrations of the digested sludge and permeate (mg/L), respectively.

2.4 Membrane cleaning

Membrane cleaning was conducted after the 221-day operation of the reactor. The membrane unit was removed from the AnMBR and the inside of the hollow fiber membrane was washed with tap water to physically remove the cake foulant. The filtration resistance of the membrane was determined by filtering tap water at a pressure of 70 kPa. Filtration resistances before and after cleaning were compared with the filtration resistance of the pristine membrane, which was measured prior to the reactor operation. The filtration

198 resistances of the membranes were obtained according to Equations 4:

199 $$R = \frac{\text{TMP (Pa)}}{\mu\ (\text{Pa} \cdot \text{s})\ \times J\ (\text{m}^3/(\text{m}^2 \cdot \text{s}))} \qquad (4)$$

200

201 where R is the filtration resistance, μ is the viscosity of water, and J is the permeate flux.

202

203 *2.5 Statistical analysis*

204 Pearson correlation coefficients were calculated to identify the significance of a correlation

205 between two parameters. The *t*-test was applied to analyze the minimum and maximum

206 acetate concentration and CH_4 content; *p*-values less than 0.05 were regarded as significant.

207 These analyses were performed using Origin Pro 9.8 software.

208

209 **3. Results and Discussion**

210 *3.1 In-situ biogas upgrading in an AnMBR*

211 The average TS and VS concentrations of the WAS were 9.9 g/L (± 2.2 g/L) and 7.9 g/L (±

212 1.8 g/L), respectively (Table 2). The VS/TS ratio remained stable at approximately 80% (±

213 2%), and the average total COD (T-COD) concentration in the WAS reached 12.4 ± 3.2 g/L.

214

215 **Table 2.** Characteristics of the raw WAS (n = 30).

Parameters (unit)	Values ± Standard deviations
TS (g/L)	9.9 ± 2.2
VS (g/L)	7.9 ± 1.8
VS/TS (%)	80 ± 2
T-COD (g/L)	12.4 ± 3.2
NH_4^+ (mg-N/L)	18 ± 11
pH (-)	6.8 ± 0.2

216

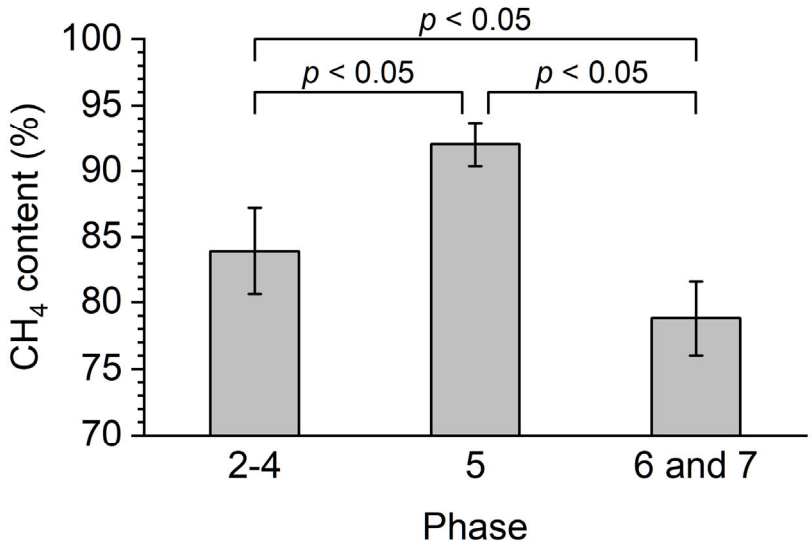
217 Table 3 summarizes the performance of the reactor during each phase. The CH_4 content in
218 the biogas was 83.1% (± 3.9%) in phase 2. Biogas recirculation began in phase 3 to
219 investigate its effect on the CH_4 content in biogas. As a result, the CH_4 content did not
220 change with biogas recirculation (85.2 ± 2.3% in phase 3). In phase 4, H_2 was introduced
221 into the reactor to achieve in-situ biogas upgrading. Dihydrogen gas was added such that a
222 4:1 stoichiometric ratio of H_2/CO_2 was obtained; the cumulative biogas volume and average
223 CO_2 content obtained in phase 3 were used to determine the added amount of H_2. It was
224 confirmed that H_2 did not remain in the biogas, but rather, it was consumed one day after

addition. As a result, the CH_4 content did not change (remained at 83.6 ± 0.5%), indicating that the biogas was not upgraded in phase 4. In phase 5, the amount of H_2 added was increased to 11 molar equivalents relative to the CO_2 generated in the reactor. This quantity was also determined based on the cumulative biogas volume and average CO_2 content obtained in phase 4. In this phase, the CH_4 content increased significantly (to 92.0 ± 1.6%) compared with that detected in phases 2, 3, and 4 ($|t| = 4.41$, $p < 0.05$) (Figure 2). The CH_4 yield and CH_4 production rate were 0.31 L/g-VS_{input} and 0.086 L/L/d, respectively. H_2 did not remain in the biogas samples. This result indicates that H_2 consumption rate was higher than 0.13 L/L/d. Although high CH_4 content (i.e., 92%) was obtained in the present study, the CH_4 production rate and H_2 consumption rare were lower than those reported in previous studies probably due to the lower OLR (Alfaro et al., 2019; Lovato et al., 2017). Energy balance analysis was conducted according to the Equations reported in previous studies (Chen et al., 2019; Cheng et al., 2021; Xiao et al., 2018). In phase 5, the energy production and net energy balance were 10.2 kJ/g-VS and 0.5 kJ/g-VS, respectively (see supplementary material).

241 **Table 3.** WAS digestion performance of the system during each phase. [a] A biogas sample was not recovered.

