# Design and Commissioning of Continuously Stirred Anaerobic Bioreactor for Upcycling Carbon Dioxide (CO$_2$) to Methane (CH$_4$) via Methanogenesis

Kusuma Rintachai[1], Tanapon Phenrat[1*], Siriwan Wichai[2], Apinun Limmongkon[2], Nusara Yinyom[2]

[1] Department of Civil Engineering, Faculty of Environmental Engineering, Naresuan University,
[2] Faculty of Medical Science, Naresuan University, Phitsanulok, 65000 Thailand

[*]Corresponding author's e-mail: pomphenrat@gmail.com

## Abstract

Carbon capture and storage (CCS) technology, especially geological storage in depleted oil and gas fields, is essential to achieving the goal of carbon net zero by 2050. Some depleted oil and gas fields contain anaerobic microbes, including methanogens that can transform CO$_2$ and hydrogen (H$_2$) to methane (CH$_4$), which can be extracted and used as a fuel. Thus, subsurface microbiological transformation via methanogens may be key to achieving the large-scale utilization of CO$_2$. While this concept is exciting and has great potential to promote a circular economy with regard to CO$_2$ and simultaneously achieve carbon neutrality, extensive research is needed to understand and to maximize methanogen performance. This research preliminary evaluates biogenic gas potential in a neighboring country. Chemical is evaluated. From chemical point of view, the analysis of $\delta^{13}C_{CH4}$ values of the biogenic gas samples from in a neighboring country reveals that the methanogenic pathway is probably dominated by biogenic carbonate reduction. Here, we reveal a design for an automated bioreactor capable of simulating deep subsurface conditions to culture strictly anaerobic methanogens obtained from a gas field in a neighboring country. To simulate deep subsurface conditions, the bioreactor contains a mixture of sediment and anaerobic microbes at an inner pressure of 8 bar and a temperature of 37°C. It has a controlled CO$_2$ and H$_2$ feeding system with real-time monitoring of pH, oxidation reduction potential, conductivity, and the transformation of CO$_2$ and H$_2$ to CH$_4$. Even without any optimization, methanogens in this reactor can transform H$_2$ and CO$_2$ to CH$_4$ at a conversion rate of 0.87 to 77.46% of theoretical yield, confirming the survival of active methanogens. This novel reactor facilitates the experimental study of anaerobic methanogenesis in deep subsurface conditions, which is very technically challenging and, to the best of our knowledge, has not previously been performed in Thailand.

**Keywords:** Automated bioreactor design, Biogenic gas, Carbon capture and storage
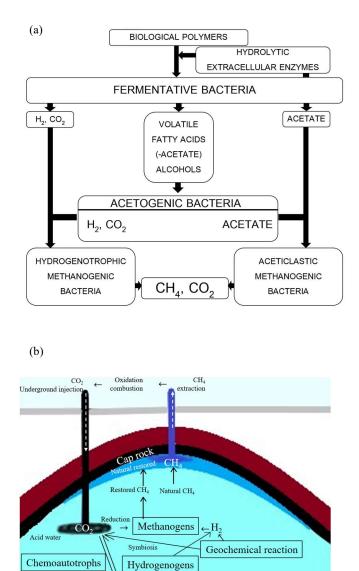
## 1. Introduction

Anaerobic digestion is an environmental engineering process that transforms the organic load in wastewater into methane (CH$_4$) with the help of various anaerobic microbes, including synthropes and methanogens (Kong et al., 2019 ). Methane can then be recovered as a fuel for on-site electricity generation or for heating (Patterson et al., 2017). In addition to wastewater treatment, methanogens also play a crucial role in subsurface biogenic gas formation, accounting for 20% of the world's natural gas reserves (Czauner, Szabó, & Mádl-Szonyi, 2023; Katz, 2011). As shown in Fig. 1(a), large organic molecules including petroleum hydrocarbons in the subsurface can be transformed into either acetate or carbon dioxide (CO$_2$) and hydrogen (H$_2$) by fermentative microbes including synthropes (Siddique et al., 2007). The acetate can then be converted to CH$_4$ by acetoclastic methanogens, while the CO$_2$ and H$_2$ can be converted to

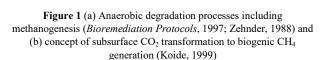CH$_4$ by hydrogenotrophic methanogenic bacteria (*Bioremediation Protocols*, 1997).

Carbon capture and storage (CCS) technology, especially geological storage in depleted oil and gas fields, is essential to achieving the goal of carbon net zero by 2050 (Holz et al., 2021). Since hydrogenotrophic methanogens may already exist in some depleted oil and gas fields, they may substantially benefit CCS projects in depleted oil and gas fields because the methanogens can transform stored CO$_2$ to CH$_4$, which can potentially be recovered and reused as a fuel; this will also result in the regeneration of geological CO$_2$ storage capacity. This transformation will take place if H$_2$ is available in the subsurface, potentially as a result of other forms of anaerobic microbial activity in the subsurface or if H$_2$ is produced by water reduction using solar energy and stored in the subsurface (Muhammed et al., 2022). This concept has also been proposed by Prof. Kodie since 1990

(Koide, 1999) as shown in Fig. 1(b). While this concept is exciting and has high potential to promote a circular economy with regard to $CO_2$ and simultaneously help achieve carbon neutrality, extensive research is needed to understand and maximize methanogen performance.

The most intensive study to understand biogeochemical factors affected biogenic gas formation was conducted by (Ni et al., 2013) for the gas field in Qaidam Basin, China (Ni et al., 2013) evaluated 143 gas samples from different biogenic gas producing locations in China, to investigate the gas origin based on the stable carbon isotopes. The analysis of $\delta^{13}C_{CH4}$ values of biogenic gases reveals that the methanogenic pathway is probably dominated by biogenic carbonate reduction ($\delta^{13}C_{CH4}$ values of biogenic gases mainly from $-55‰$ to $-75‰$).

However, since methanogens are strictly anaerobic and $CO_2$ is gaseous, the design and setup of this kind of bioreactor is challenging and such bioreactors are not currently commercially available. This research provides insight into the design, specification, and setup of a continuously stirred anaerobic bioreactor for transforming $CO_2$ to $CH_4$ via methanogenesis. This is a one-of-a-kind bioreactor capable of controlling several parameters including pressure, temperature, pH, oxidation reduction potential (ORP), dissolved oxygen and anaerobic microbial addition, as well as facilitating the continuous online monitoring of $CO_2$ depletion and $CH_4$ generation required for exploring the potential of $CO_2$ upcycling during geological CCS. In addition to the design and the setup of the bioreactor, this study demonstrates its operation without any optimization to demonstrate the baseline for $CO_2$ transformation by methanogens from a depleted oil and gas field in a neighboring country. The stable carbon isotopes of $\delta^{13}C_{CH4}$ values in the neighboring country were investigated. Out of the 13 gas samples analyzed, 6 exhibited carbon-13 isotopic values in the range of $-55.47‰$ to $-72.44‰$, indicating their biogenic origin.

(a)



(b)



**Figure 1** (a) Anaerobic degradation processes including methanogenesis (*Bioremediation Protocols*, 1997; Zehnder, 1988) and (b) concept of subsurface $CO_2$ transformation to biogenic $CH_4$ generation (Koide, 1999)

## 2. DESIGN AND SPECIFICATION OF BIOREACTOR

### 2.1 Overall Design and P&ID

The design of this bioreactor is based on the principles of continuously stirred tank reactors (CSTRs) (Ruan et al., 2019) and trickle bed reactors (TBRs) (Burkhardt et al., 2019) together with the characteristics of anaerobic subsurface environments. In the laboratory, the bioreactor was continuously stirred and operated by maintaining a continuous flow of $CO_2$ and $H_2$ into the reactor at all times. Anaerobic microbes, especially hydrogenotrophic methanogens, transformed $CO_2$ and $H_2$ to $CH_4$, and this process was monitored in real time by means of online gas chromatography. The mixing chamber of the

bioreactor was inoculated with a community of anaerobic microbes, including methanogens obtained from an active biogenic natural gas field. The system was strictly anaerobic. Temperature and pressure were monitored and controlled to mimic the subsurface environment, while aqueous chemical parameters including pH, conductivity, and ORP were also continuously monitored. The bioreactor was designed in such as way that samples could be taken from it or more chemicals or microbes added to it. Fig. 2 illustrates the piping and instrumentation diagram (P&ID) of the automated continuously stirred anaerobic bioreactor, while Fig. 3 shows the appearance of the bioreactor.