Phase	Organic loading rate (g-VS/L/d)	VS degradation (%)	Biogas yield (L/g-VS_{input})	Biogas production rate (L/L/d)	CH_4 content (%)	CH_4 yield (L/g-VS_{input})	CH_4 production rate (L/L/d)
1	0.13	47	0.22	0.024	-[a]	-	-
2	0.21	65	0.29	0.076	83.1 ± 3.9	0.24	0.063
3	0.27	63	0.22	0.059	85.2 ± 2.3	0.19	0.050
4	0.26	55	0.33	0.087	83.6 ± 0.5	0.28	0.073
5	0.28	57	0.33	0.093	92.0 ± 1.6	0.31	0.086
6	0.29	44	0.30	0.084	80.3 ± 1.5	0.24	0.068
7	0.37	37	0.36	0.134	77.7 ± 3.0	0.28	0.104

242



243

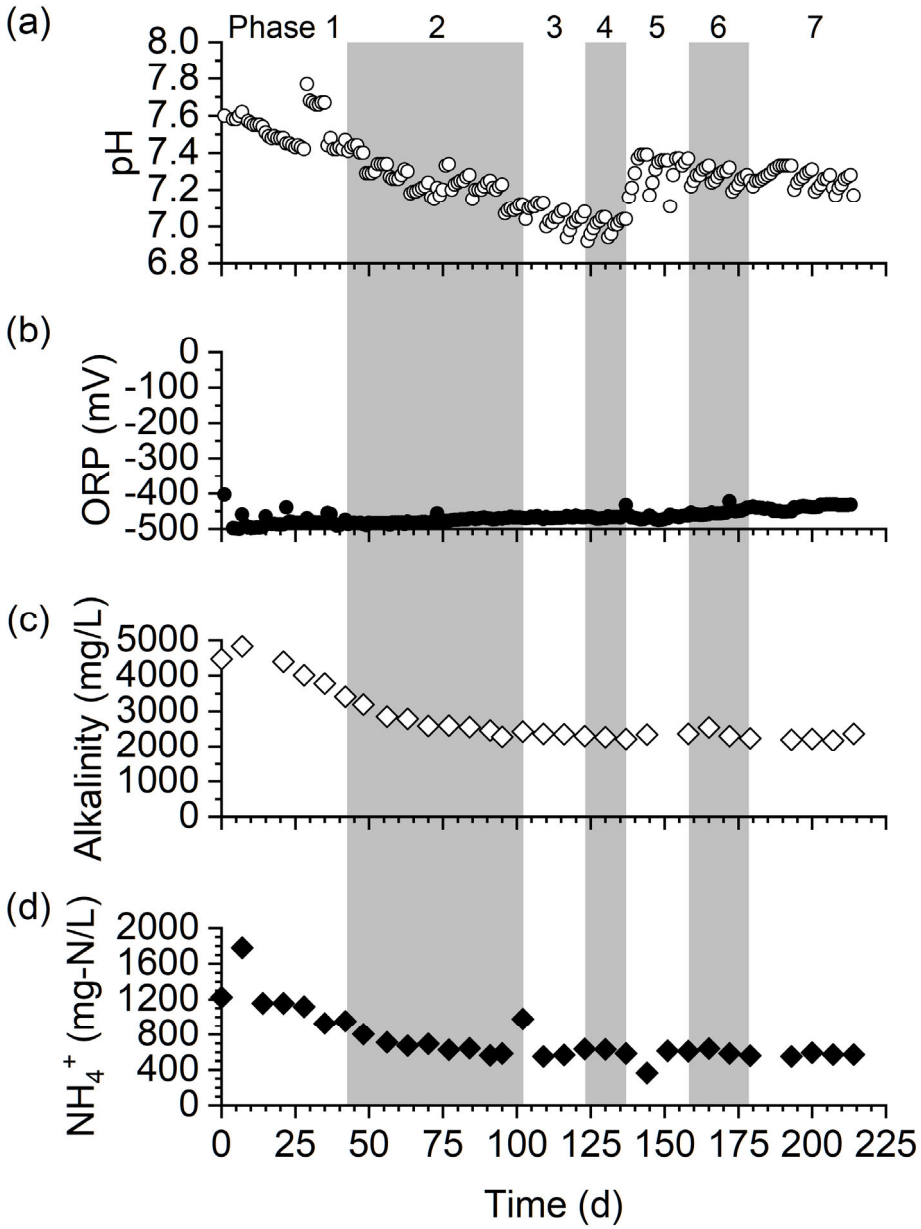
244 **Figure 2.** Comparison in CH_4 content in biogas among operational phases.

245

246 *3.2 pH, ORP, alkalinity, and NH_4^+ in an AnMBR*

247 The AnMBR investigated in this work achieved in-situ biogas upgrading following the
248 treatment of WAS, and the CH_4 content in the biogas reached 92% in phase 5 of the
249 process. There are very few studies regarding in-situ biogas upgrading using sewage sludge
250 as the substrate (Alfaro et al., 2019; Lovato et al., 2017). Compared with those studies, the
251 system evaluated in this work obtained high CH_4 content. Furthermore, in phase 5, the CO_2
252 in the biogas was converted to CH_4 following the addition of H_2; this reduced the CO_2
253 content in the biogas to 8%. This also decreased the amount of CO_2 dissolved in the liquid

254 phase, which induced the increased pH in the reactor (Figure 3a). In one operational cycle
255 (i.e., one week), the pH decreased because of the WAS input, and then it gradually
256 increased within the cycle. Compared with the pH value measured in phases 2, 3, and 4, it
257 increased significantly to 7.3 ($|t| = 5.12$, $p < 0.01$) in phase 5 following the successful in-
258 situ biogas upgrading upon adding H_2 (see supplementary material). Although a rise in pH
259 occurred during phase 5, the pH values were in the range from 7.0–7.4, which was within
260 the ideal pH range for AD (Mao et al., 2015). The ORP was below -390 mV during
261 operation, confirming AD conditions in the fermenter (Figure 3b). The alkalinity gradually
262 decreased from 5000 mg/L at the start of operation and stabilized at around 2500 mg/L
263 during phases 3–7 (Figure 3c). The NH_4^+ concentration was also stable at approximately
264 600 mg-N/L during phases 3–7 and remained below the inhibition level (Rajagopal et al.,
265 2013) (Figure 3d).
266



267

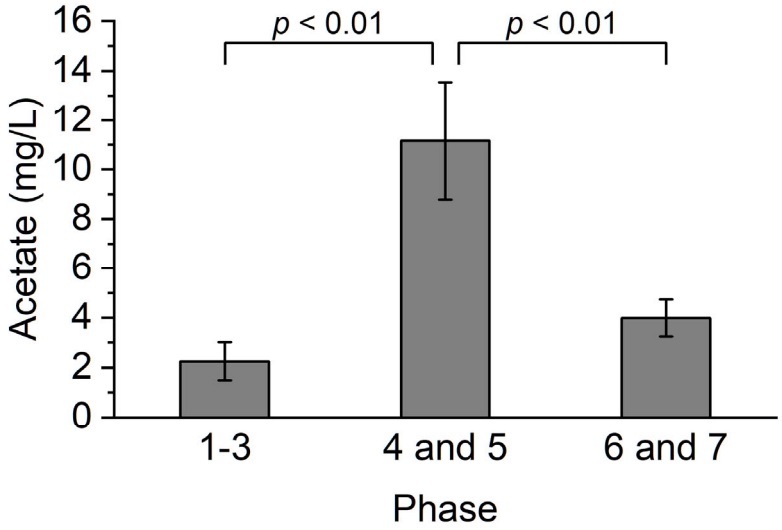
268 **Figure 3.** (a) pH, (b) ORP, (c) alkalinity, and (d) NH_4^+ concentration in the fermenter.

269

3.3 Possible contributing factors for the successful in-situ biogas upgrading in an AnMBR

Multiple factors contributed to the successful in-situ biogas upgrading demonstrated in this study. First, the amount of H_2 added (i.e., 11 molar equivalents of H_2 relative to the CO_2 generated in the reactor) in phase 5 was optimal for in-situ biogas upgrading. The H_2/CO_2 ratio of 11:1 was consistent with results obtained by Agneessens et al. (2017). They investigated the effect of H_2 addition on in-situ biogas upgrading in CSTRs and observed that with a 10:1 ratio of H_2/CO_2, the CO_2 content in the headspace of the reactor decreased to 7.9%. Second, pulsed H_2 addition in this study might be effective for in-situ biogas upgrading. Agneessens et al. (2017) also found that pulsed H_2 addition increased the H_2 uptake rates and the activity of certain hydrogenotrophic methanogens. Third, although the gas diffuser configuration and the biogas recirculation rate were not optimized in this study, biogas recirculation through the gas diffuser could facilitate H_2 gas transfer to the liquid phase. It is well known that the low gas-liquid mass transfer rate of H_2 is one of the limiting factors in the in-situ biogas upgrading process because microorganisms use the dissolved H_2 in the reactor (Bassani et al., 2016; Jensen et al., 2018; Park et al., 2021). Fourth, the relatively low OLR (0.28 g-VS/L/d) in phase 5 may prevent process failure. H_2 addition and in-situ biogas upgrading deplete the amount of dissolved CO_2 in the reactor, which

leads to the accumulation of VFAs (Mulat et al., 2017). Therefore, a high OLR might result in significant VFA accumulation during in-situ biogas upgrading, which could lead to reactor acidification and process deterioration (Franke-Whittle et al., 2014). Indeed, the acetate concentration increased in phases 4 and 5 because of H_2 addition (Figure 4). The acetate concentration remained stable at approximately 2.3 mg/L during phases 1, 2, and 3, but then it increased significantly to 11 mg/L in phases 4 and 5 ($|t| = 7.40$, $p < 0.01$). No other VFAs were detected in any of the analyzed samples. Compared with the acetate concentrations in phases 4 and 5, those in phases 6 and 7 decreased significantly ($|t| = 5.85$, $p < 0.01$) during CSTR operation without H_2 addition. Although H_2 was consumed and acetate accumulated in phase 4, the CH_4 content did not increase (see Table 3). However, the biogas yield increased from 0.22 L/g-VS_{input} in phase 3 to 0.33 L/g-VS_{input} in phase 4. These results indicated that H_2 addition might inhibit acetate consumption and promote acetate production by homoacetogens (Agneessens et al., 2017 and 2018; Liu et al., 2016; Mulat et al., 2017).



302

303 **Figure 4.** Comparison in acetate concentration in the fermenter among operational phases.

304

305 *3.4 Comparison between AnMBRs and CSTRs without H_2 addition*

306 The CH_4 contents in phases 2 and 3 were high (83.1% and 85.2%, respectively) even if H_2

307 was not added during the AnMBR mode of operation, relative to the general value (i.e., 40–

308 75%). Therefore, the CSTR mode of operation without H_2 addition was used for phases 6

309 and 7 to investigate the effect of the operational mode on the CH_4 content in biogas. As a

310 result, the CH_4 contents in phases 6 and 7 were 80.3% (± 1.5%) and 77.7% (± 3.0%),

311 respectively (see Table 3). Compared with the CH_4 contents in phases 2, 3, and 4, those in