a 5:1 ratio for highly efficient CH4 generation (Kim et al., 2013), were directed into the mixing chamber. These gases were controlled by an EL-FLOW SELECT Model Series mass

flow meter (Bronkhorst) installed at the bottom of the bioreactor to ensure thorough mixing of 5 kilograms of soil with 5 kilograms of soil and 9 liters of water (the media in the bioreactor). The bioreactor had a four-blade, equipped with a magnetic drive and magnetic couplings, manufactured by Amar (Magnetic Drives for High Pressure Reactors) and connected to a SEW-EURODRIVE geared motor (with a maximum working speed of 119 rpm and a maximum torque of 54 Nm).
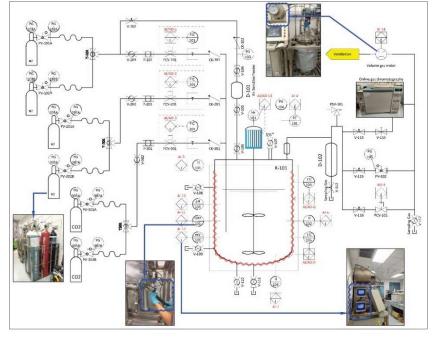


**Figure 2** P&ID of continuously stirred anaerobic bioreactor

### 2.2 Mixing Chambers

Two sizes of stainless steel (SUS304 material) mixing chambers were used in the reactor: a 10-liter vessel and a 40-liter  vessel. The equipment and fittings were designed to operate at high pressure (10 bar); Swagelok fittings (tube adapter, male connector, female connector, nut, check valve, valve, cylinder, and flexible hose) were used. The 10-L mixing chamber had a diameter of 27.30 cm and a height of 37.60 cm, with 5-L of headspace. The 40-L mixing chamber, on the other hand, had a diameter of 40.60 cm, a height of 67.76 cm, and 20-L of headspace. Both mixing chambers could be used interchangeably with the rest of the reactor components, including the frame and the gas monitoring unit. The reason for having two mixing chamber sizes was to allow evaluation of the potential for upscaling the methanogenesis process. The mixing chamber was gas-tight and pressurized to allow the creation of a strictly anaerobic environment. In the continuous bioreactor, H2 and CO2 gases, introduced at

### 2.3 Gas Sampling and Monitoring Unit

Automatic gas sampling ports at the top of the bioreactor were directly connected to a gas chromatograph (GC) for online real-time gas analysis. The gas was automatically sampled for analysis when the pressure is greater than 8 bar: when the pressure inside the bioreactor was greater than 8 bar, gas was released at the top of the bioreactor by an ASCO solenoid valve controller and the volume of gas was detected by a drum-type gas meter (Ritter, Germany). A HP6890 GC equipped with a thermal conductivity detector (TCD) was used to quantify $CH_4$, $H_2S$, $CO_2$, and $H_2$ in the released gas sample. Using capillary columns (HP-PLOT/Q) by setting conditions, the 0.5 mL gas samples were injected into the GC using the sampling valve (an automatic valve directly connected to the bioreactor). The injector temperature was 55 °C and the oven temperature was

60 °C. Argon was used as the carrier gas for the TCD, and the TCD temperature was 180 °C.

## 2.4 Data Logger and Programmable Logic Controller (PLC)

The bioreactor's parameters were monitored using a Mitsubishi touch-screen programmable logic controller (PLC) connected to a Mettler Toledo Pt4805-DXK-S8/120 ORP sensor (-2000 mV to 2000 mV (Technology, 2021)), a Mettler Toledo InPro7100i/12/120/4435 conductivity sensor (0.02 to 500 mS/cm), a Mettler Toledo InPro4260i/SG/120 pH sensor (pH 0 to 14), a WIKA temperature transmitter (-30 to 300 °C), and a WIKA pressure transmitter (-1 to 15 bar), as shown in Fig. 4. The sensor probes for ORP, conductivity, and pH measurement were calibrated using standard solutions (Thermo Fisher Scientific for ORP and conductivity, Ajax Finechem for pH). The temperature of the reactor was maintained at $37.0 \pm 1.0$ °C by a heater band operating at 3000W, 220V and 50Hz.

Moreover, the data recorded, controlled and monitored by means of the touch screen display were saved using a data logger and exported as an Excel spreadsheet.