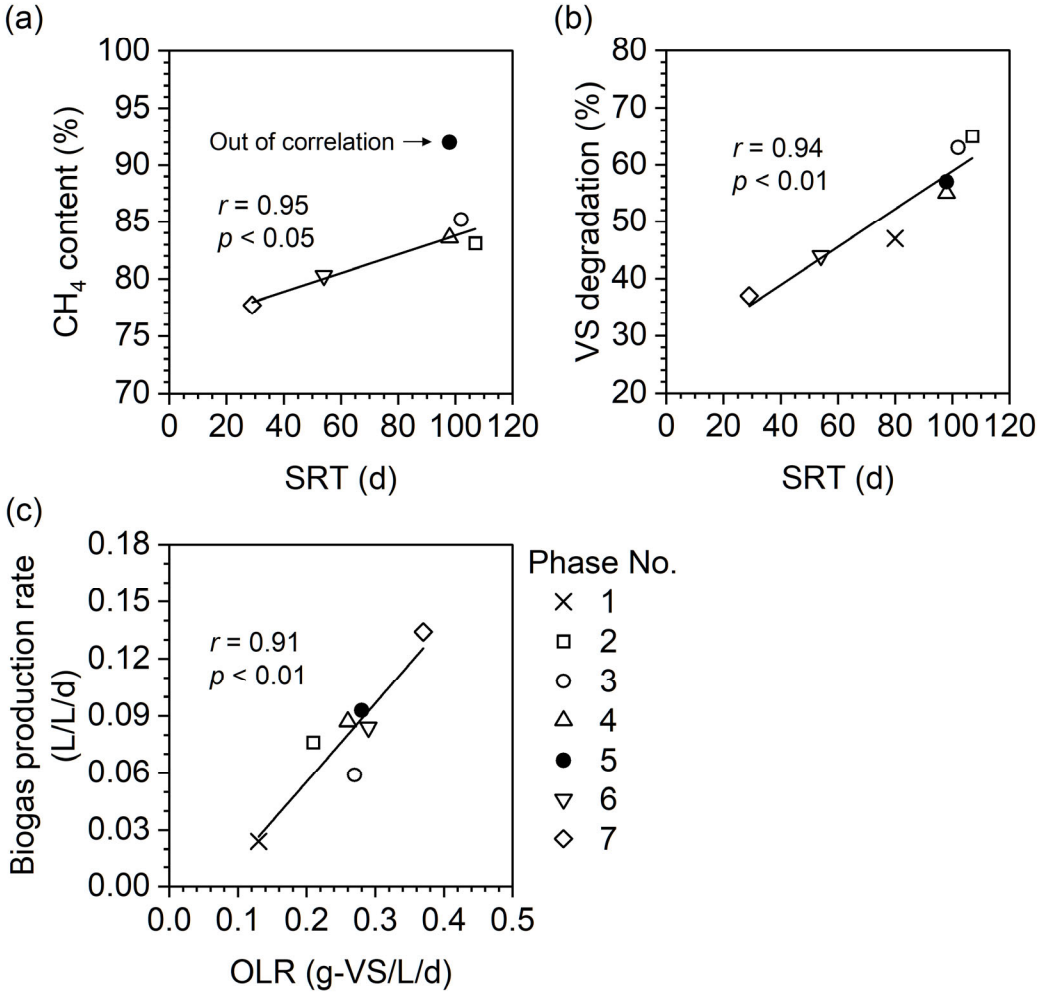
312 phases 6 and 7 were significantly lower ($|t| = 6.58$, $p < 0.05$) (see Figure 2). This result

313 indicated that the CH_4 content increased during AnMBR operation compared with CSTR
314 operation even if H_2 was not supplied to AnMBR. This suggested that it might be easier to
315 increase the CH_4 content in AnMBRs because the baseline CH_4 content was higher than
316 that in CSTRs.
317
318 There are three possible reasons for the higher CH_4 content in the AnMBR. First, the
319 membrane permeate might release dissolved inorganic carbon (i.e., dissolved CO_2 and
320 HCO_3^-) outside of the fermenter, which would lead to higher CH_4 content (Yu et al., 2018).
321 In contrast to the CSTR mode of operation, this study involved continuous membrane
322 filtration in the AnMBR mode of operation during phases 2–5. Therefore, dissolved
323 inorganic carbon in the permeate may continuously flow outward, which would promote
324 further dissolution of CO_2 into the liquid phase in the fermenter. Because the Henry's
325 constant of CO_2 is higher than that of CH_4 (Sander et al., 2015), the CH_4 content in biogas
326 might show a relative increase in an AnMBR. Both the gradual reduction in pH during
327 phases 2–5 in the AnMBR and stable pH at approximately 7.3 during phases 6 and 7 in the
328 CSTR support this explanation (see Figure 3a). However, this could be a disadvantage of
329 AnMBRs because it is associated with a lost carbon source for conversion to CH_4.

330

331 Second, the characteristics of the WAS substrate and the relatively long SRT in AnMBRs
332 may lead to higher CH_4 content in biogas. It is well known that proteins are the major
333 organic components of WAS (Xiao et al., 2017), and biogas obtained from the AD of WAS
334 has high CH_4 content (~71%) (Bougrier et al., 2007). In the present study, the SRT was
335 positively correlated with both the CH_4 content ($r = 0.95$, $p < 0.05$) and the VS degradation
336 efficiency ($r = 0.94$, $p < 0.01$) (Figure 5a and 5b). As shown in Figure 5a, the data point
337 obtained from phase 5 was out of correlation because the biogas was successfully upgraded
338 following H_2 addition. Compared with the CSTR mode, the AnMBR system developed in
339 this study achieved a higher VS degradation efficiency (see Figure 5b and Table 3).
340 Therefore, it is likely that the proteins in WAS were degraded well in the AnMBR because
341 of the longer SRT, which resulted in biogas with higher CH_4 content. The change in the
342 biogas production rate could be explained by the organic loading rate (OLR). A positive
343 correlation between the OLR and the biogas production rate ($r = 0.91$, $p < 0.01$) was
344 observed (Figure 5c). Therefore, the higher biogas production rate in phase 7 was a result of
345 the higher OLR (see Table 3).
346

347 Third, changes in the microbial community might affect the CH_4 content. Although the
348 microbial community was not investigated in the present study, Yu et al. (2016) reported
349 that hydrogenotrophic methanogenesis played a more important role in an anaerobic
350 dynamic membrane bioreactor, resulting in biogas with higher CH_4 content. Further
351 investigations are required to confirm these hypotheses.
352



353

354 **Figure 5.** Relationships between (a) SRT and CH_4 content in biogas, (b) SRT and VS

355 degradation efficiency, and (c) organic loading rate and biogas production rate.