**Figure 3** Photo of a bioreactor with a 15-L mixing chamber

**Figure 4** Monitoring and control of bioreactor

## 2.5 Liquid Feeder and Liquid and Solid Sampling

A liquid substrate feeder in the form of a cylindrical sampling bomb was used as a port through which to add substrate and anaerobic microorganisms to the bioreactor while avoiding oxygen intrusion. During substrate addition using the bomb, we flushed the bomb with nitrogen gas to replace the oxygen.

In addition, a sampling port was positioned at the bottom of the reactor to allow solids or liquids to be removed from it. This port could also be used to drain water from the reactor if necessary.
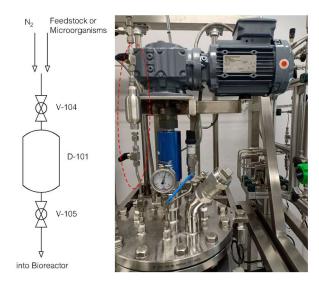
**Figure 5** Concept for liquid feeder

## 3. BIOREACTOR COMMISSIONING AND OPERATION

The bioreactor mixes $CO_2$, soil slurry, and methanogens together and monitors $CO_2$ transformation, as well as changes in geochemical parameters, in real time. To make a soil slurry, water and soil slurry were placed in the vessel before the vessel was closed using the PLC's touchscreen. After the anaerobic system was set up in the bioreactor, a vacuum was created at -0.9 bar using a vacuum pump (Vacuubrand). Next, $N_2$ was fed into the water and soil slurry to flush oxygen gas out of the system. The ORP probe showed a value of sub-zero millivolts, confirming anaerobic conditions. The mixing chamber was stirred by the motor at 87 rpm (35 Hz).

Next, a total of 190 mL of anaerobic bacteria, including hydrogenotrophic methanogens in a culture medium (tryptone soya broth, basal medium), were introduced to the bioreactor using the cylindrical sampling bomb (Fig. 5). The microorganisms were then cultured in the bioreactor for 9 days following startup. $H_2$ (99.99% purity) was fed into the bioreactor at 84 mL/min while $CO_2$ (95% purity) was fed at 17 mL/min, with a mass flow controller used to ensure a continuous flow (with real-time monitoring). When the pressure exceeded 8 bar, the system drained gas to the GC to allow quantification of gas composition and to maintain the pressure in the bioreactor. The methane yield data was thus not reported as a cumulative yield, but instead as real-time methane yield (i.e., methane yield per day). The recording parameters were saved to a memory card every 300 seconds.

## 4. PERFORMANCE EVALUATION OF BIOREACTOR

### 4.1 Control and Monitoring of Anaerobic Environment

Fig. 6 illustrates the control and monitoring of the geochemical parameters of the bioreactor, including pH, temperature, conductivity, and ORP. pH ranged from 6.16 to 7.65 (Fig.6(a)) while temperature ranged from 36.8 to 37.7 ºC (Fig.6(b)) as programmed; both these ranges are suitable for mesophilic methanogens (Schiraldi & Rosa, 2014). The conductivity ranged from 10.42 mS/cm to 14.98 mS/cm (Fig. 6(c)), representing the content of water-soluble inorganic compounds such as $SO_4^{2-}$ and $Fe^{3+}$ (Agency, 2012; Authority, 2014). Moreover, during the 21 days of the test, ORP was in the range of -439.0 mV to -537.8 mV (Fig.6(d)), which is clear evidence of a strictly anaerobic system favorable for methanogenesis (below -330 mV) (Hungate, 1969; Mauerhofer et al., 2019). In sum, the commission of the bioreactor was successful in providing suitable conditions for methanogenesis.

### 4.2 Monitoring $CO_2$ Transformation and $CH_4$ Formation

Using a 10-L mixing chamber, Fig. 7 (a) illustrates the flow of $CO_2$ and $H_2$ being fed into the bioreactor (at a $H_2$-to-$CO_2$ ratio of 5: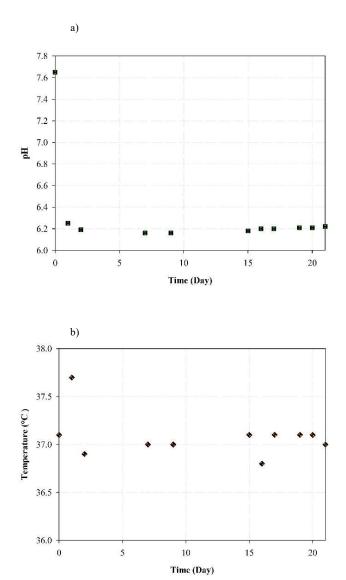1) as well as the outlet flow from the bioreactor. Notably, both inlet and outlet flows were stable over the experimental period. Fig. 7(b) shows the results of monitoring the major gas components as a function of time. In addition to $CO_2$ and $H_2$, which were the inlet gas components, a substantial portion of $CH_4$ was continuously detected at the outlet over the experimental period. This confirms the continuous formation of $CH_4$ from $CO_2$ and $H_2$ 9 days after the initial incubation of anaerobic microbes. Fig. 7(c) illustrates the kinetics of $CH_4$ formation as $CH_4$ yield (% of theoretical yield) and $CH_4$ concentration (% volume) in the bioreactor. Utilizing $CO_2$ and $H_2$ at a ratio of 1:5 as the feedstock, the theoretical $CH_4$ yield is 0.0167 mole. During 21 days of continuous operation in the bioreactor, the maximum yield of $CH_4$ formation by methanogens, without any optimization or manipulation, ranged from 0.87 to 77.46% of the theoretical yield per day. This corresponded to a volume percentage of 0.072% to 6.47%. in comparison, the control group, which did not receive $CO_2$ and $H_2$, showed $CH4$ formation at a volume percentage of 0.014% to 0.57%, as illustrated in Fig.7(d). Concentrations of $CO_2$ and $H_2$ were 0.073 to 18.05% by volume and 0 to 75.25% by volume, respectively (see Fig.7(a)) while the total gas outflow rate was 9.52 to 80.42 mL/min (see Fig.7(a)). In sum, the bioreactor generated anaerobic conditions suitable for incubating anaerobic microbes, which produced up to 77.46% of the maximum theoretical $CH_4$ yield.
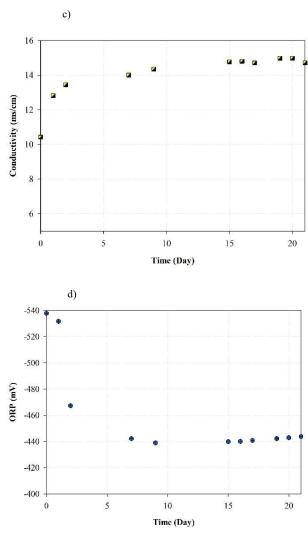
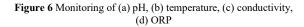## 5. CONCLUSION AND RECOMMENDATIONS

Table 1 summarizes the specifications and working conditions of the bioreactor developed in this study. The design and commissioning of the bioreactor was successful in producing anaerobic conditions and incubating anaerobic microbes, especially methanogens, for $CO_2$ upcycling. The bioreactor controlled and monitored the inlet gases, the products, and the geochemical parameters in the mixing chamber effectively. Using anaerobic microbes, including hydrogenotropic methanogens, with $CO_2$ and $H_2$ as the feedstock, the $CH_4$ yield of the 10-L bioreactor was 77.46% of the theoretical yield. This reactor serves as an effective tool with which to study the subsurface transformation of $CO_2$. This represents an emerging technology that not only facilitates the geological storage of $CO_2$ but can also turn it into fuel, generating significant value added from $CO_2$ and thus forming an important component of a circular economy and an essential incentive for $CO_2$ management.
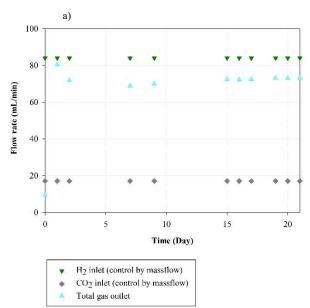
The recommendations from this research regarding the competition in hydrogen gas utilization between hydrogenotrophic methanogens and other microbial groups, such as iron-reducing bacteria and sulfate-reducing bacteria, emphasize the necessity of a balanced and excessive supply of hydrogen gas. This balance is
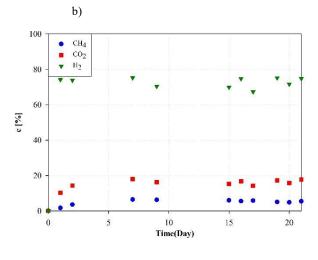
crucial to counteract carbon dioxide in hydrogenotrophic methanogenesis by microorganisms. For larger-scale applications, directly introducing high-grade hydrogen gas may not be suitable. On the contrary, exploring ways to cultivate bacteria capable of generating hydrogen gas, thriving in subsurface environments, and proliferating offers a sustainable approach for hydrogen gas production.
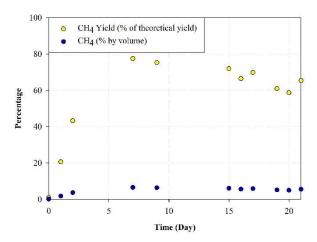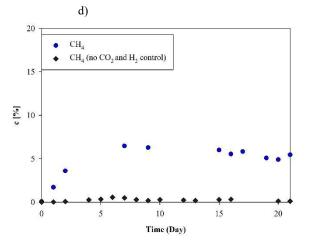
a)