356

357 *3.5 Performance of the membrane unit*

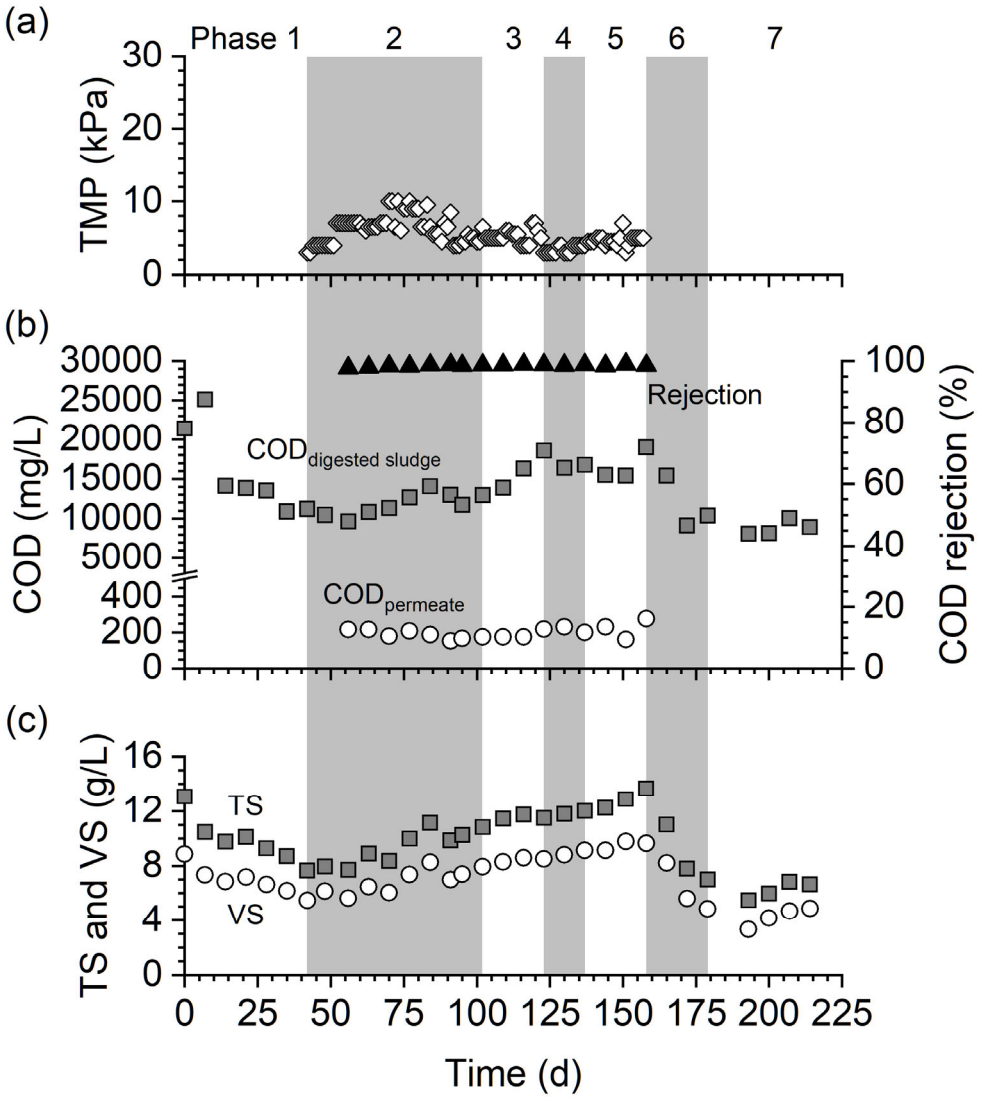
358 Although membrane fouling is an inevitable challenge in membrane-based treatment

processes (Meng et al., 2017; Wang et al., 2014), severe membrane fouling was not observed in this study (Figure 6a). The TMP was stable under 10 kPa for 117 d of continuous filtration without cleaning the membrane (phases 2–5). There are two reasons for the successful mitigation of membrane fouling. One is that the reactor configuration incorporated external cross-flow. There are two types of AnMBR configurations: external cross-flow and submerged configurations (Abdelrahman et al., 2020). In external cross-flow configurations, the membrane unit is outside of the digester, whereas in the submerged configurations, membranes are immersed in the digester or external sludge tank. The high shear force on the membranes in a cross-flow configuration can better control membrane fouling (Abdelrahman et al., 2020). In addition, the use of an external membrane unit outside the digester can provide ease of membrane maintenance since the head space of the digester cannot be opened frequently in order to maintain anaerobic conditions. Although cross-flow velocity of 0.5 m/s in the present study require additional energy (to power the cross-flow pumps), they are proven to mitigate membrane fouling, and energy can be recovered from the AD system in the form of biogas. Second, the relatively low filtration flux (i.e., 1.25 LMH ≈ 0.03 m/d) effectively prevented a drastic increase in the TMP in this study. AnMBRs treating WAS have relatively long HRTs compared with aerobic or

anaerobic membrane bioreactors treating low-strength wastewater. Therefore, a relatively low flux is often sufficient for WAS digestion in AnMBRs, which could mitigate membrane fouling (Hafuka et al., 2019).

In the AnMBR, the COD concentration in the membrane permeate stabilized at 210 mg/L during phases 2–5, which suggested that the effluent quality was higher than that of a conventional CSTR (Figure 6b). The COD concentration in the digested sludge was in the range from 8000 to 25,200 mg/L, and it increased gradually from 10,500 to 19,000 mg/L during phases 2–5, while membrane filtration continued. The same trend was observed for the concentrations of TS and VS (Figure 6c). Overall, the efficiency of COD rejection by the membrane was greater than 95%. This result was attributed to the membrane's highly efficient rejection of suspended COD. The COD, TS, and VS concentrations in digested sludge gradually increased during phases 2–5 because of the solid-liquid separation by the membrane. In addition, these concentrations were higher than those in the WAS (see Table 2). These results suggested that the membrane thickened the WAS and helped retain the digested sludge inside the reactor (Hafuka et al., 2016); these factors led to a higher content of microbial biomass inside the reactor. In the present study, the AnMBR demonstrated a

393 high VS degradation efficiency compared to that of the CSTR because of the longer SRT
394 (100 d; see Figure 5b). This result originated from the distinctive feature of AnMBRs, i.e.,
395 the decoupling of the HRT and SRT. AnMBRs can prolong the SRT without simultaneously
396 extending the HRT, which shortens the overall treatment time and reduces the footprint of
397 the reactor (Cheng et al., 2021).
398



399

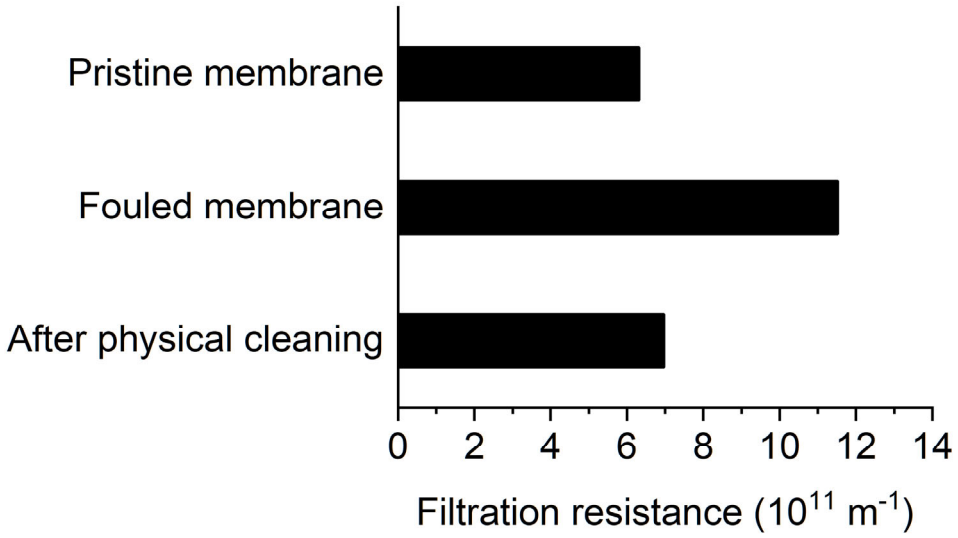
400 **Figure 6.** (a) TMP of the membrane unit over time; (b) COD concentrations of the digested

401 sludge and membrane permeate, as well as the efficiency of COD rejection by the

402 membrane; (c) TS and VS concentrations in the fermenter.

403

404 The filtration resistance of the pristine membrane was 6.3×10^{11} m^{-1} (Figure 7). The
405 resistance increased to 11.5×10^{11} m^{-1} due to membrane fouling after the 221-day operation
406 of the reactor. Hydraulic physical cleaning was effective in removing membrane foulants
407 and the resistance decreased to 7.0×10^{11} m^{-1}. This result suggests that physically reversible
408 fouling had high contribution to the membrane fouling in the present study (Hafuka et al.,
409 2019).
410



411
412 **Figure 7.** Filtration resistances of the pristine membrane, the fouled membrane, and the
413 membrane after physical cleaning.
414

4. Conclusions

In this study, an AnMBR was employed to digest waste activated sludge in a semi-batch mode, and the system demonstrated successful in-situ biogas upgrading. The CH_4 content reached 92% when 11 equivalents of H_2 were added (relative to the CO_2 generated by the fermenter). To our knowledge, this represents the first report detailing in-situ biogas upgrading in an AnMBR digesting WAS. The addition of H_2 increased the acetate concentration, and successful biogas upgrading led to an increased pH. Compared with the CSTR mode of operation, the AnMBR mode of operation without added H_2 obtained higher CH_4 contents. It was possible to conduct continuous membrane filtration of the digested sludge over 117 d without cleaning the membrane. No appreciable rise in the TMP was observed because of the low filtration flux and cross-flow filtration. The present study has some limitations such as the low OLR and lacks an energy balance analysis. Further research to solve these problems is now under way.

Acknowledgments

This research was financially supported by Takuma Co., Ltd., Japan. We also thank Suzanne Adam, PhD, from Edanz (https://jp.edanz.com/ac) for editing a draft of this

manuscript.

Appendix A. Supplementary Material

Supplementary materials are available.

References

Abdelrahman, A.M., Ozgun, H., Dereli, R.K., Isik, O., Ozcan, O.Y., van Lier, J.B., Ozturk, I., Ersahin, M.E., 2021. Anaerobic membrane bioreactors for sludge digestion: Current status and future perspectives. Crit. Rev. Environ. Sci. Technol. 51, 2119–2157. https://doi.org/10.1080/10643389.2020.1780879.

Agneessens, L.M., Ottosen, L.D.M., Andersen, M., Olesen, C.B., Feilberg, A., Kofoed, M.V.W., 2018. Parameters affecting acetate concentrations during in-situ biological hydrogen methanation. Bioresour. Technol. 258, 33–40. https://doi.org/10.1016/j.biortech.2018.02.102.

Agneessens, L.M., Ottosen, L.D.M., Voigt, N.V., Nielsen, J.L., de Jonge, N., Fischer, C.H., Kofoed, M.V.W., 2017. In-situ biogas upgrading with pulse H_2 additions: The relevance of methanogen adaption and inorganic carbon level. Bioresour. Technol. 233,

256–263. https://doi.org/10.1016/j.biortech.2017.02.016.

Ahmad, T., Ahmad, K., Alam, M., 2016. Sustainable management of water treatment sludge through 3'R' concept. J. Clean. Prod. 124, 1–13. https://doi.org/10.1016/j.jclepro.2016.02.073.

Alfaro, N., Fdz-Polanco, M., Fdz-Polanco, F., Diaz, I., 2019. H_2 addition through a submerged membrane for in-situ biogas upgrading in the anaerobic digestion of sewage sludge. Bioresour. Technol. 280, 1-8. https://doi.org/10.1016/j.biortech.2019.01.135.

Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H., Kougias, P.G., 2018. Biogas upgrading and utilization: Current status and perspectives. Biotechnol. Adv. 36, 452–466. https://doi.org/10.1016/j.biotechadv.2018.01.011.

Appels, L., Baeyens, J., Degreve, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755–781. https://doi.org/10.1016/j.pecs.2008.06.002.

Ardolino, F., Cardamone, G.F., Parrillo, F., Arena, U., 2021. Biogas-to-biomethane upgrading: A comparative review and assessment in a life cycle perspective. Renew. Sust. Energ. Rev. 139, 110588. https://doi.org/10.1016/j.rser.2020.110588.

Bassani, I., Kougias, P.G., Angelidaki, I., 2016. In-situ biogas upgrading in thermophilic

466 granular UASB reactor: key factors affecting the hydrogen mass transfer rate.
467 Bioresour. Technol. 221, 485–491. https://doi.org/10.1016/j.biortech.2016.09.083.
468 Bassani, I., Kougias, P.G., Treu, L., Angelidaki, I., 2015. Biogas Upgrading via
469 Hydrogenotrophic Methanogenesis in Two-Stage Continuous Stirred Tank Reactors at
470 Mesophilic and Thermophilic Conditions. Environ. Sci. Technol. 49, 12585–12593.
471 https://doi.org/10.1021/acs.est.5b03451.
472 Bougrier, C., Delgenes, J.P., Carrere, H., 2007. Impacts of thermal pre-treatments on the
473 semi-continuous anaerobic digestion of waste activated sludge. Biochem. Eng. J. 34,
474 20–27. https://doi.org/10.1016/j.bej.2006.11.013.
475 Chen, R., Wen, W., Jiang, H.Y., Lei, Z., Li, M.Z., Li, Y.Y., 2019. Energy recovery potential
476 of thermophilic high-solids co-digestion of coffee processing wastewater and waste
477 activated sludge by anaerobic membrane bioreactor. Bioresour. Technol. 274, 127–133.
478 https://doi.org/10.1016/j.biortech.2018.11.080.
479 Cheng, H., Li, Y.M., Hu, Y.S., Guo, G.Z., Cong, M., Xiao, B.Y., Li, Y.Y., 2021. Bioenergy
480 recovery from methanogenic co-digestion of food waste and sewage sludge by a high-
481 solid anaerobic membrane bioreactor (AnMBR): Mass balance and energy potential.
482 Bioresour. Technol. 326, 124754. https://doi.org/10.1016/j.biortech.2021.124754.

Deng, L.Y., Hagg, M.B., 2010. Techno-economic evaluation of biogas upgrading process using CO_2 facilitated transport membrane. Int. J. Greenh. Gas Control. 4, 638–646. https://doi.org/10.1016/j.ijggc.2009.12.013.

Deschamps, L., Imatoukene, N., Lemaire, J., Mounkaila, M., Filali, R., Lopez, M., Theoleyre, M.A., 2021. In-situ biogas upgrading by bio-methanation with an innovative membrane bioreactor combining sludge filtration and H_2 injection. Bioresour. Technol. 337, 125444. https://doi.org/10.1016/j.biortech.2021.125444.

Franke-Whittle, I.H., Walter, A., Ebner, C., Insam, H., 2014. Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. Waste Manage. 34, 2080–2089. https://doi.org/10.1016/j.wasman.2014.07.020.

Fu, S.F., Angelidaki, I., Zhang, Y.F., 2021. In situ Biogas Upgrading by CO_2-to-CH_4 Bioconversion. Trends Biotechnol. 39, 336–347. https://doi.org/10.1016/j.tibtech.2020.08.006.

Götz, M., Lefebvre, J., Mors, F., Koch, A.M., Graf, F., Bajohr, S., Reimert, R., Kolb, T., 2016. Renewable Power-to-Gas: A technological and economic review. Renew. Energ. 85, 1371–1390. https://doi.org/10.1016/j.renene.2015.07.066.

Hafuka, A., Mashiko, R., Odashima, R., Yamamura, H., Satoh, H., Watanabe, Y., 2019. Digestion performance and contributions of organic and inorganic fouling in an anaerobic membrane bioreactor treating waste activated sludge. Bioresour. Technol. 272, 63–69. https://doi.org/10.1016/j.biortech.2018.09.147.

Hafuka, A., Mimura, K., Ding, Q., Yamamura, H., Satoh, H., Watanabe, Y., 2016. Performance of anaerobic membrane bioreactor during digestion and thickening of aerobic membrane bioreactor excess sludge. Bioresour. Technol. 218, 476–479. https://doi.org/10.1016/j.biortech.2016.06.124.

Jensen, M.B., Kofoed, M.V.W., Fischer, K., Voigt, N.V., Agneessens, L.M., Batstone, D.J., Ottosen, L.D.M., 2018. Venturi-type injection system as a potential H_2 mass transfer technology for full-scale in situ biomethanation. Appl. Energy 222, 840–846. https://doi.org/10.1016/j.apenergy.2018.04.034

Lai, C.Y., Zhou, L., Yuan, Z., Guo, J., 2021. Hydrogen-driven microbial biogas upgrading: Advances, challenges and solutions. Water Res. 197, 117120. https://doi.org/10.1016/j.watres.2021.117120.

Liu, R.B., Hao, X.D., Wei, J., 2016. Function of homoacetogenesis on the heterotrophic methane production with exogenous H_2/CO_2 involved. Chem. Eng. J. 284, 1196–1203.

https://doi.org/10.1016/j.cej.2015.09.081.

Lovato, G., Alvarado-Morales, M., Kovalovszki, A., Peprah, M., Kougias, P.G., Rodrigues, J.A.D., Angelidaki, I., 2017. In-situ biogas upgrading process: Modeling and simulations aspects. Bioresour. Technol. 245, 332–341. https://doi.org/10.1016/j.biortech.2017.08.181.

Luo, G., Johansson, S., Boe, K., Xie, L., Zhou, Q., Angelidaki, I., 2012. Simultaneous hydrogen utilization and in situ biogas upgrading in an anaerobic reactor. Biotechnol. Bioeng. 109, 1088–1094. https://doi.org/10.1002/bit.24360.

Mao, C.L., Feng, Y.Z., Wang, X.J., Ren, G.X., 2015. Review on research achievements of biogas from anaerobic digestion. Renew. Sust. Energ. Rev. 45, 540–555. https://doi.org/10.1016/j.rser.2015.02.032.

Meng, F.G., Zhang, S.Q., Oh, Y., Zhou, Z.B., Shin, H.S., Chae, S.R., 2017. Fouling in membrane bioreactors: An updated review. Water Res. 114, 151–180. https://doi.org/10.1016/j.watres.2017.02.006.

Mulat, D.G., Mosbaek, F., Ward, A.J., Polag, D., Greule, M., Keppler, F., Nielsen, J.L., Feilberg, A., 2017. Exogenous addition of H_2 for an in situ biogas upgrading through biological reduction of carbon dioxide into methane. Waste Manage. 68, 146–156.

https://doi.org/10.1016/j.wasman.2017.05.054.

Nguyen, L.N., Kumar, J., Vu, M.T., Mohammed, J.A.H., Pathak, N., Commault, A.S., Sutherland, D., Zdarta, J., Tyagi, V.K., Nghiem, L.D., 2021. Biomethane production from anaerobic co-digestion at wastewater treatment plants: A critical review on development and innovations in biogas upgrading techniques. Sci. Total Environ. 765, 142753. https://doi.org/ 10.1016/j.scitotenv.2020.142753.

Park, J.G., Kwon, H.J., Cheon, A.I., Jun, H.B., 2021. Jet-nozzle based improvement of dissolved H_2 concentration for efficient in-situ biogas upgrading in an up-flow anaerobic sludge blanket (UASB) reactor. Renew. Energ. 168, 270–279. https://doi.org/10.1016/j.renene.2020.12.019.

Rajagopal, R., Masse, D.I., Singh, G., 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour. Technol. 143, 632–641. https://doi.org/10.1016/j.biortech.2013.06.030.

Ryckebosch, E., Drouillon, M., Veruaeren, H., 2011. Techniques for transformation of biogas to biomethane. Biomass Bioenergy 35, 1633–1645. https://doi.org/10.1016/j.biombioe.2011.02.033.

Sander, R., 2015. Compilation of Henry's law constants (version 4.0) for water as solvent,

551 Atmospheric Chem. Phys. 15, 4399–4981. https://doi.org/10.5194/acp-15-4399-2015.
552 Skouteris, G., Hermosilla, D., Lopez, P., Negro, C., Blanco, A., 2012. Anaerobic membrane
553 bioreactors for wastewater treatment: A review. Chem. Eng. J. 198, 138–148.
554 https://doi.org/10.1016/j.cej.2012.05.070.
555 Sun, Q., Li, H.L., Yan, J.Y., Liu, L.C., Yu, Z.X., Yu, X.H., 2015. Selection of appropriate
556 biogas upgrading technology-a review of biogas cleaning, upgrading and utilization.
557 Renew. Sust. Energ. Rev. 51, 521–532. https://doi.org/10.1016/j.rser.2015.06.029.
558 Thema, M., Bauer, F., Sterner, M., 2019. Power-to-Gas: Electrolysis and methanation status
559 review. Renew. Sust. Energ. Rev. 112, 775–787.
560 https://doi.org/10.1016/j.rser.2019.06.030.
561 Wang, H., Zhang, Y.F., Angelidaki, I., 2016. Ammonia inhibition on hydrogen enriched
562 anaerobic digestion of manure under mesophilic and thermophilic conditions. Water
563 Res. 105, 314–319. https://doi.org/10.1016/j.watres.2016.09.006.
564 Wang, Z.W., Ma, J.X., Tang, C.Y.Y., Kimura, K., Wang, Q.Y., Han, X.M., 2014. Membrane
565 cleaning in membrane bioreactors: A review. J. Membr. Sci. 468, 276–307.
566 https://doi.org/10.1016/j.memsci.2014.05.060.
567 Xia, A., Cheng, J., Murphy, J.D., 2016. Innovation in biological production and upgrading

of methane and hydrogen for use as gaseous transport biofuel. Biotechnol. Adv. 34, 451–472. https://doi.org/10.1016/j.biotechadv.2015.12.009.

Xiao, B.Y., Qin, Y., Qu, J., Chen, H., Yu, P.F., Liu, J.X., Li, Y.Y., 2018. Comparison of single-stage and two-stage thermophilic anaerobic digestion of food waste: Performance, energy balance and reaction process. Energy Convers. Manag. 156, 215–223. https://doi.org/10.1016/j.enconman.2017.10.092.

Xiao, K.K., Chen, Y., Jiang, X., Seow, W.Y., He, C., Yin, Y., Zhou, Y., 2017. Comparison of different treatment methods for protein solubilisation from waste activated sludge. Water Res. 122, 492–502. https://doi.org/10.1016/j.watres.2017.06.024.

Xu, H., Wang, K.J., Zhang, X.Q., Gong, H., Xia, Y., Holmes, D.E., 2020. Application of in-situ H_2-assisted biogas upgrading in high-rate anaerobic wastewater treatment. Bioresour. Technol. 299, 122598. https://doi.org/10.1016/j.biortech.2019.122598.

Yu, D.W., Meng, X.S., Liu, J.B., Dian, L., Sui, Q.W., Zhang, J.Y., Zhong, H., Wei, Y.S., 2018. Formation and characteristics of a ternary pH buffer system for in-situ biogas upgrading in two-phase anaerobic membrane bioreactor treating starch wastewater. Bioresour. Technol. 269, 57–66. https://doi.org/10.1016/j.biortech.2018.08.072.

Yu, H.G., Wang, Z.W., Wu, Z.C., Zhu, C.W., 2016. Enhanced waste activated sludge

digestion using a submerged anaerobic dynamic membrane bioreactor: performance, sludge characteristics and microbial community. Sci. Rep. 6, 20111. https://doi.org/10.1038/srep20111.

Zhao, J., Li, Y., Dong, R.J., 2021. Recent progress towards in-situ biogas upgrading technologies. Sci. Total Environ. 800, 149667. https://doi.org/ 10.1016/j.scitotenv.2021.149667.

Zhu, X.P., Cao, Q., Chen, Y.C., Sun, X.Y., Liu, X.F., Li, D., 2019. Effects of mixing and sodium formate on thermophilic in-situ biogas upgrading by H_2 addition. J. Clean. Prod. 216, 373–381. https://doi.org/10.1016/j.jclepro.2019.01.245.