b)



c)



d)



**Figure 6** Monitoring of (a) pH, (b) temperature, (c) conductivity, (d) ORP

a)



▼ $H_2$ inlet (control by massflow)
◆ $CO_2$ inlet (control by massflow)
▲ Total gas outlet

b)



c)



d)



**Figure 7** (a) monitoring of inlet and outlet flow rates, (b) gas components of the outlet, (c) kinetics of $CH_4$ yield and $CH_4$ concentration as a result of bioreactor testing, and (d) $CH_4$ concentration as a result of $CO_2$ and $H_2$ as the feedstock and no $CO_2$ and $H_2$ control bioreactor testing

**Table 1** The summary specification and working range of the bioreactor

| Item | Specification | Monitoring and controlling work |
|---|---|---|
| Pressure (headspace of the bioreactor) | 0 to 10 bar | Working at 8 bar by means of continuous system |
| Oxidation reduction potential (ORP) | -2000 to 2000 mV (Technology, 2021) | ORP was in the range of -439.0 mV to -537.8 mV, which is suitable for anaerobic conditions. |
| GC /HP-PLOT/Q Column | Detect nonpolar and polar compounds in samples, such as hydrocarbons, $CH_4$, $CO_2$, $H_2$, and $H_2S$ (Technologies, 2022; Zhou & Wang, 2003) | $CH_4$, $CO_2$, and $H_2$ levels monitored as 0.072 to 6.47% vol, 0.073 to 18.05% vol, and 0 to 75.25% vol, respectively. Monitored by gas chromatography (GC), as shown in section 2 on materials and methods (6). |
| Mass flow Controller | Flow rates of mass flows of gases are 10 to 500 ml/min for $CO_2$, 20 to 1000 ml/min for $N_2$, and 10 to 500 ml/min for $H_2$. | Working at 100 ml/min for $N_2$, 17 ml/min for $CO_2$, and 84 ml/min for $H_2$. |
| Heating | Maximum 120 °C | Working at 37 °C |
| Mechanical pressure resistance for pH, ORP, and conductivity sensor | Mechanical pressure resistance at 15 bar for ORP and pH and 10 bar for conductivity (25 °C) | Working at 8 bar |
| Volume gas meter | Measuring range is 6 to 360 l/hr | Monitored via the touch screen every 0.1 liters |
| Data logging | Parameters saved every 5 to 1200 seconds. | Recording parameters saved every 300 seconds |
| Relief valve | The safety device is set to a pressure of 12.5 bar. | When the pressure in the bioreactor exceeds 12.6 bar, the system will release the pressure and trigger an audible alert to notify the operator. |

### 7. REFERENCES

Agency, U. S. E. P. (2012). Water: Monitoring & Assessment. Retrieved 03/31/2022, 2022, from https://archive.epa.gov/water/archive/web/*html*/vms59.html

Authority, M. W. (2014). Conductivity. from https://www.mwa.co.th/ewt_news.php?nid=13321

Bedoić, R., Dorotić, H., Rolph Schneider, D., Čuček, L., Ćosić, B., Pukšec, T., & Duić, N. (2021). Synergy between feedstock gate fee and power-to-gas: An energy and economic analysis of renewable methane production in a biogas plant. *Renewable Energy, 173*, 12-23. doi: https://doi.org/10.1016/j.renene.2021.03.124

*Bioremediation Protocols*. (1997). (D. Sheehan Ed.): Humana Press.

Burkhardt, M., Jordan, I., Heinrich, S., Behrens, J., Ziesche, A., & Busch, G. (2019). Long term and demand-oriented biocatalytic synthesis of highly concentrated methane in a trickle bed reactor. *Applied Energy, 240*, 818-826. doi: 10.1016/j.apenergy.2019.02.076

Czauner, B., Szabó, Z., & Mádl-Szonyi, J. (2023). Basin-Scale Hydraulic Evaluation of Groundwater Flow Controlled Biogenic Gas Migration and Accumulation in the Central Pannonian Basin. *MDPI, 15*(18). doi: 10.3390/w15183272

Fernández-Prini, R., Aires, B., Alvarez, J. L., Aires, B., & Harvey, A. H. (2003). Henry's Constants and Vapor–Liquid Distribution Constants for Gaseous Solutes in H2O and D2O at High Temperatures. *Physical and Chemical*. doi: https://doi.org/10.1063/1.1564818

Holz, F., Scherwath, T., Granado, P. C. d., Skar, C., Olmos, L., Ploussard, Q., . . . Herbst, A. (2021). A 2050 perspective on the role for carbon capture and storage in the European power system and industry sector. *Energy Economics, 104*. doi: https://doi.org/10.1016/j.eneco.2021.105631

Hungate, R. E. (1969). Chapter IV A Roll Tube Method for Cultivation of Strict Anaerobes. *Methods in Microbiology, 3*, 117-132.doi:https://doi.org/10.1016/S0580-9517(08)70503-8

Katz, B. J. (2011). Microbial Processes and Natural Gas Accumulations. *The Open Geology Journal,, 5*, 75-83. doi: 10.2174/1874262901105010075

Kim, S., Choi, K., & Chung, J. (2013). Reduction in carbon dioxide and production of methane by biological reaction in the electronics industry. *International Journal of Hydrogen Energy, 38*(8), 3488-3496. doi: 10.1016/j.ijhydene.2012.12.007

Koide, H. (1999). GEOLOGICAL SEQUESTRATION AND MICROBIOLOGICAL RECYCLING OF CO2 IN AQUIFERS. *Paper presented at the Greenhouse Gas Control Technologies, Proc. 4th Int. Conf. on Greenhouse Gas Control Technologies.*

Kong, D., Zhang, K., Junfeng Liang, Wenxuan Gao, & author, L. D. (2019 ). Methanogenic community during the anaerobic digestion of different substrates and organic loading rates. *Microbiologyopen, 8*(5). doi: 10.1002/mbo3.709

Magnetic Drives for High Pressure Reactor. Retrieved 03/31/2022, 2022, from https://amarequip.com/magnetic-drives-for-high-pressure-reactor

Mauerhofer, L.-M., Pappenreiter, P., Paulik, C., Seifert, A. H., ernacchi, S., & Rittmann, S. K.-M. R. (2019). Methods for quantification of growth and productivity in anaerobic microbiology and biotechnology. *Folia Microbiologica*, 321–360. doi: https://doi.org/10.1007/s12223-018-0658-4

Muhammed, N. S., Haq, B., Shehri, D. A., Al-Ahmed, A., Rahman, M. M., & Zaman, E. (2022). A review on underground hydrogen storage: Insight into geological sites, influencing factors and future outlook. *Energy Reports, 8*, 461-499. doi: https://doi.org/10.1016/j.egyr.2021.12.002

Ni, Y., Dai, J., Zou, C., Liao, F., Shuai, Y., & Zhang, Y. (2013). Geochemical characteristics of biogenic gases in China. *International Journal of Coal Geology, 113*, 76-87. doi: 10.1016/j.coal.2012.07.003

Patterson, T., Savvas, S., Chong, A., Law, I., Dinsdale, R., & Esteves, S. (2017). Integration of Power to Methane in a waste water treatment plant – A feasibility study. *Bioresour Technol, 245*, 1049-1057. doi: https://doi.org/10.1016/j.biortech.2017.09.048

Rehman, A., Ma, H., Ahmad, M., Irfan, M., Traore, O., & Chandio, A. A. (2021). Towards environmental Sustainability: Devolving the influence of carbon dioxide emission to population growth, climate change, Forestry, livestock and crops production in Pakistan. *Ecological Indicators, 125*. doi: https://doi.org/10.1016/j.ecolind.2021.107460

Rice, D. D., & Claypool, G. E. (1981). Generation, Accumulation, and Resource Potential of Biogenic Gas1. *AAPG Bulletin, 65*(1), 5–25. doi: https://doi.org/10.1306/2F919765-16CE-11D7-8645000102C1865D

Ruan, D., Zhou, Z., Pang, H., Yao, J., Chen, G., & Qiu, Z. (2019). Enhancing methane production of anaerobic sludge digestion by microaeration: Enzyme activity stimulation, semi-continuous reactor validation and microbial community analysis. *Bioresour Technol, 289*, 121643. doi: 10.1016/j.biortech.2019.121643

Sander, R. (2015). Compilation of Henry's law constants (version 4.0) for water as solvent. *Atmospheric Chemistry and Physics, 15*(8). doi: https://doi.org/10.5194/acp-15-4399-2015, 2015.

Sander, S. P., Abbatt, J., Barker, J. R., Burkholder, J. B., Friedl, R. R., Golden, D. M., Wine, P. H. (2011). Chemical Kinetics and Photochemical Data for Use in Atmospheric Studies, Evaluation No. 17.

Schiraldi, C., & Rosa, M. D. (2014). *Encyclopedia of Membranes* (E. Drioli & L. Giorno Eds.): Springer, Berlin, Heidelberg.

Seifert, A. H., Rittmann, S., & Herwig, C. (2014). Analysis of process related factors to increase volumetric productivity and quality of biomethane with Methanothermobacter marburgensis. *Applied Energy, 132*, 155–162. doi:http://dx.doi.org/10.1016/j.apenergy.2014.07.002

Show, K.-Y., Lee, D.-J., & Chang, J.-S. (2011). Bioreactor and process design for biohydrogen production. *Bioresour Technol, 102*, 8524–8533. doi: 10.1016/j.biortech.2011.04.055

Singh, J., Kaushik, N., & Biswas, S. (2014). Bioreactors – Technology & Design Analysis. *THE SCITECH, 01*(06).

Susilawati, R., Papendick, S. L., Gilcrease, P. C., Esterle, J. S., Golding, S. D., & Mares, T. E. (2013). Preliminary investigation of biogenic gas production in Indonesian low rank coals and implications for a renewable energy source. *Asian Earth Sciences*. doi: 10.1016/j.jseaes.2013.08.024

Technologies, A. (2022). HP-PLOT Q Columns. Retrieved 03/31/2022, 2022, from https://www.agilent.com/en/product/gc-columns/plot-gc-columns/hp-plot-q-columns

Technology, S. S. (2021). Mettler Toledo – Redox sensor. Retrieved 03/31/2022, 2022, from https://www.sea-sun-tech.com/product/mettler-redox/Wellinger, A.,

Murphy, J., & Baxter, D. (2013). *The biogas handbook Science, production and applications*: Woodhead Publishing.

Wolfgang, D., Lauckner, J., Liu, Z., Svensson, U., & Buhmann, D. (1996). The kinetics of the reaction $CO_2 + H_2O + H^+ + HCO_3^-$, as one of the rate limiting steps for the dissolution of calcite in the system $H_2O-CO_2-CaCO_3$. *Geochimica et Cosmochimica Acta, 60*. doi: 10.1016/0016-7037(96)00181-0

Zehnder, A. J. B. (1988). *Biology of Anaerobic Microorganisms (Wiley Series in Ecological and Applied Microbiology) 99th Edition* (A. J. B. Zehnder Ed.): Wiley-Interscience; 99th edition (September 7, 1988).

Zhou, Y., & Wang, C. (2003). Analysis of Permanent Gases and Methane with the Agilent 6820 Gas Cromatograph.

Zinder, S. H. (1993). *Physiological Ecology of Methanogens*.

## 8. BIOGRAPHIES

Miss Kusuma Rintachai

Department of Civil Engineering, Faculty of Environmental Engineering, Naresuan University, Phitsanulok, Thailand

Associate Professor Dr. Tanapon Phenrat

Department of Civil Engineering, Faculty of Environmental Engineering, Naresuan University, Phitsanulok, Thailand

Dr. Siriwan Wichai

Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

Associate Professor Dr. Apinun Limmongkon

Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

Miss Nusara Yinyom

Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand