Final Report

Project Title: Biological conversion of CO_2 into CH_4 by anaerobic sludge with an addition of zero valent iron (ZVI)

By Dr. Maneerat Khemkhao

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Abstract

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Carbon dioxide (CO_2) can be used as a carbon source by a wide range of microorganisms those can convert it into gas biofuel, including methane (CH_4). In this study, biogas was upgraded by a reduction of CO_2 into CH_4 in batch experiments. Anaerobic sludge was fed with mineral medium and supplemented with CO_2, which is the only carbon source. Zero valent iron (ZVI) was added into an anaerobic digestion system for accelerating methane production. Process performances on the conversion of CO_2 into CH_4 was evaluated. Enhancement of biological conversion of CO_2 into CH_4 by anaerobic sludge with an addition of ZVI was achieved in this study. The results showed that 96 g/l ZVI with 0.57 gVSS/l anaerobic sludge at initial pH of 7 contributed to substantial CH_4 production. In addition, the study of a generation of H_2 was investigate with a pressure of CO_2 150 kPa at different ZVI dosages. The obtained results showed a simultaneous reduction of CO_2 with accumulation of H_2 in the headspace. At the end of the experiment characterized by SEM, indicated many scraggy and prismatic crystals on the anaerobic corrosive ZVI surface and smooth texture on the original ZVI. It was pointed out that the addition of zero ZVI as an alternative H_2 donor via ZVI corrosion.

Keywords: anaerobic condition, bio-methanation, CO_2 bio-conversion, zero valent iron

บทคัดย่อ

รหัสโครงการ: MRG

ชื่อโครงการ: การเติมโลหะเหล็กประจุศูนย์เพื่อช่วยเปลี่ยนคาร์บอนไดออกไซด์เป็นมีเทนของกากตะกอนหัวเชื้อ

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ระยะเวลาโครงการ: 2 ปี

คาร์บอนไดออกไซด์สามารถใช้เป็นแหล่งคาร์บอนโดยจุลินทรีย์ ที่สามารถเปลี่ยนให้เป็นก๊าซเชื้อเพลิงชีวภาพรวมทั้งมีเทนได้ ในการศึกษานี้เป็นการทดลองปรับปรุงคุณภาพของก๊าซชีวภาพด้วยการลดปริมาณคาร์บอนไดออกไซด์โดยเปลี่ยนให้มีเทน โดยตะกอนหัวเชื้อแบบไม่ใช้อากาศถูกเลี้ยงในอาหารที่มีแร่ธาตุและมีเพียงคาร์บอนไดออกไซด์เป็นแหล่งคาร์บอน การเติมโลหะเหล็กประจุศูนย์เพื่อเป็นการเร่งการผลิตมีเทนในระบบการย่อยสลายแบบไม่ใช้อากาศ จากการประเมินผลของการเปลี่ยนเปลี่ยนคาร์บอนไดออกไซด์เป็นมีเทนพบว่าสามารถเพิ่มการเปลี่ยนคาร์บอนไดออกไซด์เป็นมีเทนด้วยการเติมโลหะเหล็กประจุศูนย์ได้ ผลการทดลองแสดงให้เห็นว่า การเติมโลหะเหล็กประจุศูนย์ 96 กรัมต่อลิตร ต่อตะกอนเชื้อ 0.57 กรัมของแข็งแขวนลอยระเหยได้ต่อลิตร ที่มีค่าพีเอชเริ่มต้นเท่ากับ 7 ทำให้ได้มีการผลิตมีเทนมากที่สุด ซึ่งการศึกษาการผลิตไฮโดรเจนด้วยการใช้คาร์บอนไดออกไซด์ที่ความดัน 150 kPa ที่มีการเติมโลหะเหล็กประจุศูนย์ความเข้มข้นต่างๆ พบว่าเป็นการกระตุ้นให้เกิดการลดลงของคาร์บอนไดออกไซด์และเกิดการสะสมของไฮโดรเจนเพิ่มมากขึ้น เมื่อสิ้นสุดการทดลอง ผลจากการวิเคราะห์ด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดพบว่า โลหะเหล็กประจุศูนย์มีลักษณะเล็กลงและมีลักษณะพื้นผิวเป็นลักษณะปริซึม เนื่องจากการถูกกัดกร่อน ในขณะที่โลหะเหล็กประจุศูนย์เริ่มต้นมีผิวที่เรียบ ผลการทดลองชี้ให้เห็นว่าการเติมโลหะเหล็กประจุศูนย์เป็นทางเลือกหนึ่งที่ใช้เป็นผู้ให้ไฮโดรเจนผ่านการกัดก่อนของโลหะเหล็กประจุศูนย์ได้

คำสำคัญ: สภาวะไร้อากาศ, การผลิตไบโอมีเทน, การเปลี่ยนคาร์บอนไดออกไซด์ทางชีวภาพ, การปรับปรุงก๊าซชีวภาพที่แหล่งกำเนิด, โลหะเหล็กประจุศูนย์

Executive summary

Several studies have applied ZVI to stimulate the anaerobic digestion of waste and wastewater. However, little research has been studied on the use of ZVI and anaerobic granular sludge for the conversion of CO_2 as a sole carbon substrate to CH_4. Enhanced biological conversion of CO_2 into CH_4 by anaerobic sludge with an addition of ZVI was achieved in this study. The results observed that 96 g/l ZVI with 0.57 gVSS/l anaerobic sludge at initial pH of 7 contributed to substantial CH_4 production. Optimization of the ZVI dosage is important for the biomethanation of CO_2 because ZVI can bilaterally affect (contribute or inhibit) depending on its concentration.

Hydrogen production study was conducted with a pressure of CO_2 150 kPa at different ZVI concentration. The results evidenced a simultaneous reduction of CO_2 with accumulation of H_2 in the headspace. At the end of the experiment examined by SEM, observed many scraggy and rough hollows-like prismatic crystals on the anaerobic corrosive ZVI surface and smooth texture on the original ZVI. It was confirmed that the addition of zero ZVI as an alternative H_2 donor via ZVI corrosion. This study provides an alternative method for CO_2 bio-transformation to CH_4 for energy or for value-added and a potential CO_2 reducing for clean bioenergy recovery.

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1. Introduction to research problem and its significance

Concerning with an environmental issue and a climate change, the alternative energy sources are an increasing interest in biofuels. That is, bio-alcohols and biodiesel are considered for auto-motion while biomass and biogas are considered for electrical power generation (Huertas et al., 2011).

It is generally known that biogas is made by a process of anaerobic digestion of sludge in a wastewater treatment plant of organic wastes or residual crops. Biogas is a renewable energy source that composes with various gases such as combustible methane (CH_4) of 50-75%, non-combustible carbon dioxide (CO_2) of 25-50% and traces of nitrogen (N_2) <1% and hydrogen sulfide (H_2S) ranging from 10 to 2,000 ppm. H_2S can cause corrosion resulting in a damage of energy co-generation equipment or other installations (Osorio and Torres, 2009; Mulat et al., 2017). Biogas composition depends on a type of feed stocks and operating conditions (Harasimowicz et al, 2007).

In biogas composition, biogas mainly consists of CH_4 and CO_2.CH_4 is an odourless, colourless or inflammable gas that is lighter than air. Its calorific value is 37,781.6 kJ/Nm^3 and its energy generation capacity is 5 kWh/Nm^3 (Osorio and Torres, 2009). In order to be used as fuel for vehicles or for internal combustion engines, CH_4 concentration should be greater than 90% (Harasimowicz et al, 2007) because biogas calorific power is proportional to CH_4 content.

If a percentage of non-combustible CO_2 in biogas is high approximately 40%, caloric or heating value of biogas will be low. This leads to a reduction of an engine power output resulting in a limit of the use of biogas in electrical power plants or in combustion engines (Osorio and Torres, 2009; Mulat et al., 2017). Not only CO_2 decreases an energy content of biogas but also the condensate water accumulates in the pipe line (Osorio and Torres, 2009). Therefore, biogas should be purified and its pollutants should be eliminated in order to obtain a high percentage of CH_4 and to reach a standard natural gas or car fuel.

Biogas upgrading needs to increase the heating value and to standardize the biogas quality. There are several methods of CO_2 removal such as physical absorption, chemical absorption, biological absorption, pressure swing adsorption with activated carbon or molecular sieves, use of organic solvents, cryogenic separation and membrane purification (Apples et al., 2008; Osorio and Torres, 2009). However, CO_2 from these processes contributes to greenhouse effect.

Recently, CO_2 is considered as substrate or chemical feedstock but is not as a waste that needs to be treated (Appels et al., 2013). In order to reduce CO_2 emission into the atmosphere, there are several strategies to utilize CO_2 including a production of urea (~70 Mt CO_2/y), inorganic carbonates and pigments (~30 Mt CO_2/y), salicylic acid (~20 Mt CO_2/y), methanol (~6 Mt CO_2/y) and propylene carbonate (a few kt CO_2/y) (Jajesniak et al., 2014).

In biological process, CH_4 is a handy energy source that can produce by hydrogenotrophic methanogens using CO_2 or CO as carbon source and H_2 as energy source (Kawaguchi et al., 2010; Sato et al., 2013). In addition, several studies had focused on a methanation reaction because of its important implications for energy and environment (Frontera et al., 2017). Therefore, CO_2 methanation driven by microbial metabolism is a highly attractive option for renewable source. CO_2 methanation requires a H_2/CO_2 ratio of 4 for yielding a complete CH_4 of 100% (Miguel et al., 2015). However, a required H_2, which is supplemented to CO_2 methanation, is an expensive and H_2 storage is problematic because of safety issues and requires new infrastructure (Luo et al., 2012). Therefore, H_2 should be produced from renewable resources. For example, H_2 can be generated from water electrolysis and the energy required for electrolysis should be also renewable (Miguel et al., 2015).

Another option of H_2 source, which is cheaper compared to water electrolysis, is an addition of zero valent iron (ZVI), which can produce H_2 and release Fe^{2+} from Fe^0 (Zhang et al., 2012). In previous study, adding ZVI was found to enhance CH_4 production in an anaerobic digestion of waste sludge. That is, CH_4 production was raised up to 21% at 10 g/L of iron scrap added into the anaerobic sludge digester (Feng et al., 2014). It is generally known that methanogens are sensitive to the environment, thus CH_4 yield in anaerobic sludge digestion depends on the variation of environment factors. However, an addition of ZVI could be efficiently recovered the methanogenic activity suppressed by heat-pretreatment and alkali condition. In addition, ZVI provided a short lag time of CH_4 production, and increased the CH_4 yield by 91.5% (Zhang et al., 2015).

Although an addition of ZVI to enhance CH_4 production in anaerobic digestion of sludge were previously studied (Feng et al., 2014; Liu et al., 2015; Zhang et al., 2015), there is no report for an addition of ZVI to enhance CH_4 production from a conversion of CO_2. In addition, the predominant microorganism relating with a mechanism CO_2 reduction into CH_4 by the addition of ZVI still remain unknown. In this study, ZVI will be added into anaerobic sludge in order to promote CH_4 production from CO_2 reduction. In addition, microorganisms particularly involved in CO_2 reduction will be also monitored.

2. Literature reviews

In an anaerobic digestion step, complex organic substance, i.e. lipids, carbohydrates and proteins, are digested into CH_4, CO_2 and water in the absence of oxygen. The sequence of reactions consists of (1) hydrolysis of complex polymers, (2) fermentation of the hydrolysis end-products to short chain fatty acids by acidogens (SCFAs), (3) conversion of SCFAs to acetate by acetogens and (4) production of CH_4 by methanogens as shown in Fig. 1.

Lipid or triglyceride is hydrolyzed to long chain fatty acids (LCFAs) and glycerol. After hydrolysis, LCFAs and glycerol are oxidized via β-oxidation to acetate or propionate. Carbohydrate is converted into simple sugars via hydrolysis and subsequently fermented to VFAs. Protein is hydrolyzed to amino acids and then degraded to VFAs either via anaerobic oxidation linked to hydrogen production or through fermentation according to the Stickland reaction (Ahmad et al., 2011; Miron et al., 2000; Ramsay and Pullammanappallil, 2001). Therefore, this process needs a good association among bacteria types.

Anaerobic digestion composes of a series of complex metabolic interactions between various types of microorganisms. Microorganisms mainly relate with CH_4 production includes of hydrolytic, acidogenic or fermentatvie and acetogenic bacteria and methanogen archaea.

Biogas consists of various gases, i.e. CH_4 of 50-75%, CO_2 of 25-50% and traces of N_2less than 1% andH_2S around 10-2,000 ppm (Osorio and Torres, 2009; Mulat et al., 2017). Biogas composition depends on a type of feed stocks and operating conditions (Harasimowicz et al., 2007). CH_4 is an odourless, colourless or inflammable gas, which is lighter than air. Its calorific value is 37,781.6 kJ/Nm^3 and its energy generation capacity is 5 kWh/Nm^3 (Osorio and Torres, 2009).

CH_4 content should be greater than 90% if used as fuel (Harasimowicz et al., 2007). That is, biogas calorific power is proportional to CH_4 content. If CO_2 content is higher than 40% an engine power output will reduce. Then, the use of biogas in electrical power plants or in combustion engines will limit (Osorio and Torres, 2009; Mulat et al., 2017). Therefore, in order to reach a high heating value or high percentage of CH_4, biogas should be upgraded and its pollutants should be removed.

The utilizing CO_2 as a carbon source is currently interesting. Not only CO_2 is transformed into value-added chemicals or fuels but also CO_2 emission into the atmosphere is reduced. Usually, carbon atoms exhibit oxidation states from +4 to -4 and the most reduced form of carbon is CH_4 with an oxidation number of -4 (Chang, 2010).

It had been reported that CH_4 is occurred by two main processes, i.e. biogenic and abiogenic. The former is microbial methanogenesis while the latter includes thermal cracking of kerogen and pyrolysis or inorganic reaction of water with hot ultramafic rocks and metals (Sato et al., 2013). Methanogens are a phylogenetically diverse group of obligate anaerobic microorganisms belonging to the Euryarchaeota phylum of the Archaea domain (Kotelnikova, 2002). Normally, methanogens compete with three other major anaerobic metabolic groups for their substrates in natural habitats such as sulfate-reducing bacteria, acetogens, and ferric iron reducers (Sato et al., 2013).

When only CO_2 is available as an electron acceptor, methanogens and acetogens dominate habitats. At low H_2 contents, a pH of less than 7, and high temperatures, acetogenic bacteria channel acetic acid into H_2 and CO_2 formation. Then, methanogens convert acetate, H_2 and CO_2 into CH_4 (Thauer, et al., 2008). Energy metabolism of methanogens has been considered to be restricted to the formation of methane from CO_2/H_2, formate, methanol, methylamines, and/or acetate. To promote CO_2 reduction in Eq. (1), molecular hydrogen needs to be sustainably supplied. In addition, the proportion of CO_2 converted to methane depends upon hydrogen flux (Sato et al., 2013).

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \quad \Delta G^0 = -131 \text{ KJ/mol} \qquad (1)$$

Hydrogenotrophic methanogens utilize the H_2/CO_2 pathway for CH_4 production. That is, H_2 and CO_2 are converted into methane by some microorganisms such as *Methanosarcina formicicum* (Kimmel et al., 1991) as presented in Eq. (1). It is generally known that hydrogenotrophic methanogenesis are responsible for the conversion of 1 mole of CO_2 into 1 mole of CH_4 with the use of 4 moles of H_2 as a source of electrons (Luo et al., 2012). However, these methanogens require a long retention time in a digester, because their growth rate are very low. And, they are very sensitive to harsh conditions. Therefore, their cells can easily be washed out from the digester at high dilution rates in a continuous process (Westman et al., 2016).

Other methanogenic pathway, methanogenic archaea reduce CO_2 by using electrical current as a reducing power source. The hypothesis is that methanogenic archaea can utilize protons and electrons as shown in Eq. (2). This process may involve de novo formation of molecular hydrogen presented in Eq. (3), which is then immediately utilized by hydrogenotrophic methanogen for CO_2 reduction as shown in Eq. (1).

$$CO_2 + 8H^+ + 8^{e-} \longrightarrow CH_4 + 2H_2O \qquad (2)$$

$$2H^+ + 8^{e-} \longrightarrow H_2 \qquad (3)$$

However, electrochemical conversion of CO_2 into CH_4 requires an electrical current to generate H_2. Zero valent iron (ZVI) is a reductive material (Fig.2) and has been widely applied in wastewater treatment, groundwater purification and soil remediation (Jiang et al., 2011). ZVI can decline an oxidation–reduction potential to create a more favorable environment for anaerobic wastewater treatment when it has been added into anaerobic systems (Liu et al., 2012). In Eq. (4), H_2 may produce from the chemical corrosion of Fe^0 or from the hydrolysis-acidification (Feng et al., 2014).

$$Fe^0 + 2H^+ \longrightarrow Fe^{2+} + H_2 \qquad (4)$$

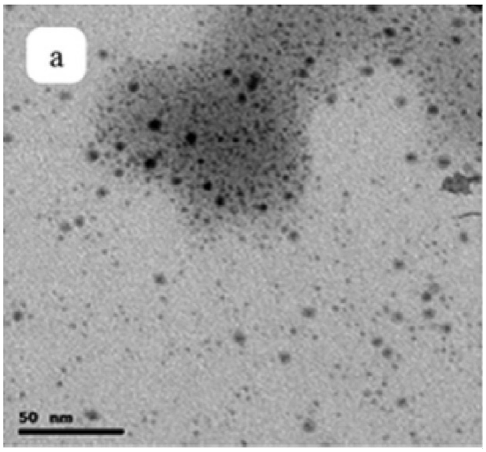

Figure 1: Transmission electron microscope (TEM) image of Fe nanoparticle (Abdelsalam et al., 2017)

It had been reported that Fe could directly serve as an electron donor for reducing CO_2 into CH_4 through autotrophic methanogenesis (Feng et al., 2014) as shown in Eq. (5).

$$CO_2 + 4Fe^0 + 8H^+ \longrightarrow CH_4 + 4Fe^{2+} + 2H_2O \quad \Delta G^0 = -150.5 \text{ kJ/mol } CH_4 \qquad (5)$$

Therefore, there were previous works that studied on the adding ZVI in an anaerobic digestion of waste sludge. For examples, due to low hydrolysis-acidification of activated sludge, Feng et al. (2014) applied ZVI to enhance anaerobic process, especially hydrolysis-acidification process. The results indicated that ZVI was effectively enhanced the decomposition of protein and cellulose, two main components of a sludge. That is, the degradation of protein increased 21.9% and the volatile fatty acids (VFAs) production increased 37.3% with adding ZVI compared to control without ZVI. In addition, more acetate and less propionate are found during the hydrolysis-acidification with ZVI. ZVI made the CH_4 production raise 43.5% and sludge reduction ratio increase 12.2 percent points. Fluorescence in situ hybridization analysis (FISH) showed that the abundances of hydrogen-consuming microorganisms including homoacetogens and hydrogenotrophic methanogens with ZVI were higher than the control, which reduced the H_2 accumulation to create a beneficial condition for the sludge digestion in thermodynamics.

Wu et al. (2015) applied ZVI for promoting organic removal and methane production of swine wastewater. The short-term exposure with different ZVI concentrations was performed to investigate the effects of ZVI on the anaerobic system for treating swine wastewater. As shown in the results, an increase ZVI dose had an effect on both COD removal and CH_4 production. Because corrosion-induced H_2 and dissolved ferrous ions was high, the methanogenesis and the biodegradation could stimulate. In addition, the abiotic corrosion reactions, i.e. flocculation, adsorption and precipitation, were removed some suspended COD. However, high ZVI doses had a potential risk on microorganism because of the large numbers of solid iron species in sludge. It could encapsulate the cells and then damaged the cellular structure. Therefore, Wu et al. (2015) recommended that the most enhancing effect induced by ZVI was observed at the ratio of ZVI and sludge ($r_{ZVI/VSS}$) of 2.63, while the maximum utilization of per ZVI adding occurred at the $r_{ZVI/VSS}$ of 0.74. However, the $r_{ZVI/VSS}$ of 5.26 was proved not suitable to microorganism and thus weaken the enhancing effect.

In addition, Liu et al. (2015) developed model to describe the enhanced CH_4 production and sulfate reduction processes with consideration of physiochemical and microbial interactions between ZVI and microorganisms in ZVI-anaerobic granular sludge reactors. These results confirmed that ZVI improved propionate degradation and methanogenesis together with alleviates the inhibition of undissociated H_2S on acetogens, methanogenic archaea and sulfate-reducing bacteria in the reactor, which are responsible for the better performances in terms of methane production and sulfate reduction. In addition, the significant promotion from ZVI was observed under relative low COD/SO_4^2 ratio condition.

In general, heat or alkali pretreatment is an effective method to improve hydrolysis of waste sludge and to enhance anaerobic sludge digestion. Nevertheless, these pretreatments may suppress the activity of methanogens in the sludge. Therefore, Zhang et al. (2015) studied on the addition of ZVI to enhance the methanogenic activity in anaerobic sludge digester under two methanogens-suppressing conditions, i.e. heat-pretreatment and alkali condition, respectively. They found that ZVI could efficiently recover the methanogenic activity in anaerobic sludge digester even if methanogens were totally inhibited in the pretreatment or removed. In addition, ZVI provided a short the lag time of methane production, and increased the methane yield by 91.5%. ZVI could accelerate the decomposition of acetate and hydrogen. This indicated that the acetoclastic methanogenesis and H_2-utilizing methanogenesis were enhanced.

The effects of ZVI on the anaerobic sludge and related microbial community shifts are still unclear. Therefore, Antwi et al. (2017) studied the effect of ZVI addition on the microbial community structure during methanation from potato starch processing wastewater. In comparing with the 16S rRNA gene sequencing results, when ZVI was added, the amount of Chloroflexi, Firmicutes and Euryarchaeota increased by 2.46, 1 and 3.53 times, respectively, whereas the amount of Bacteroidetes, Synergistetes, Proteobacteria and Caldiserica decreased. Growth trend was similar for populations of the genus *Methanosaeta*, *Levilinea*, *Longilinea* and *Syntrophomonas*. Microbial community shift was most observed in 10 and 20 g/L ZVI. The results revealed that ZVI had impacted positively on the microbial community structure to favor and enhance both hydrolysis-acidification and methanogenesis processes during a digestion.

Suanon et al. (2017) investigated the effect of nano zero valent iron (nZVI) and iron power (IP) on methane production and pharmaceutical and personal care products removal during sludge anaerobic digestion. Results showed that the use of nZVI and IP during anaerobic digestion could enhance methane yield by 25.2% at dose rate of 0.1% wet weight for nZVI and 40.8% at the dose rate of 1.6% wet weight for IP. COD removal efficiencies were 54.4% and 66.2% in the presence of nZVI and IP, respectively, which were higher compared to the control group (44.6%).

Wang et al. (2016) examined the effects of metal nanoperticles, i.e. nZVI, Ag, Fe_2O_3 and MgO, on methane production and microorganism community shift in the anaerobic digestion of sludge. The results showed that when the dosage of 10 mg nZVI/gTSS and 100 mg Fe_2O_3/gTSS, methane production increased to 120% and 117% compared with the control, respectively, whereas when the dosage of 500 mg Ag/gTSS and 500 mg MgO/g TSS generated lower levels of methane production (73.52% and 1.08% that of the control, respectively). These results revealed that low concentrations of nZVI and Fe_2O_3 promoted the amount of Bacteria and Archaea domains and activities of key enzymes but that higher concentrations of Ag and MgO inhibited them.

In addition, Abdelsalam et al. (2017) investigated the effects of the nanoparticles of Iron (Fe) and Iron Oxide (Fe_3O_4) with different concentrations on biogas and methane production. The results showed that the addition of Fe and Fe_3O_4 magnetic at 20 mg/L significantly increased the biogas volume ($p < 0.05$) by 1.45 and 1.66 times the biogas volume produced by the control, respectively. That is, the highest specific biogas and methane production of 20 mg/L Fe_3O_4 magnetic were 584 ml biogas/gVS and 351.8 ml CH_4/gVS, respectively compared with the control which yielded only 352.6 ml biogas/gVS and 179.6 ml CH_4/gVS.

Generally, bacteria have negatively charged surfaces under usual pH conditions. Electro static repulsion between bacteria is unfavorable to the aggregation of microbial communities. Therefore, the cation has been used to accelerate anaerobic granulation process. That is, multivalent positive ions, i.e. Fe^{2+}, Ca^{2+} or Al^{3+}, reduce electro static repulsion (Schmidt and Ahring, 1993). The multivalent metal ions will bind to EPS of sludge to form more stable complexes (Shen et al., 1993). However, continuous dosage with high concentration may cause potential toxicity to microorganisms (Oleszkiewicz and Sharma, 1990). The results of Zhang et al. (2011) confirmed that the circulation increased Fe^{2+} leaching and decreased the oxidation–reduction potential in the reactor. Fe^{2+} increased EPS production that benefited sludge aggregation. Fluorescence in situ hybridization test revealed that the abundance of methanogens of the sludge increased with applying the circulation.

Huang et al. (2017) studied the reaction mechanism of ZVI coupling with microbe to degrade tetracycline (TC) in permeable reactive barrier (PRB). Three permeable reactive barrier columns packed with ZVI (Fe), ZVI and microorganism (M + Fe) and microorganism (M) were employed. The results showed that the removal efficiencies of three PRB were approximately 50%, 40% and 10% respectively. ZVI degraded TC by adsorption and reduction. ZVI was converted into Fe^{2+} and Fe^{3+} while TC was degraded. With the presence of Fe^{2+} and Fe^{3+}, EPS increased obviously and microbial community structure changed distinctly. In addition, Comamonadaceae, Oxalobacteraceae and Chromatiaceae increased obviously in contrast to no ferric situation, and microbial removal efficiency of TC increased at the same time.

3. Objective

1) To gain better understandings on the effect of zero valent iron (ZVI) on the conversion of CO_2 into CH_4 by methanogens using microbial activity and extracellular polymer substance concentration data.

2) To obtain an optimal concentration of ZVI for the conversion of CO_2 to CH_4 by methanogens.

4. Research methodology

1) Anaerobic sludge source and its characteristics

Anaerobic sludge used in this study was taken from Ngaung-Khaem water quality control plant (Bangkok, Thailand). The sludge was washed and sieved to remove fine particles and excess water. The content of volatile suspended solids (VSS) was analyzed. The maximum methanogenic activity of the sludge in assays utilizing acetate and hydrogen as substrate was determined as mg CH_4-COD per g VSS per day, respectively. The anaerobic sludge was stored at 4 ^{O}C before further test.

2) Anaerobic basal mineral medium

The anaerobic medium pH 7.2 for methanogenic bioassay contained (per liter) 280 mg of NH_4Cl, 5,000 mg $NaHCO_3$, 250 mg of K_2HPO_4, 10 mg of $CaCl_2.2H_2O$, 83 mg of $MgCl_2.6H_2O$, 100 mg of yeast nitrogen base and 1 ml of trace elements (Karri et al., 2006; Gonzalez-Estrella et al., 2013). The pH of anaerobic basal medium was adjusted to 7.2. The trace element solution contained (per liter) 50 mg of H_3BO_3, 2,000 mg of $FeCl_2.4H_2O$, 50 mg of $ZnCl_2$, 50 mg of $MnCl_2.4H_2O$, 50 mg of $(NH_4)_6Mo_7O_{24}.4H_2O$, 90 mg of $AlCl_3.6H_2O$, 2,000 mg of $CoCl_2.6H_2O$, 50 mg of $NiCl_2.6H_2O$, 30 mg of $CuCl_2.2H_2O$, 100 mg of $NaSeO_3.5H_2O$, 1,000 mg of EDTA, 200 mg of resazurin as a redox indicator and 1 ml of 36%HCl (Tanner, 1989; Karri et al., 2006). The anaerobic medium was boiled for 5-10 min in a microwave to release O_2 and then flushed with N_2/CO_2 (80:20, v/v) to make it anoxic. 27 ml of the anaerobic medium was dispensed into 160 ml serum bottles under N_2/CO_2. The serum bottles was sealed with butyl rubber stopper and aluminum cap.

3) Effect of ZVI on a conversion of CO_2 into CH_4

Initially, anaerobic inoculum (1.5 g VSS per liter) was added to 160 ml serum bottles containing with 15 ml of 1.1× concentrated medium. Subsequently, all serum bottles were flushed with the gas mixture N_2/CO_2 (80:20, v/v). The sludge was tested for it capacity to convert CO_2 directly into CH_4. Hydrogen gas was used as electron donors. That is, H_2/CO_2 gas mixture was supplied by pressurizing bottles to a final headspace with 152 kPa of H_2/CO_2 (80:20, v/v). This made an overpressure after first flushing the assay bottles with the $N_2/$

CO_2 gas mixture. After that, all bottles were pre-incubated overnight at 37 ± 2 ^{O}C in an incubator shaker at 120 rpm for the adaptation of the inoculum to the medium conditions (Gonzalez-Estrella et al., 2013).

On the following day, the headspace of all bottles were flushed with N_2/CO_2 to eliminate methane accumulated and trace of gas mixture H_2/CO_2. For the negative control, 3 ml of DI water were added into the serum bottles and was flushed with a gas mixture of N_2/ CO_2. For the positive control, the gas mixture of H_2/CO_2 was also added again. In assay with ZVI on methane production from CO_2, 3 ml of ZVI stock solution was added giving a final concentration 1, 2, 4, 8 and 16 g/l and then was flushed with CO_2 gas. All assays were performed in triplicate and were incubated at 37 ±2 ^{O}C with a constant agitation speed of 120 rpm.

In order to determine methane production, 100 µl of gas samples in the head space of each assay were withdrawn at hourly intervals. Each experiment was considered terminated when the substrates were consumed and when products became stable or reached stationary stage.

4) Analytical procedures

To determine methane content, 100 µl of headspace was injected to a gas chromatograph (GC) with a thermal conductivity detector (TCD). Each liquid sample was centrifuged at 14,500 rpm for 5 min and submitted to analyses. Acids and alcohols (if any) were determined using a gas chromatograph (GC) equipped with a flame ionization detector (FID). For VFA determination, 0.4 ml of the supernatant was mixed with 0.4 ml of internal standard VFAs, and then injected to a 1 m x 2 mm, 60-80 mesh Carbopack C glass column (Supelco) coating with 0.3% Carbowa x 20 M and 0.1% H_3PO_4. For solvent analyses, 0.1 ml of the supernatant was transferred into a 20 ml headspace vial crimped with Teflon coated septum, and then injected into a 30 m x 530 µm x 2 µm DB-ACL2 capillary column (Agilent J&W GC column, Agilent Technologies). Total solids (TS) and volatile solids (VS) of inoculums before and after anaerobic batch process were analyzed as described in standard methods (APHA, 2005). All measurements were performed in triplicate.

The concentration of Fe (II, III) ions in the liquid effluent was determined by ortho phenanthroline spectrophotometry at 510 nm. Each liquid sample was filtered using 0.45 µm membrane filter and diluted to appropriate concentration levels (Huang et al., 2017). All measurements were performed in triplicate.

Scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDS) was applied to determine the chemical composition of anaerobic sludge at the end of batch experiment. The sample was directly examined with a SEM-EDS analysis. Images of the sample were taken at a voltage between 7 and 10 keV under a high vacuum mode of operation.

EPS extraction and analysis, a heat extraction method was followed Morgan et al. (1990). An extraction of the Loosely bound (LB)-EPS and Tightly bound (TB)-EPS from the anaerobic sludge was conducted according to Li and Yang (2007). The sludge was resuspended into 15 mL of 0.05% NaCl solution and then diluted its original volume of 50 mL. The NaCl solution for dilution was pre-heated to 70 ^{O}C to ensure that the sludge suspension reached an immediate warm temperature of 50 ^{O}C. After that, sludge suspension was sheared by a vortex mixer for 1 min, followed by centrifugation at 4000 xg for 10 min. The organic matter in the supernatant was readily extractable EPS, and was regarded as the LB-EPS of the biomass. For the extraction of the TB-EPS, the sludge pellet left in the centrifuge tube was resuspended in 0.05% NaCl solution to its original volume of 50 mL. The sludge suspension was heated to 60 ^{O}C in a water bath for 30 min, and the sludge mixture was then centrifuged at 4000 xg for 15 min. The supernatant that was collected was regarded as the TB-EPS extraction of the sludge. Both the LB-EPS and TB-EPS extractions were analysed for proteins and polysaccharides.

Protein was analysed by a UV/VIS spectrophotometer following the modified Lowry method using bovine serum albumin as the standards. Polysaccharide content was determined with the phenol-sulphuric acid method using glucose as the standard.

5. Results and Discussion

1) Effect of ZVI concentrations on CO_2 transformation to CH_4 by anaerobic sludge

Fig. 2 presents the percentage of carbon dioxide and methane under different ZVI dosages on CH_4 generation from CO_2 under batch conditions. The highest CH_4 content over time was found at 96 and 224 g/l of ZVI in 14 days followed by ZVI concentrations of 64 and 32 g/l in 22 days (Fig. 2b). For the control, the CO_2 in the headspace was not eliminated (Fig. 2a) and no amount of H_2 was detected during the experiment, except in the sample of H_2/CO_2 (in the first 4 days). It was due to the fact that H_2 released from ZVI was a direct electron transfer to methanogens (Uchiyama et al., 2010; Mori et al., 2010). At concentration of ZVI at 96 g/l, the CO_2 in the headspace was eliminated after 11 days while CH_4 continues to rise probably due to dissolved CO_2 and/or through the biodegradation of acetic acid (Vyrides et al., 2018).

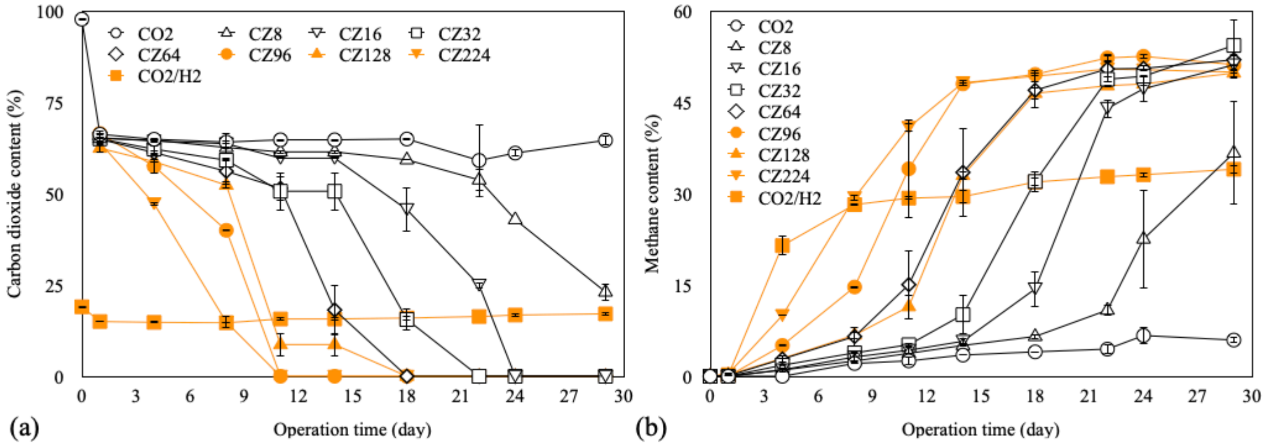

Figure 2: Concentration of (a) CO_2 and (b) CH_4 in the headspace during the anaerobic batch experiment of CO_2 conversion to CH_4 depending on ZVI concentration.

The highest CO_2 utilisation over time was detected with ZVI dosage of 224 g/l while the CH_4 production from ZVI dosage of 224 and 96 g/l was relatively the same amount (Fig. 3). The CO_2 concentration in the headspace was dropped in all the bottles, except the control, by dissolution into water. The highest CH_4 production over time was found at 96 g/l of ZVI in 22 days followed by ZVI concentrations of 224 g/l (Fig. 3b).

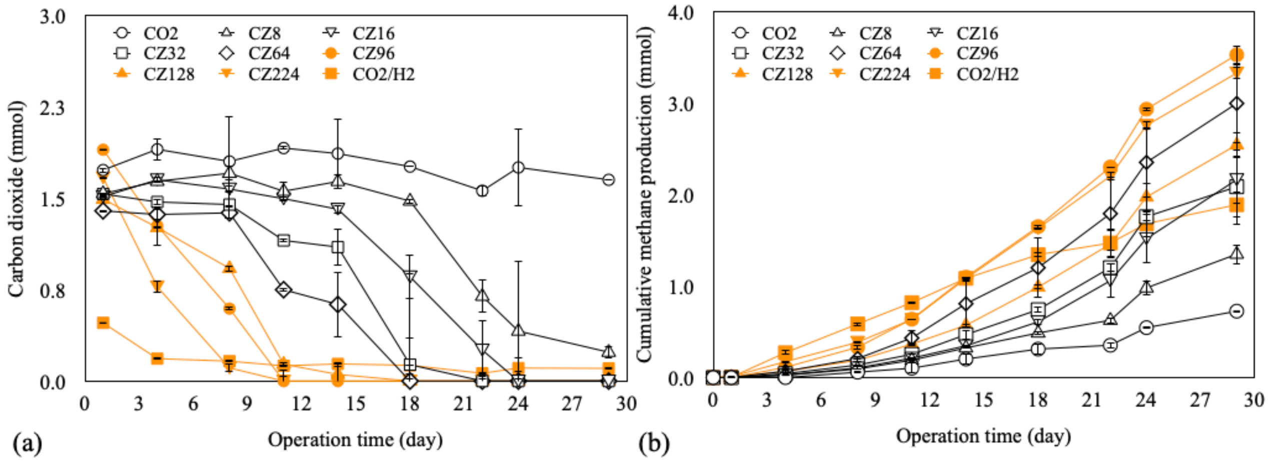

Figure 3: Temporal change of (a) carbon utilisation and (b) cumulative methane production.

2) Effect of ZVI concentrations on pH variation during a CO_2 conversion to CH_4

Fig. 4 shows a pH value on CO_2 transformation to CH_4 with a presence of ZVI by anaerobic sludge. At the end of batch experiment, the pH value increases with an increase ZVI dosage. The final pH value with ZVI addition was higher than the control conditions (no ZVI addition) in all the tests. At ZVI concentrations of 96 g/l after 14 days, the pH has risen to 9.17 and this slowed down the abiotic production of H_2. ZVI, elemental iron, can be readily oxidized in acidic condition and it produce soluble ferrous ion. Hydroxyl ion is also produced as reaction by-product, which lead to pH increase (Hwang et al., 2018).

In consumption of CO_2 and H_2, hydrogenotrophic methanogens required 4 mol of H_2 per 1 mol of CO_2 can function without any substrate limitation (Vyrides et al., 2018). At ZVI concentrations of 96 g/l, there was adequate amounts of H_2 and CO_2 to be converted by hydrogenotrophic methanogens to CH_4 and thus, even at pH 9.17 after 14 days the reaction was not hindered.

Boopathy and Daniels (1991) were explained that in the system of CO_2 with the present of ZVI, the abiotic consumption of H^+ for the generation of H_2 ($Fe^\circ + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^- \ \Delta G^\circ = -5.02$ kJ mol/Fe) and the consumption of CO_2 and H_2 by microorganisms ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ ($\Delta G^\circ = -131$ kJ/mol) and ($4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2\ H_2O$ ($\Delta G^\circ = -95$ kJ/mol) led to pH increase.

Boopathy and Daniels (1991) reported that that the optimum initial pH for pure methanogens under exposure in these conditions was in the range of 5.4–6.5. The hydrogenotrophic methanogens can adjust to moderate acidophilic pH. Therefore, the hydrogenotrophic methanogens performance under initial pH of 5 and 6 (Li et al., 2018).

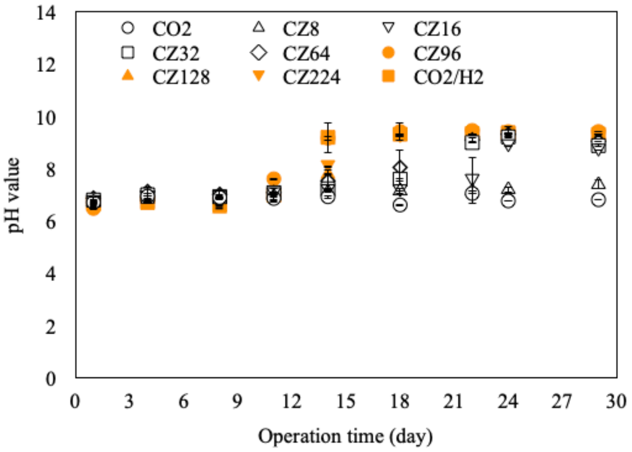

Figure 4: pH value over time under various ZVI concentrations

3) Effect of ZVI concentrations on EPS content during a CO_2 conversion to CH_4

EPS are biopolymers that extend from the cell surface to the water solution. Excessive EPS production under certain conditions is therefore likely to cause the outer portion of a thick EPS layer to be loosely bound to the cells. The LB-EPS content in this study is represented by the proportion of easily extractable EPS. Similar to TB-EPS, LB- EPS should be able to bridge neighbouring cells in floc formation.

The EPS of anaerobic sludge from a batch experiment are listed in Table 1. Protein is regarded as the substance hard to be extracted. Polysaccharide and protein were two main components of EPS. That is, protein was more than polysaccharide in all extracted EPS, which confirmed previous studies that the predominant EPS material was protein (Huang et al., 2017). The predominance of protein in the EPS may be due to the presence of a large quantity of exoenzymes (FrØlund et al., 1995). It was found that quantity of LB-EPS was much greater than the quantity of TB-EPS in both polysaccharides and proteins.

Fe^{2+} is a chelating agent of EPS generating. Low concentration of ZVI can stimulate microbial self protection mechanism, microbe secrete more EPS to prevent introverted transfer of toxic materials (He et al., 2014; Wang et al., 2015).

Shen et al. (1993) had been reported that Fe^{2+} enable to bond with EPS of sludge to form stable complexes, which is crucial to maintain an intact structure of anaerobic sludge. In addition, the interaction between Fe^{2+} and EPS could help the formation of granulation (Shen et al., 1993). It was believed that Fe^{2+} could decrease the surface charge of microorganisms by double layer compression, which could strengthen the aggregation of microorganisms (Yu et al., 2000).

Table 1: Content of EPS in anaerobic sludge

Sample	Polysaccharides (mg/g SS)		Proteins (mg/g SS)	
	Loosely bound EPS	Tightly bound EPS	Loosely bound EPS	Tightly bound EPS
original sludge	1.68	0.62	32.39	16.27
CO_2	5.86	2.28	55.69	29.83
CZ 16	7.06	2.12	62.13	32.39
CZ 32	4.74	1.41	52.53	21.78
CZ 64	6.10	1.76	57.97	28.22
CZ 96	2.93	0.79	40.31	19.90
CZ 224	5.78	2.48	49.31	23.52
CO_2/H_2	7.08	4.20	63.94	34.00

4) Effect of ZVI concentration on VFA levels during methanation from CO_2

The changes in the composition of VFAs in different assays are shown in Fig. 5. Acetate was the the predominant in the CO_2 conversion to CH_4 production depending on ZVI concentration, followed by propionate. It was found that as the ZVI concentration increased, acetate concentration increased after day 18. After that, acetate content in the experiment of 64 g/l ZVI adding was rapidly raised up and become the predominant. The changes in propionate, butyrate and valerate concentrations were showed a similar trend with acetate.

Dong et al. (2019) explained that the conversion of CO_2 to acetic acid, known as homoacetogenesis ($2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$ $\Delta G° = -95$ kJ/mol), dominantly occurs in an H_2-rich environment. Therefore, acetate build-up in a conversion of CO_2 to CH_4 might result from the competition between methanogens and homoacetogens. Normally, hydrogenotrophic methanogenesis is thermodynamically more possible than homoacetogenesis. However, under a higher H_2 partial pressure condition, homoacetogens are more competitive with better kinetics for H_2 utilization than methanogens (Molenaar et al., 2017).

In addition, Kato et al. (2015) found that acetate originating from only CO_2 accumulated in the presence of ZVI when methanogens were inhibited. This result is in a good accordance with the inhibited biomethanation of CO_2 at the highest ZVI concentration as previously mentioned.

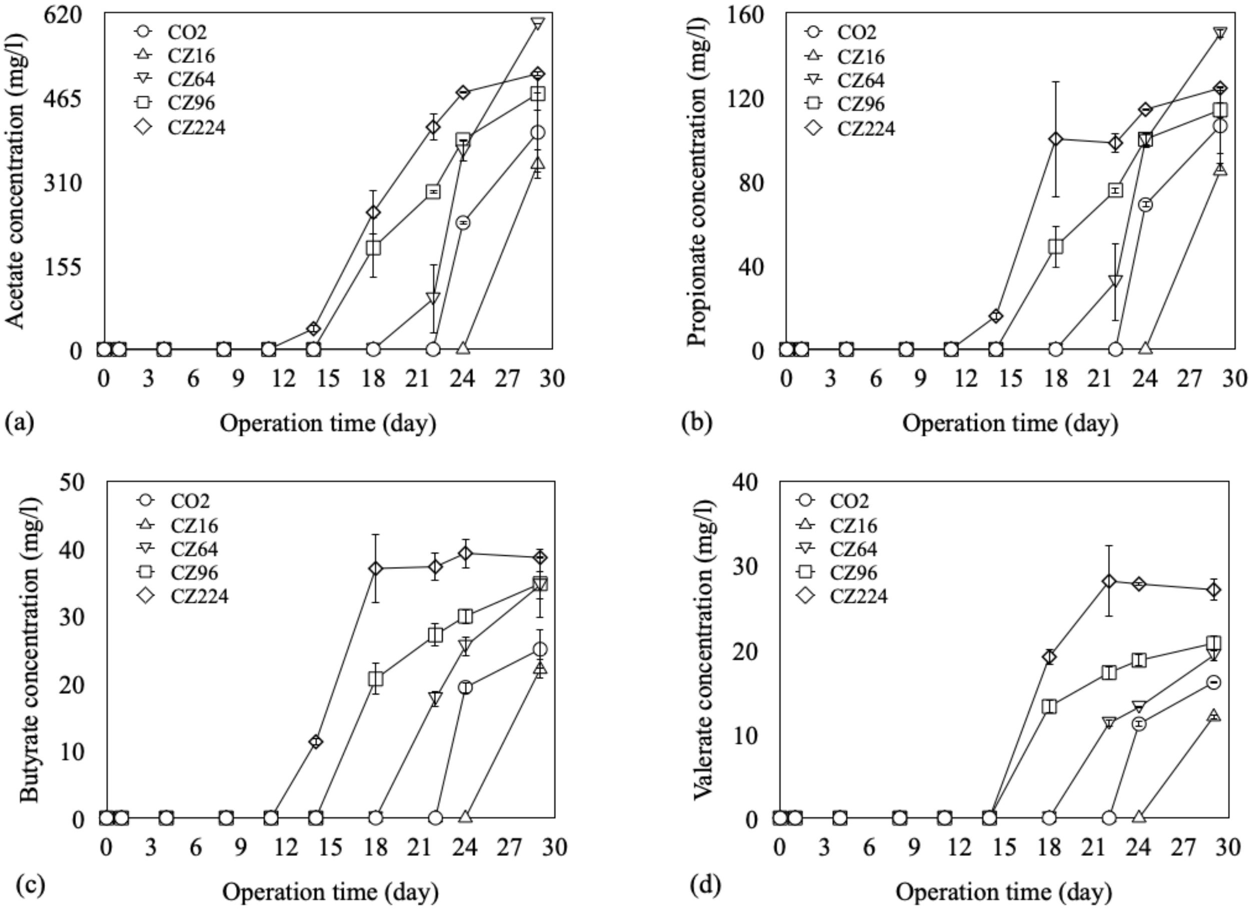

Figure 5: VFAs production by anaerobic sludge when CO_2 was used as a sole carbon source under various ZVI dosages (a) Acetate (b) Propionate (c) Butyrate and (c) Valerate concentrations.

5) H_2 production with various dosage of ZVI

Cumulative H_2 production profiles in the presence of ZVI at different concentrations without anaerobic sludge are shown in Fig. 6. H_2 production potential from ZVI corrosion was conducted for 31 days. It was found that H_2 concentration transiently increased in proportion to the ZVI concentration. It was expected that H_2 content might increase with an increase of ZVI dosage and a shaking because the reaction of ZVI was intensified. Even though the amount of hydrogen released increases with the ZVI dose, the cumulative H_2 of 96 g/l ZVI increased faster than that of 224 g/l ZVI. Therefore, it is concluded that the addition of adequate amount ZVI caused positive condition to achieve faster H_2 production as well as more production of H_2. It may be attributed to the accumulation of iron hydroxide on the particle surface as a result of OH^- formation.

The conversion of CO_2 to CH_4 via a well-known corrosion process: $2H_2O + Fe \rightarrow H_2 + Fe^{2+} + 2OH^-$ $\Delta G° = -5.02$ kJ/mol (Dong et al., 2019). The evolution of H_2 from ZVI corrosion substituted CO_2 in the headspace, probably resulting in rapid dissolution of the CO_2 into water. The dissolution rate of CO_2 seems to be highly related to the ZVI concentration. Rapid (<1 day) dissolution of CO_2 continued until the end of experiment of ZVI addition; however, CO_2 dissolution slightly slowed down at 8 g/l of

ZVI addition. CO_2 and H_2 molecules are separated into hydrate and gas phases by preferential uptake of CO_2 molecules into the hydrate from the H_2 + CO_2 gas mixture.

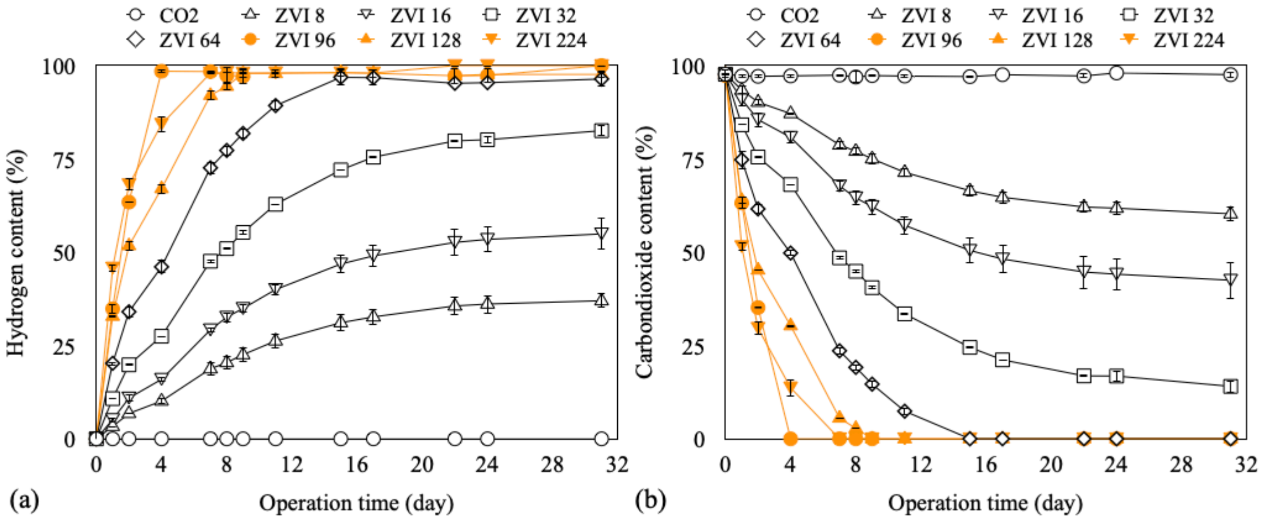

Figure 6: The content of (a) H_2 and CO_2 in the headspace under various ZVI dosages in basal medium without anaerobic sludge.

SEM image of the ZVI before and after hydrogen release after anaerobic incubation is illustrated in Fig. 7. The original ZVI powder had a clear-cut surface and smooth texture (Fig. 7a). Meanwhile, ZVI adding into aqueous solution, prismatic and needle-like crystals was observed. It was found that the smaller shape of prismatic and crystals occurred with an increase dose of ZVI addition (Fig. 7b-f). Many scraggy and rough hollows on the anaerobic corrosive ZVI surface were observed after anaerobic incubation. Uchiyama et al. (2010) and Yadav et al. (2012) explained that the prismatic and crystals observed in the SEM images might comprised of iron (hydr)oxides clusters (e.g., $FeCO_3$, Fe_3O_4, Fe_2O_3, $Fe(OH)_3$ and FeOOH). Charge and intermediate form H_2/[H] transfer through generated conductive iron compounds would occur more easily and directly. In addition, Ma et at. (2018) demonstrated that ZVI might be considered as CO_2 sink under the strictly anaerobic conditions in petroleum reservoir production waters.

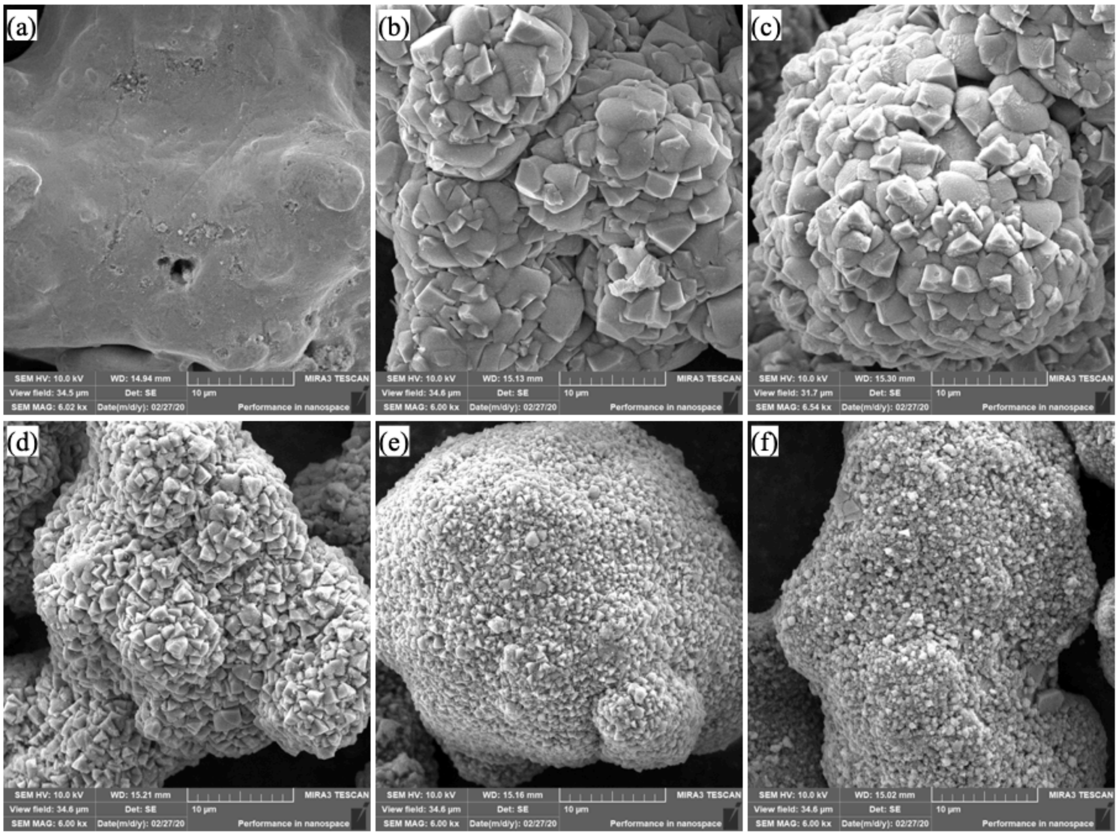

Figure 7: SEM of ZVI corrosion (a) original ZVI without anaerobic incubation and ZVI of (b) 16 g/l (c) 64 g/l (d) 96 g/l (e) 128 g/l (f) 224 g/l after anaerobic incubation.

6. Conclusion

Enhancement of biological conversion of CO_2 into CH_4 by anaerobic sludge with an addition of ZVI was achieved in this study. The biomethane production was highly affected by the ZVI concentration due to difference in H_2 production potential. The highest CH_4 content over time was found at 96 and 224 g/l of ZVI while the highest CH_4 production over time was found at 96 g/l of ZVI. At the end of batch experiment, the pH value increases with an increase ZVI dosage. The quantity of LB-EPS was much greater than the quantity of TB-EPS in both polysaccharides and proteins. Protein was more than polysaccharide in all extracted EPS. Acetate was the the predominant in the CO_2 to CH_4 production depending on ZVI concentration, followed by propionate. It may explained that an excessive addition of ZVI promoted acetate production by homoacetogenesis rather than a conversion of CO_2 into CH_4 by methanogenesis, due to the inhibition by ZVI.

Hydrogen production study was performed with a pressure of CO_2 150 kPa at different ZVI dosages. The obtained results evidenced a simultaneous reduction of CO_2 with accumulation of H_2 in the headspace. At the end of the experiment, characterized by SEM, indicated many scraggy and rough hollows-like prismatic crystals on the anaerobic corrosive ZVI surface and smooth texture on the original ZVI. This study demonstrated the potential for the biomethanation of gaseous CO_2 by using H_2 generated from the nZVI corrosion process.

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   1) International Journal Publication:

Maneerat Khemkhao, Vichai Domrongpokkaphan, Sasikarn Nuchdang, Chantaraporn Phalakornkule. Effects of zero valent iron on an acceleration CO_2 reduction to CH_4 with acclimated sludge.

   2) International conference: Extended abstract in ICCEIB 2020

V. Domrongpokkaphan, C. Phalakornkule, M. Khemkhao. Enhancement of anaerobic palm oil mill effluent digestion by zero valent iron addition as hydrogen donor. *The 5th International Conference of Chemical Engineering and Industrial Biotechnology (ICCEIB 2020)* on 9th –11th August 2020 in Kuala Lumpur, Malaysia.

Effects of zero valent iron on a acceleration CO_2 reduction to CH_4 with acclimated sludge

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Abstract

This study presents a new approach for carbon dioxide (CO_2) bio-transformation to methane (CH_4) using a combined Zero valent iron (ZVI) and an acclimated anaerobic sludge. An anaerobic sludge was acclimated with a diluted decanted palm oil mill effluent (POME) under mesophilic conditions. The enriched microbial culture under the mesophilic condition increased the methane production 2 fold-time higher than that of the original sludge. Enhancement of biological conversion of CO_2 into CH_4 by anaerobic sludge with an addition of ZVI was achieved. The results showed that 96 g/l ZVI with 0.57 gVSS/l anaerobic sludge at initial pH of 7 contributed to substantial CH_4 production. In addition, the study of a generation of H_2 was investigate with a pressure of CO_2 150 kPa at different ZVI dosages. The obtained results showed a simultaneous reduction of CO_2 with accumulation of H_2 in the headspace. At the end of the experiment characterized by SEM, indicated many scraggy and prismatic crystals on the anaerobic corrosive ZVI surface and smooth texture on the original ZVI. It was pointed out that the addition of zero ZVI as an alternative H_2 donor via ZVI corrosion.

Keywords: Anaerobic condition; Acclimation; bio-methanation; CO_2 bio-conversion; palm oil mill effluent; zero valent iron

Statement of novelty

The novel finding of this work is a CO_2 bio-transformation to CH_4 using ZVI addition combined with an acclimated anaerobic sludge.

1. Introduction

In biological process, CH_4 is a handy energy source that can produce by hydrogenotrophic methanogens using CO_2 or CO as carbon source and H_2 as energy source (Kawaguchi et al., 2010; Sato et al., 2013). In addition, several studies had focused on a methanation reaction because of its important implications for energy and environment (Frontera et al., 2017). Therefore, CO_2 methanation driven by microbial metabolism is a highly attractive option for renewable source. CO_2 methanation requires a H_2/CO_2 ratio of 4 for yielding a complete CH_4 of 100% (Miguel et al., 2015). However, a required H_2, which is supplemented to CO_2 methanation, is an expensive and H_2 storage is problematic because of safety issues and requires new infrastructure (Luo et al., 2012). Therefore, H_2 should be produced from renewable resources. For example, H_2 can be generated from water electrolysis and the energy required for electrolysis should be also renewable (Miguel et al., 2015).

Another option of H_2 source, which is cheaper compared to water electrolysis, is an addition of zero valent iron (ZVI), which can produce H_2 and release Fe^{2+} from Fe^0 (Zhang et al., 2012). In previous study, adding ZVI was found to enhance CH_4 production in an anaerobic digestion of waste sludge. That is, CH_4 production was raised up to 21% at 10 g/L of iron scrap added into the anaerobic sludge digester (Feng et al., 2014). It is generally known that methanogens are sensitive to the environment, thus CH_4 yield in anaerobic sludge digestion depends on the variation of environment factors. However, an addition of ZVI could be efficiently recovered the methanogenic activity suppressed by heat-pretreatment and alkali condition. In addition, ZVI provided a short lag time of CH_4 production, and increased the CH_4 yield by 91.5% (Zhang et al., 2015).

Although an addition of ZVI to enhance CH_4 production in anaerobic digestion of sludge were previously studied (Feng et al., 2014; Liu et al., 2015; Zhang et al., 2015), there is no report for an addition of ZVI to enhance CH_4 production from a conversion of CO_2. In addition, the predominant microorganism relating with a mechanism CO_2 reduction into CH_4 by the addition of

ZVI still remain unknown. In this study, ZVI was added into anaerobic sludge in order to promote CH_4 production from CO_2 reduction. In addition, microorganisms particularly involved in CO_2 reduction was also monitored.

2. Materials and methods

2.1 Anaerobic sludge source and preparation of acclimated sludge

Anaerobic sludge used in this study was taken from Ngaung-Khaem water quality control plant (Bangkok, Thailand). The sludge was washed and sieved to remove fine particles and excess water. The content of volatile suspended solids (VSS) was analyzed. The maximum methanogenic activity of the sludge in assays utilizing acetate and hydrogen as substrate was determined as mg CH_4-COD per g VSS per day, respectively. The anaerobic sludge was stored at 4 ^{O}C before further test. The acclimation process was carried out in a CSTR operated under mesophilic condition. The CSTR was fed with POME (2 gCOD/l.d) for 30 d. A pH adjustment was not required.

2.2 Anaerobic basal mineral medium

The anaerobic medium pH 7.2 for methanogenic bioassay contained (per liter) 280 mg of NH_4Cl, 5,000 mg $NaHCO_3$, 250 mg of K_2HPO_4, 10 mg of $CaCl_2.2H_2O$, 83 mg of $MgCl_2.6H_2O$, 100 mg of yeast nitrogen base and 1 ml of trace elements (Karri et al., 2006; Gonzalez-Estrella et al., 2013). The pH of anaerobic basal medium was adjusted to 7.2. The trace element solution contained (per liter) 50 mg of H_3BO_3, 2,000 mg of $FeCl_2.4H_2O$, 50 mg of $ZnCl_2$, 50 mg of $MnCl_2.4H_2O$, 50 mg of $(NH_4)_6Mo_7O_{24}.4H_2O$, 90 mg of $AlCl_3.6H_2O$, 2,000 mg of $CoCl_2.6H_2O$, 50 mg of $NiCl_2.6H_2O$, 30 mg of $CuCl_2.2H_2O$, 100 mg of $NaSeO_3.5H_2O$, 1,000 mg of EDTA, 200 mg of resazurin as a redox indicator and 1 ml of 36%HCl (Tanner, 1989; Karri et al., 2006). The anaerobic medium was boiled for 5-10 min in a microwave to release O_2 and then flushed with N_2/CO_2 (80:20, v/v) to make it anoxic. 27 ml of the anaerobic medium was dispensed into 160 ml serum bottles under N_2/CO_2. The serum bottles was sealed with butyl rubber stopper and aluminum cap.

2.3 Experiments on the influence of ZVI on CO_2 reduction to CH_4

Initially, anaerobic inoculum (1.5 g VSS per liter) was added to 160 ml serum bottles containing with 15 ml of 1.1× concentrated medium. Subsequently, all serum bottles were flushed with the gas mixture N_2/CO_2 (80:20, v/v). The sludge was tested for it capacity to convert CO_2 directly into CH_4. Hydrogen gas was used as electron donors. That is, H_2/CO_2 gas mixture was supplied by pressurizing bottles to a final headspace with 152 kPa of H_2/CO_2 (80:20, v/v). This made an overpressure after first flushing the assay bottles with the N_2/CO_2 gas mixture. After that, all bottles were pre-incubated overnight at 37 ± 2 ^{O}C in an incubator shaker at 120 rpm for the adaptation of the inoculum to the medium conditions (Gonzalez-Estrella et al., 2013).

On the following day, the headspace of all bottles were flushed with N_2/CO_2 to eliminate methane accumulated and trace of gas mixture H_2/CO_2. For the negative control, 3 ml of DI water were added into the serum bottles and was flushed with a gas mixture of N_2/CO_2. For the positive control, the gas mixture of H_2/CO_2 was also added again. In assay with ZVI on methane production from CO_2, 3 ml of ZVI stock solution was added giving a final concentration 1, 2, 4, 8 and 16 g/l and then was flushed with CO_2 gas. All assays were performed in triplicate and were incubated at 37 ±2 ^{O}C with a constant agitation speed of 120 rpm.

In order to determine methane production, 100 μl of gas samples in the head space of each assay were withdrawn at hourly intervals. Each experiment was considered terminated when the substrates were consumed and when products became stable or reached stationary stage.

2.4 H_2 release from ZVI dissolution

To investigate ZVI dissolution and H_2 production, a batch assay was conducted in a series of 45 mL serum bottles by adding ZVI to the final concentrations of 8, 16, 32, 64, 96, 128 and 224 g/l suspension in DI water. All treatments were conducted in a dark room in triplicate under mesophilic conditions (37 ^{O}C). After the addition of ZVI, the serum bottle was capped tightly. While the mixed liquor in the bottle was magnetically stirred at 120 rpm, H_2 produced from ZVI dissolution was monitored by CG-TCD. At the end of experiment, pH value was measured. ZVI in liquor was dried and examined with SEM-EDS.

2.5 Analytical procedures

To determine methane content, 100 µl of headspace was injected to a gas chromatograph (GC) with a thermal conductivity detector (TCD). Each liquid sample was centrifuged at 14,500 rpm for 5 min and submitted to analyses. Acids and alcohols (if any) were determined using a gas chromatograph (GC) equipped with a flame ionization detector (FID). For VFA determination, 0.4 ml of the supernatant was mixed with 0.4 ml of internal standard VFAs, and then injected to a 1 m x 2 mm, 60-80 mesh Carbopack C glass column (Supelco) coating with 0.3% Carbowa x 20 M and 0.1% H_3PO_4. For solvent analyses, 0.1 ml of the supernatant was transferred into a 20 ml headspace vial crimped with Teflon coated septum, and then injected into a 30 m x 530 µm x 2 µm DB-ACL2 capillary column (Agilent J&W GC column, Agilent Technologies). Total solids (TS) and volatile solids (VS) of inoculums before and after anaerobic batch process were analyzed as described in standard methods (APHA, 2005). All measurements were performed in triplicate.

The concentration of Fe (II, III) ions in the liquid effluent was determined by ortho phenanthroline spectrophotometry at 510 nm. Each liquid sample was filtered using 0.45 µm membrane filter and diluted to appropriate concentration levels (Huang et al., 2017). All measurements were performed in triplicate.

Scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDS) was applied to determine the chemical composition of anaerobic sludge at the end of batch experiment. The sample was directly examined with a SEM-EDS analysis. Images of the sample were taken at a voltage between 7 and 10 keV under a high vacuum mode of operation.

EPS extraction and analysis, a heat extraction method was followed Morgan et al. (1990). An extraction of the Loosely bound (LB)-EPS and Tightly bound (TB)-EPS from the anaerobic sludge was conducted according to Li and Yang (2007). The sludge was resuspended into 15 mL of 0.05% NaCl solution and then diluted its original volume of 50 mL. The NaCl solution for dilution was pre-heated to 70 ^{O}C to ensure that the sludge suspension reached an immediate warm temperature of 50 ^{O}C. After that, sludge suspension was sheared by a vortex mixer for 1 min, followed by centrifugation at 4000 xg for 10 min. The organic matter in the supernatant was readily extractable EPS, and was regarded as the LB-EPS of the biomass. For the extraction of the TB-EPS, the sludge pellet left in the centrifuge tube was resuspended in 0.05% NaCl solution to its original volume of 50 mL. The sludge suspension was heated to 60 ^{O}C in a water bath for 30 min, and the sludge mixture was then centrifuged at 4000 xg for 15 min. The supernatant that was collected was regarded as the TB-EPS extraction of the sludge. Both the LB-EPS and TB-EPS extractions were analysed for proteins and polysaccharides. Protein was analysed by a UV/VIS spectrophotometer following the modified Lowry method using bovine serum albumin as the standards. Polysaccharide content was determined with the phenol-sulphuric acid method using glucose as the standard.

3. Results and discussion

3.1 CO_2 conversion to CH_4 using ZVI

Effect of ZVI concentrations on CO2 transformation to CH4 by anaerobic sludge presents in Fig. 1. The highest CO_2 utilisation over time was detected with ZVI dosage of 224 g/l while the CH4 production from ZVI dosage of 224 and 96 g/l was relatively the same amount (Fig. 1). The

CO_2 concentration in the headspace was dropped in all the bottles, except the control, by dissolution into water. The highest CH_4 production over time was found at 96 g/l of ZVI in 22 days followed by ZVI concentrations of 224 g/l (Fig. 1b). The highest CH_4 content was found at 96 and 224 g/l of ZVI in 14 days followed by ZVI concentrations of 64 and 32 g/l in 22 days. For the control, the CO_2 in the headspace was not eliminated and no amount of H_2 was detected during the experiment, except in the sample of H_2/CO_2 (in the first 4 days).

It was due to the fact that H_2 released from ZVI was a direct electron transfer to methanogens (Uchiyama et al., 2010; Mori et al., 2010). At concentration of ZVI at 96 g/l, the CO_2 in the headspace was eliminated after 11 days while CH_4 continues to rise probably due to dissolved CO_2 and/or through the biodegradation of acetic acid (Vyrides et al., 2018). The final pH value with ZVI addition was higher than the control conditions (no ZVI addition) in all the tests. At ZVI concentrations of 96 g/l after 14 days, the pH has risen to 9.17 and this slowed down the abiotic production of H_2. ZVI, elemental iron, can be readily oxidized in acidic condition and it produce soluble ferrous ion. Hydroxyl ion is also produced as reaction by-product, which lead to pH increase (Hwang et al., 2018).

In consumption of CO_2 and H_2, hydrogenotrophic methanogens required 4 mol of H_2 per 1 mol of CO_2 can function without any substrate limitation (Vyrides et al., 2018). At ZVI concentrations of 96 g/l, there was adequate amounts of H_2 and CO_2 to be converted by hydrogenotrophic methanogens to CH_4 and thus, even at pH 9.17 after 14 days the reaction was not hindered. Boopathy and Daniels (1991) were explained that in the system of CO_2 with the present of ZVI, the abiotic consumption of H^+ for the generation of H_2 ($Fe^\circ + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^- \ \Delta G^\circ = -5.02$ kJ mol/Fe) and the consumption of CO_2 and H_2 by microorganisms ($4H_2$ +

$CO_2 \rightarrow CH_4 + 2H_2O$ ($\Delta G°= -131$ kJ/mol) and ($4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2\ H_2O$ ($\Delta G° =$ -95 kJ/mol) led to pH increase. The optimum initial pH for pure methanogens under exposure in these conditions was in the range of 5.4–6.5 (Boopathy and Daniels, 1991). The hydrogenotrophic methanogens can adjust to moderate acidophilic pH. Therefore, the hydrogenotrophic methanogens performance under initial pH of 5 and 6 (Li et al., 2018).

EPS are biopolymers that extend from the cell surface to the water solution. Excessive EPS production under certain conditions is therefore likely to cause the outer portion of a thick EPS layer to be loosely bound to the cells. The LB-EPS content in this study is represented by the proportion of easily extractable EPS. Similar to TB-EPS, LB-EPS should be able to bridge neighbouring cells in floc formation.

The EPS of anaerobic sludge from a batch experiment are listed in Table 1. Protein is regarded as the substance hard to be extracted. Polysaccharide and protein were two main components of EPS. That is, protein was more than polysaccharide in all extracted EPS, which confirmed previous studies that the predominant EPS material was protein (Huang et al., 2017). The predominance of protein in the EPS may be due to the presence of a large quantity of exoenzymes (FrØlund et al., 1995). It was found that quantity of LB-EPS was much greater than the quantity of TB-EPS in both polysaccharides and proteins.

Fe^{2+} is a chelating agent of EPS generating. Low concentration of ZVI can stimulate microbial self protection mechanism, microbe secrete more EPS to prevent introverted transfer of toxic materials (He et al., 2014; Wang et al., 2015). Shen et al. (1993) had been reported that Fe^{2+} enable to bond with EPS of sludge to form stable complexes, which is crucial to maintain an intact structure of anaerobic sludge. In addition, the interaction between Fe^{2+} and EPS could help the formation of granulation (Shen et al., 1993). It was believed that Fe^{2+} could decrease the surface charge of microorganisms by double layer compression, which could strengthen the aggregation of microorganisms (Yu et al., 2000).

The changes in the composition of VFAs in different assays are shown in Fig. 2. Acetate was the the predominant in the CO_2 conversion to CH_4 production depending on ZVI concentration, followed by propionate. It was found that as the ZVI concentration increased, acetate concentration increased after day 18. After that, acetate content in the experiment of 64 g/l ZVI adding was rapidly raised up and become the predominant. The changes in propionate, butyrate and valerate concentrations were showed a similar trend with acetate.

Dong et al. (2019) explained that the conversion of CO_2 to acetic acid, known as homoacetogenesis ($2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$ $\Delta G° = -95$ kJ/mol), dominantly occurs in an H_2-rich environment. Therefore, acetate build-up in a conversion of CO_2 to CH_4 might result from the competition between methanogens and homoacetogens. Normally, hydrogenotrophic methanogenesis is thermodynamically more possible than homoacetogenesis. However, under a higher H_2 partial pressure condition, homoacetogens are more competitive with better kinetics for H_2 utilization than methanogens (Molenaar et al., 2017).

In addition, Kato et al. (2015) found that acetate originating from only CO_2 accumulated in the presence of ZVI when methanogens were inhibited. This result is in a good accordance with the inhibited biomethanation of CO_2 at the highest ZVI concentration as previously mentioned.

3.2 CO_2 conversion to CH_4 using ZVI combined with acclimated sludge

CO_2 conversion to CH_4 was successful with 96 g/l ZVI addition. Microbial consortium in the sludge is important for a transformation of CO_2 to CH_4. Therefore, an enrichment of active microbial culture will enhance the process of CO_2 conversion to CH_4 by ZVI adding. After acclimation, the CO_2 concentration in the headspace was dropped in all the bottles, except the control, by dissolution into water. The highest CH_4 concentration over time was found at 96 g/l of ZVI in 20 days followed by ZVI concentrations of 32, 16 g/l (Fig. 3). The highest CH_4 content was found at 96 g/l of ZVI, followed by ZVI concentrations of 32 g/l. For the control, the CO_2 in the headspace was not eliminated and no amount of H_2 was detected during the experiment, except in the sample of H_2/CO_2. The highest CO_2 utilisation over time was detected with ZVI dosage of 96 g/l, followed by ZVI concentrations of 32 and 16 g/l while the control was slightly decreased. The CH_4 production from ZVI dosage of 96 g/l was the highest while other assays CH_4 production were relatively the same amount (Fig. 4).

The methanogenic activities test and concentration of ferrous and ferric are shown in Table 2. It can be seen that microbial enrichment helped enhance both acetoclastic and hydrogenotrophic methanogenic activities of the sludge compared with the original sludge. In addition, the contents of Fe^{2+} and Fe^{3+} in liquor was decreased. ZVI is converted into Fe^{2+} and Fe^{3+}. Huang et al. (2017) concluded that an ionic ion was the necessary element to microorganism growth and a chelating agent of EPS generating. With the presence of Fe^{2+} and Fe^{3+}, microbial community structure changed obviously.

3.3 H_2 production from a dissolution of ZVI

Cumulative H_2 production profiles in the presence of ZVI at different concentrations without anaerobic sludge are shown in Fig. 5. H_2 production potential from ZVI corrosion was conducted for 31 days. It was found that H_2 concentration transiently increased in proportion to the ZVI concentration. It was expected that H_2 content might increase with an increase of ZVI dosage and a shaking because the reaction of ZVI was intensified. Even though the amount of hydrogen released increases with the ZVI dose, the cumulative H_2 of 96 g/l ZVI increased faster than that of 224 g/l ZVI. Therefore, it is concluded that the addition of adequate amount ZVI caused positive condition to achieve faster H_2 production as well as more production of H_2. It may be attributed to the accumulation of iron hydroxide on the particle surface as a result of OH^{-} formation.

The conversion of CO_2 to CH_4 via a well-known corrosion process: $2H_2O + Fe \rightarrow H_2 + Fe^{2+} + 2OH^{-}$ $\Delta G° = -5.02$ kJ/mol (Dong et al., 2019). The evolution of H_2 from ZVI corrosion substituted CO_2 in the headspace, probably resulting in rapid dissolution of the CO_2 into water. The dissolution rate of CO_2 seems to be highly related to the ZVI concentration. Rapid (<1 day) dissolution of CO_2 continued until the end of experiment of ZVI addition; however, CO_2 dissolution slightly slowed down at 8 g/l of ZVI addition. CO_2 and H_2 molecules are separated into hydrate and gas phases by preferential uptake of CO_2 molecules into the hydrate from the H_2 + CO_2 gas mixture.

SEM image with EDS analysis of the ZVI before and after hydrogen release after anaerobic incubation is illustrated in Fig. 6. The original ZVI powder had a clear-cut surface and smooth texture (Fig. 6a). Meanwhile, ZVI adding into aqueous solution, prismatic and needle-like crystals was observed (Fig. 6b). It was found that the smaller shape of prismatic and crystals occurred with a ZVI dose of 96 g/l (Fig. 6c). Many scraggy and rough hollows on the anaerobic corrosive ZVI surface were observed after anaerobic incubation. Uchiyama et al. (2010 and Yadav et al. (2012) explained that the prismatic and crystals observed in the SEM images might comprised of iron (hydr)oxides clusters (e.g., $FeCO_3$, Fe_3O_4, Fe_2O_3, $Fe(OH)_3$ and FeOOH).

EDS analysis indicated that there were significant iron peaks in ZVI 96 g/l and original background control. For the original ZVI, only iron peaks was observed (Fig 6a). Meanwhile, the peak elements of C, O and Na were found in a dissolution of ZVI 96 g/l. That is, the O peak was relatively large area in element composition profiles, followed by the C peak (Fig. 6d). Charge and intermediate form H_2/[H] transfer through generated conductive iron compounds would occur more easily and directly. In addition, Ma et at. (2018) demonstrated that ZVI might be considered as CO_2 sink under the strictly anaerobic conditions in petroleum reservoir production waters.

Conclusions

A new approach for CO_2 bio-transformation to CH_4 using a combined ZVI and an acclimated anaerobic sludge was achieved in this study. An anaerobic sludge was acclimated with a diluted decanted POME under mesophilic conditions. The enriched microbial culture under the mesophilic condition increased the methane production 2 fold-time higher than that of the original sludge. Biological conversion of CO_2 into CH_4 by anaerobic sludge was accelerated with ZVI. The results showed that 96 g/l ZVI with 0.57 gVSS/l anaerobic sludge at initial pH of 7 contributed to substantial CH_4 production. In addition, the study of a generation of H_2 was investigate with a pressure of CO_2 150 kPa at different ZVI dosages. The obtained results showed a simultaneous reduction of CO_2 with accumulation of H_2 in the headspace. At the end of the experiment characterized by SEM, indicated many scraggy and prismatic crystals on the anaerobic corrosive ZVI surface and smooth texture on the original ZVI. It was pointed out that the addition of zero ZVI as an alternative H_2 donor via ZVI corrosion.

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Figure Legend

Fig 1 Change of (a) carbon utilisation and (b) cumulative methane production.

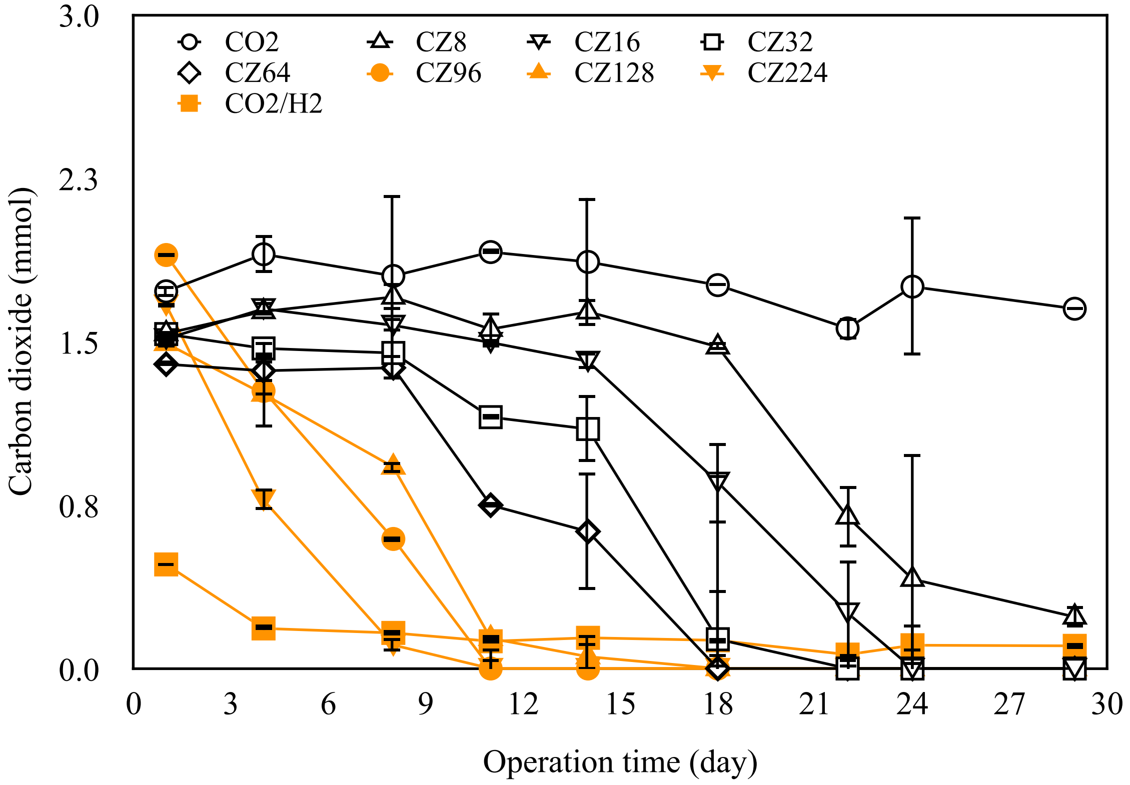
Fig 2 VFAs production by anaerobic sludge when CO_2 was used as a sole carbon source under various ZVI dosages (a) Acetate (b) Propionate (c) Butyrate and (c) Valerate concentrations.

Fig 3 Concentration of (a) CO_2 and (b) CH_4 in the headspace during a CO_2 conversion to CH_4 various ZVI concentrations of acclimated anaerobic sludge.

Fig 4 Change of (a) carbon utilisation and (b) cumulative methane production of acclimated anaerobic sludge.

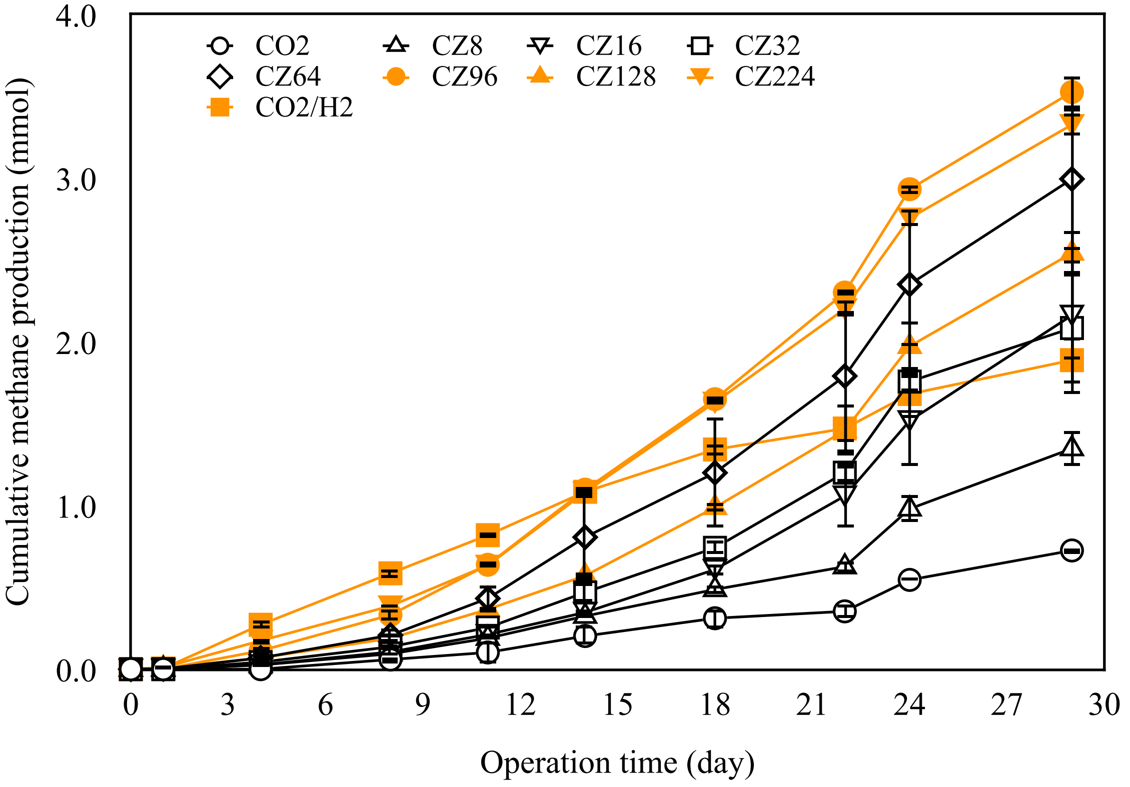
Fig 5 The content of (a) H_2 and CO_2 in the headspace under various ZVI dosages in basal medium without anaerobic sludge.

Fig 6 Scanning electron microscopic micrographs and EDS analysis of (a-b) original ZVI and (c-d) ZVI of 96 g/l after anaerobic incubation.



3.0
2.3
1.5
0.8
0.0
Carbon dioxide (mmol)
0
3
6
9
12
15
18
21
24
27
30
Operation time (day)
CO2
CZ8
CZ16
CZ32
CZ64
CZ96
CZ128
CZ224
CO2/H2

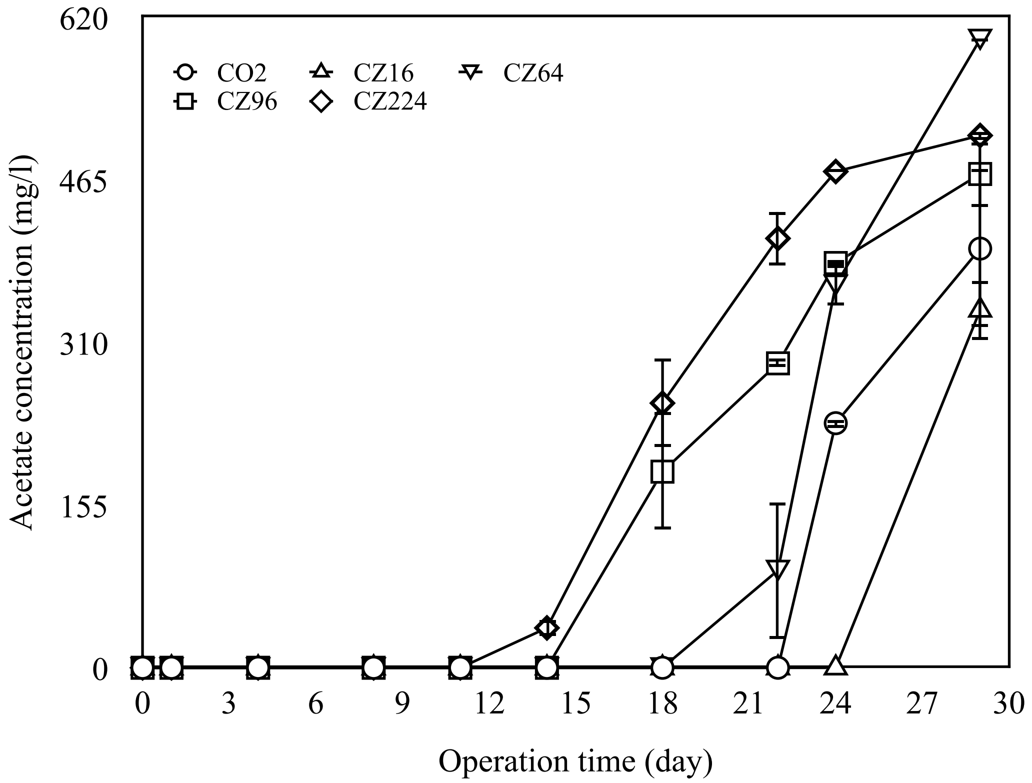
a)



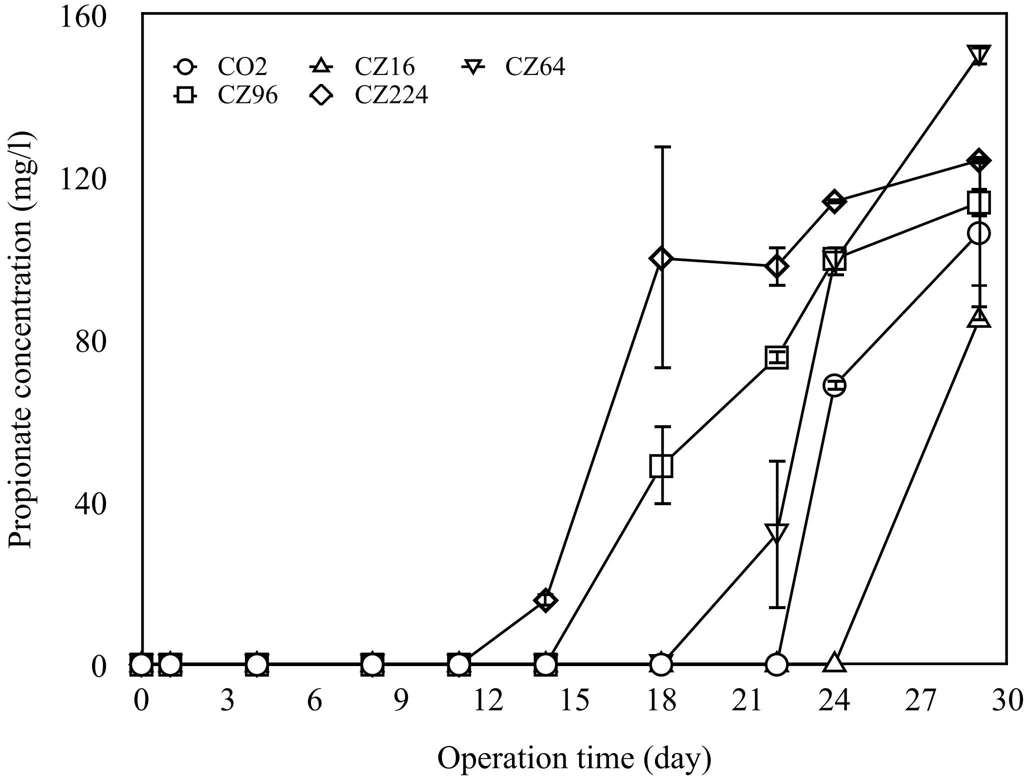
4.0
3.0
2.0
1.0
0.0
Cumulative methane production (mmol)
0
3
6
9
12
15
18
21
24
27
30
Operation time (day)
CO2
CZ8
CZ16
CZ32
CZ64
CZ96
CZ128
CZ224
CO2/H2

b)

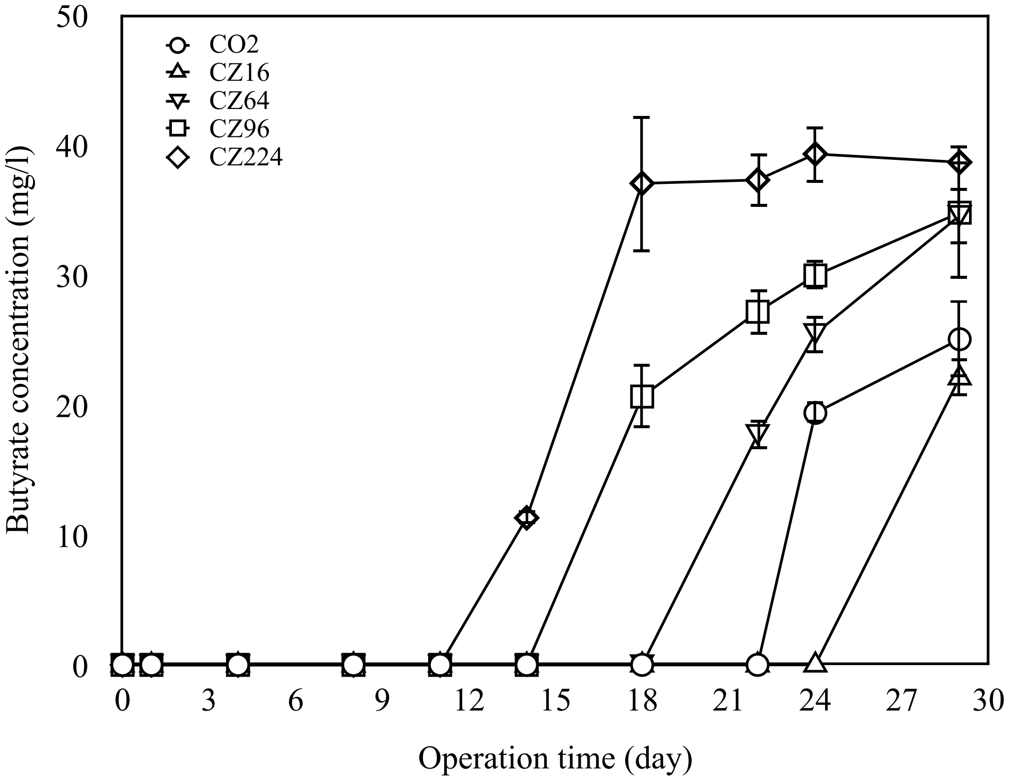
Fig 1


620
465
310
155
0
Acetate concentration (mg/l)
0
3
6
9
12
15
18
21
24
27
30
Operation time (day)
CO2
CZ16
CZ64
CZ96
CZ224

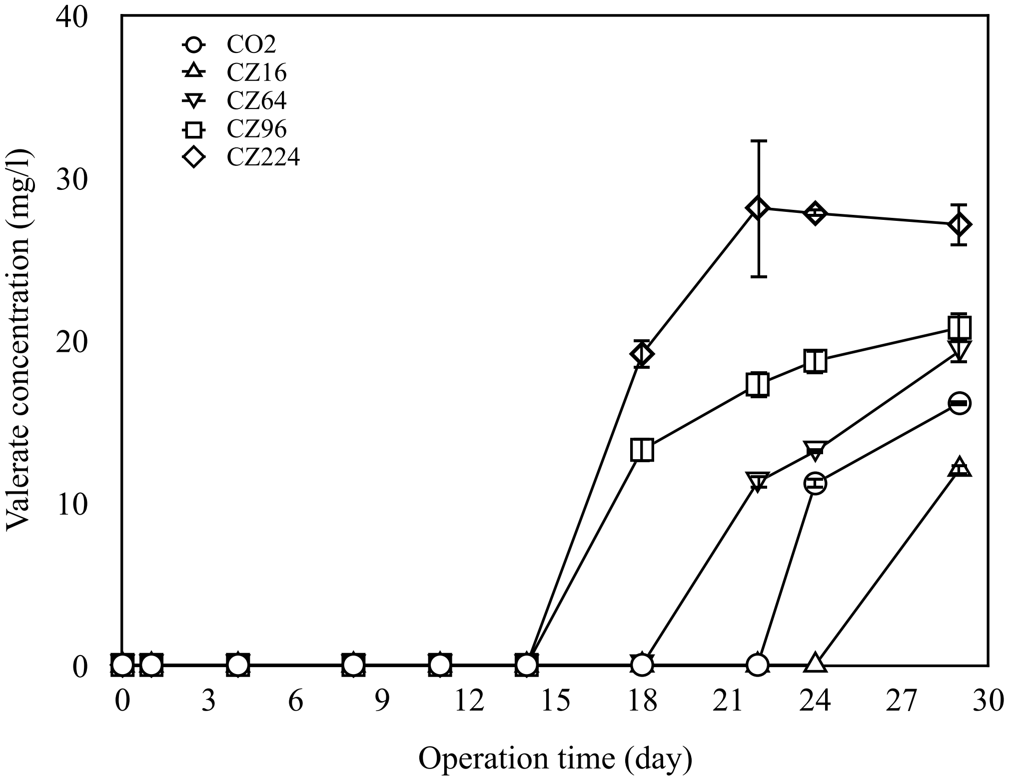
a)


160
120
80
40
0
Propionate concentration (mg/l)
0
3
6
9
12
15
18
21
24
27
30
Operation time (day)
CO2
CZ16
CZ64
CZ96
CZ224

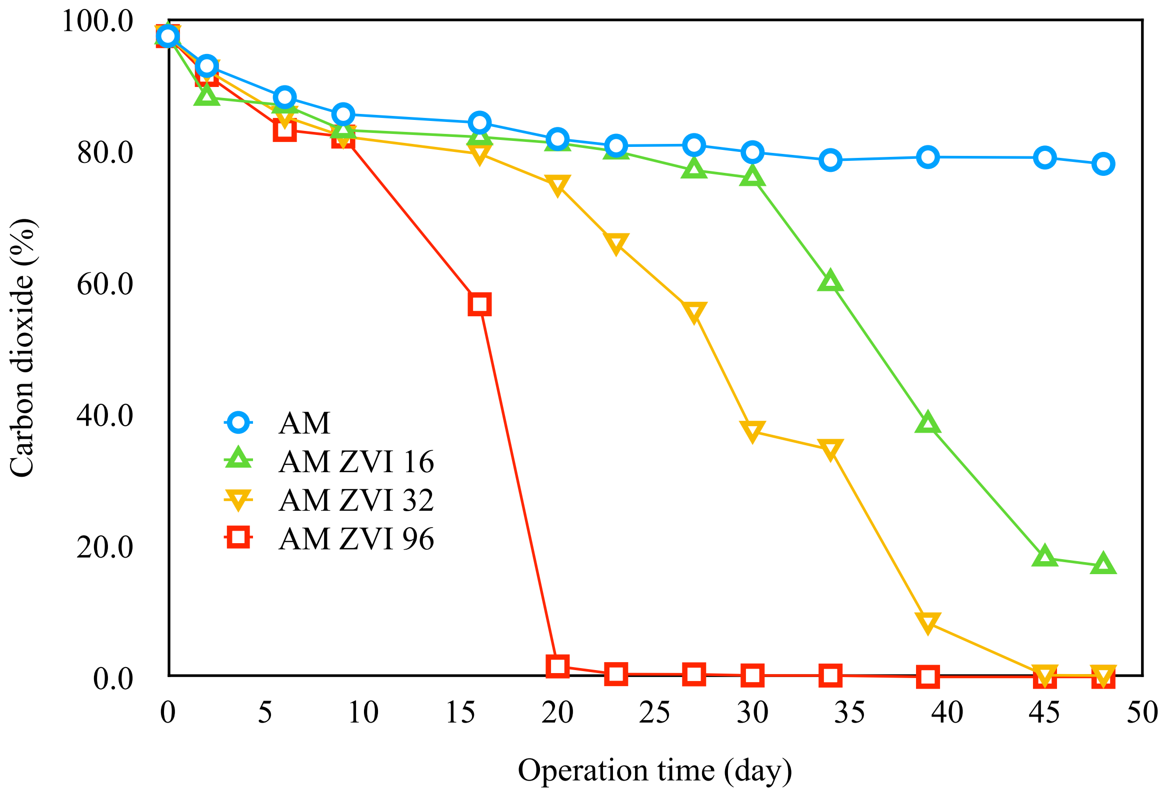
b)


CO2
CZ16
CZ64
CZ96
CZ224
Butyrate concentration (mg/l)
0
10
20
30
40
50
0
3
6
9
12
15
18
21
24
27
30
Operation time (day)

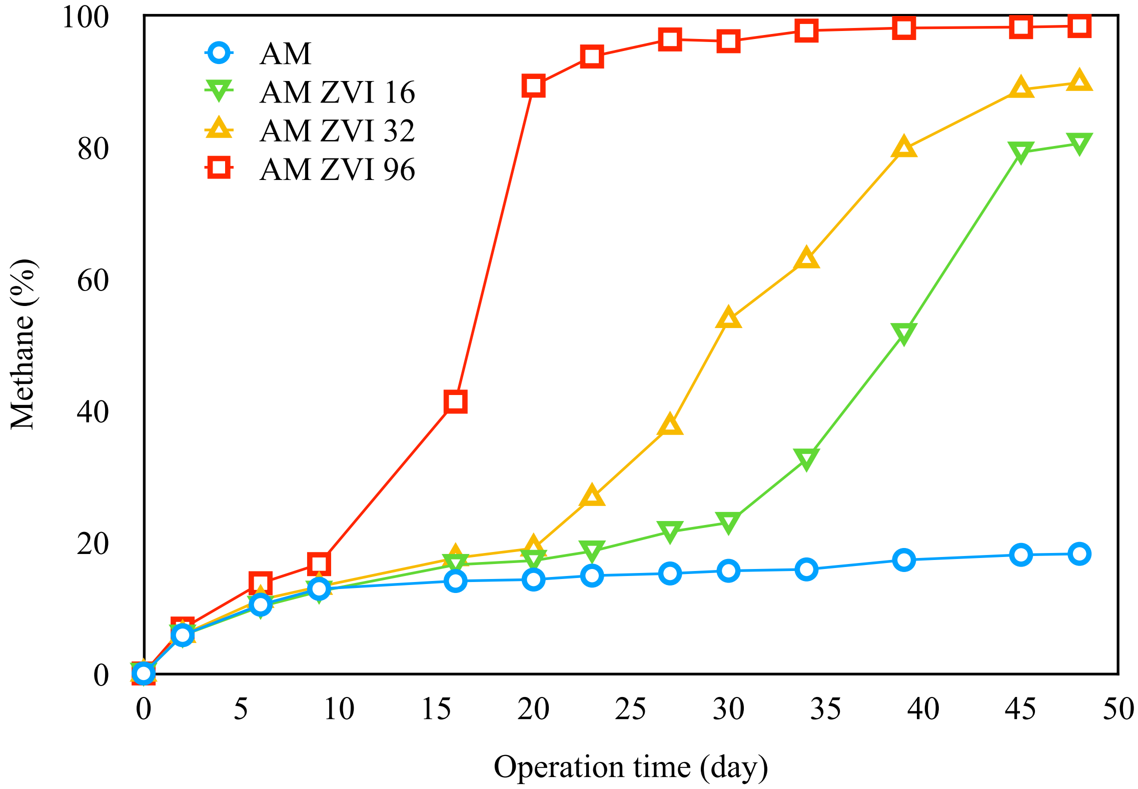
c)


CO2
CZ16
CZ64
CZ96
CZ224
Valerate concentration (mg/l)
0
10
20
30
40
0
3
6
9
12
15
18
21
24
27
30
Operation time (day)

d)
Fig 2

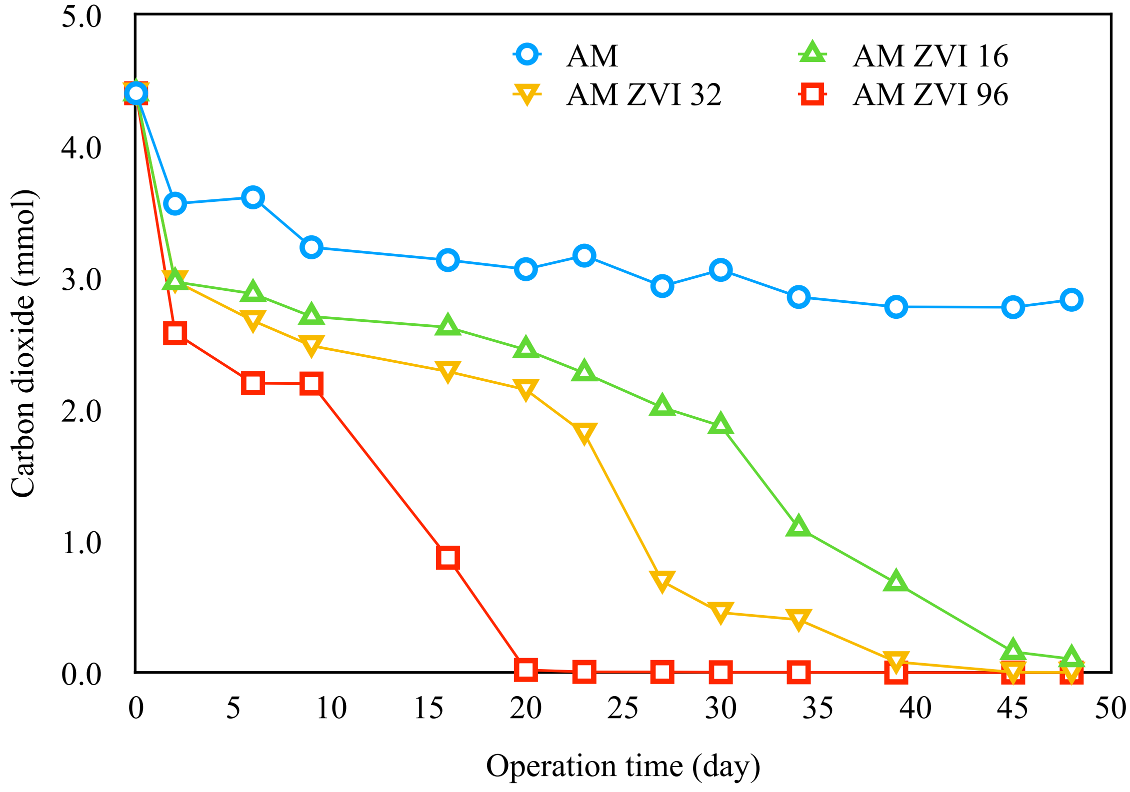

100.0
80.0
60.0
40.0
20.0
0.0
Carbon dioxide (%)
0
5
10
15
20
25
30
35
40
45
50
Operation time (day)
AM
AM ZVI 16
AM ZVI 32
AM ZVI 96

a)

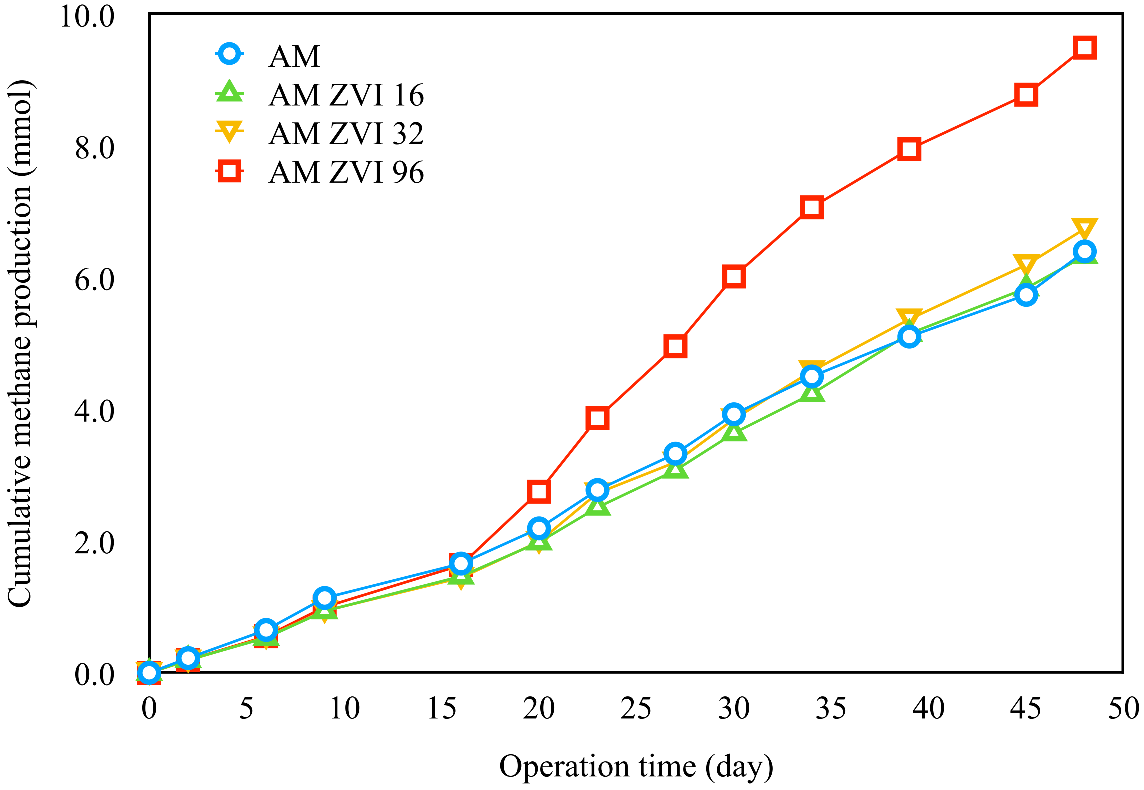

100
80
60
40
20
0
Methane (%)
0
5
10
15
20
25
30
35
40
45
50
Operation time (day)
AM
AM ZVI 16
AM ZVI 32
AM ZVI 96

b)

Fig 3

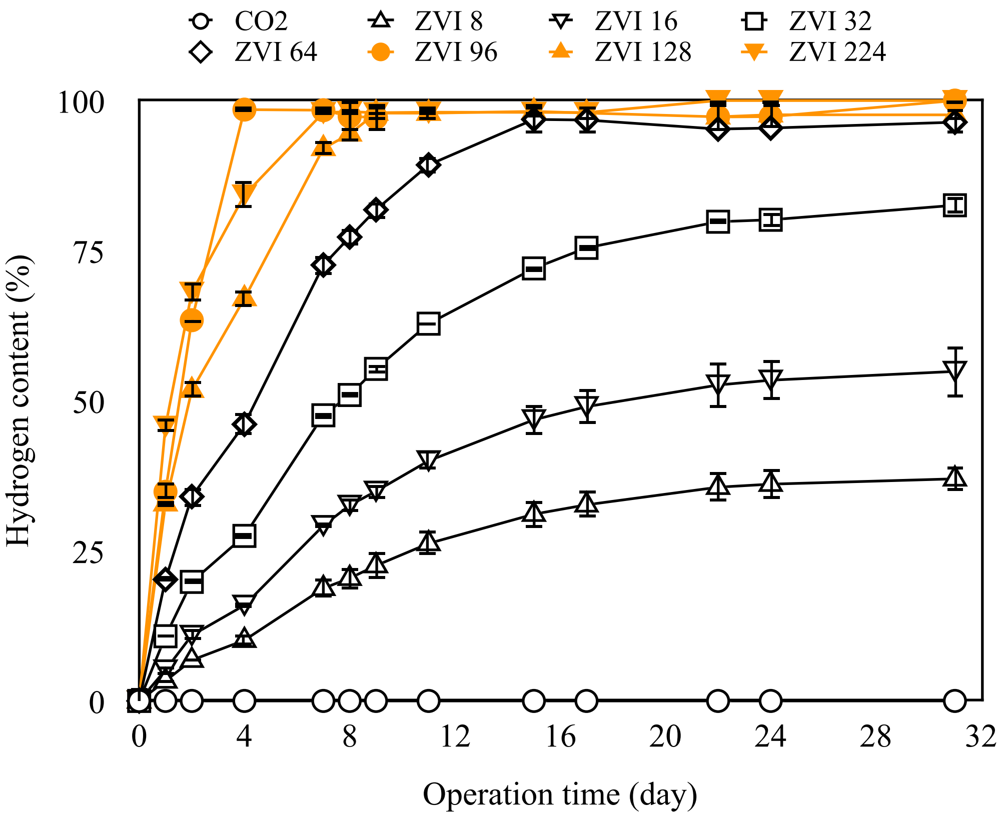

5.0
4.0
3.0
2.0
1.0
0.0
Carbon dioxide (mmol)
0
5
10
15
20
25
30
35
40
45
50
Operation time (day)
AM
AM ZVI 16
AM ZVI 32
AM ZVI 96

a)

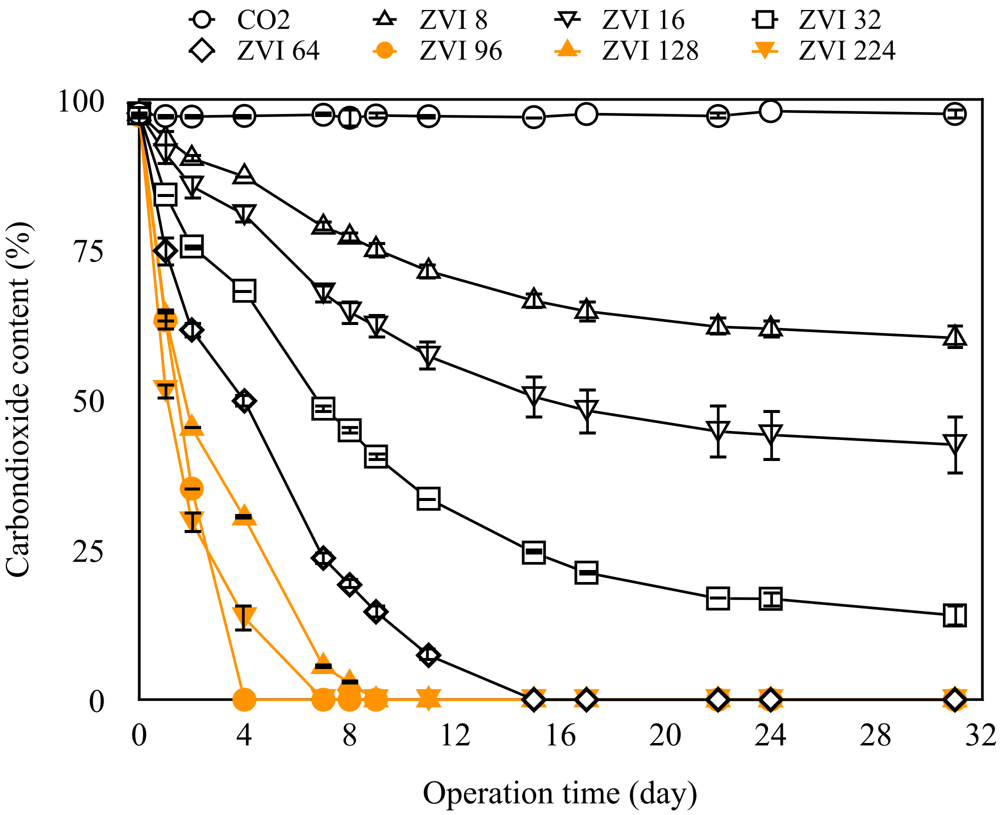

10.0
8.0
6.0
4.0
2.0
0.0
Cumulative methane production (mmol)
0
5
10
15
20
25
30
35
40
45
50
Operation time (day)
AM
AM ZVI 16
AM ZVI 32
AM ZVI 96

b)

Fig 4

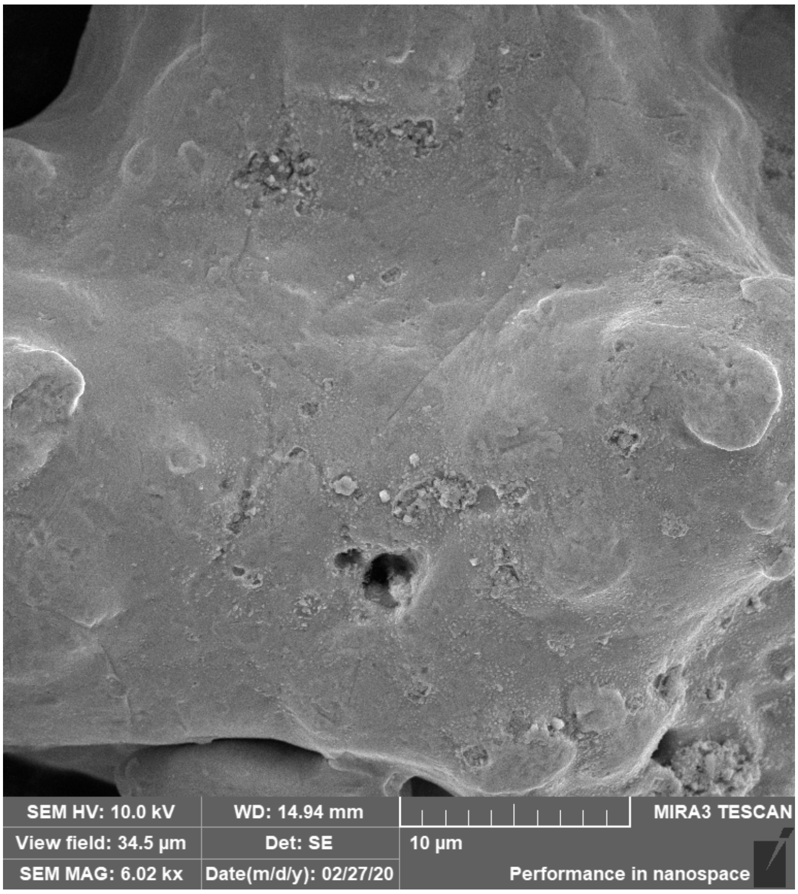

CO2
ZVI 8
ZVI 16
ZVI 32
ZVI 64
ZVI 96
ZVI 128
ZVI 224
Hydrogen content (%)
100
75
50
25
0
0
4
8
12
16
20
24
28
32
Operation time (day)

a)

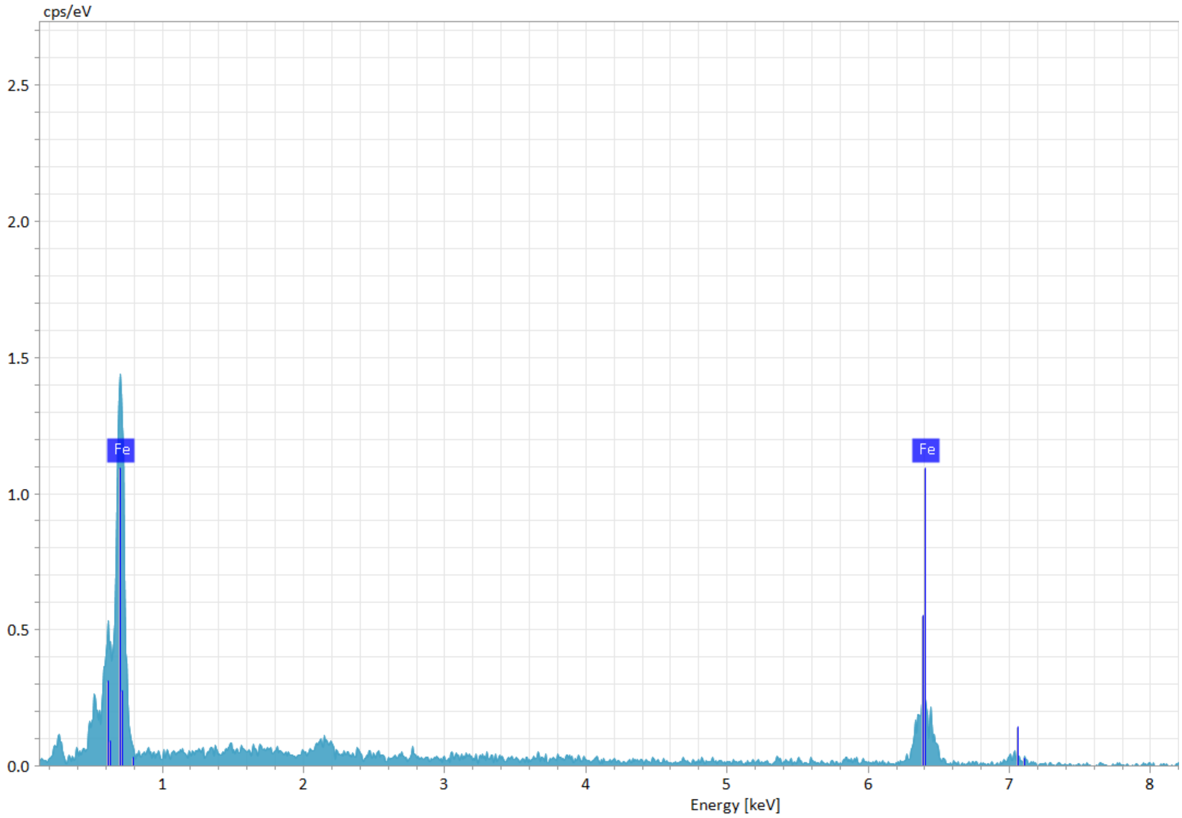

CO2
ZVI 8
ZVI 16
ZVI 32
ZVI 64
ZVI 96
ZVI 128
ZVI 224
Carbondioxide content (%)
100
75
50
25
0
0
4
8
12
16
20
24
28
32
Operation time (day)

b)

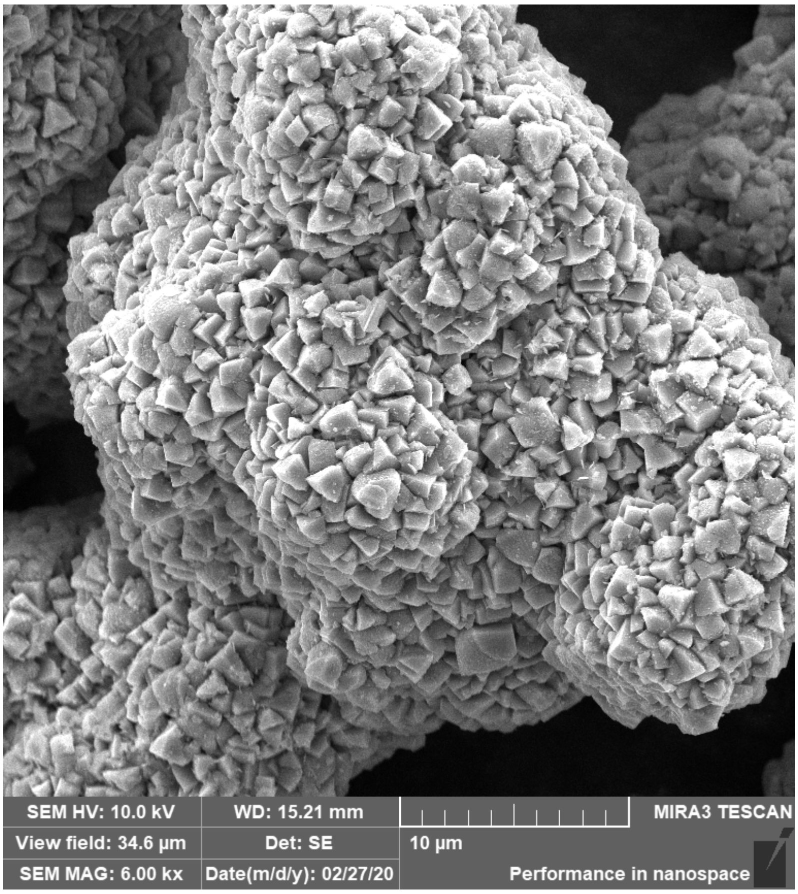
Fig 5


SEM HV: 10.0 kV
WD: 14.94 mm
MIRA3 TESCAN
View field: 34.5 μm
Det: SE
10 μm
SEM MAG: 6.02 kx
Date(m/d/y): 02/27/20
Performance in nanospace

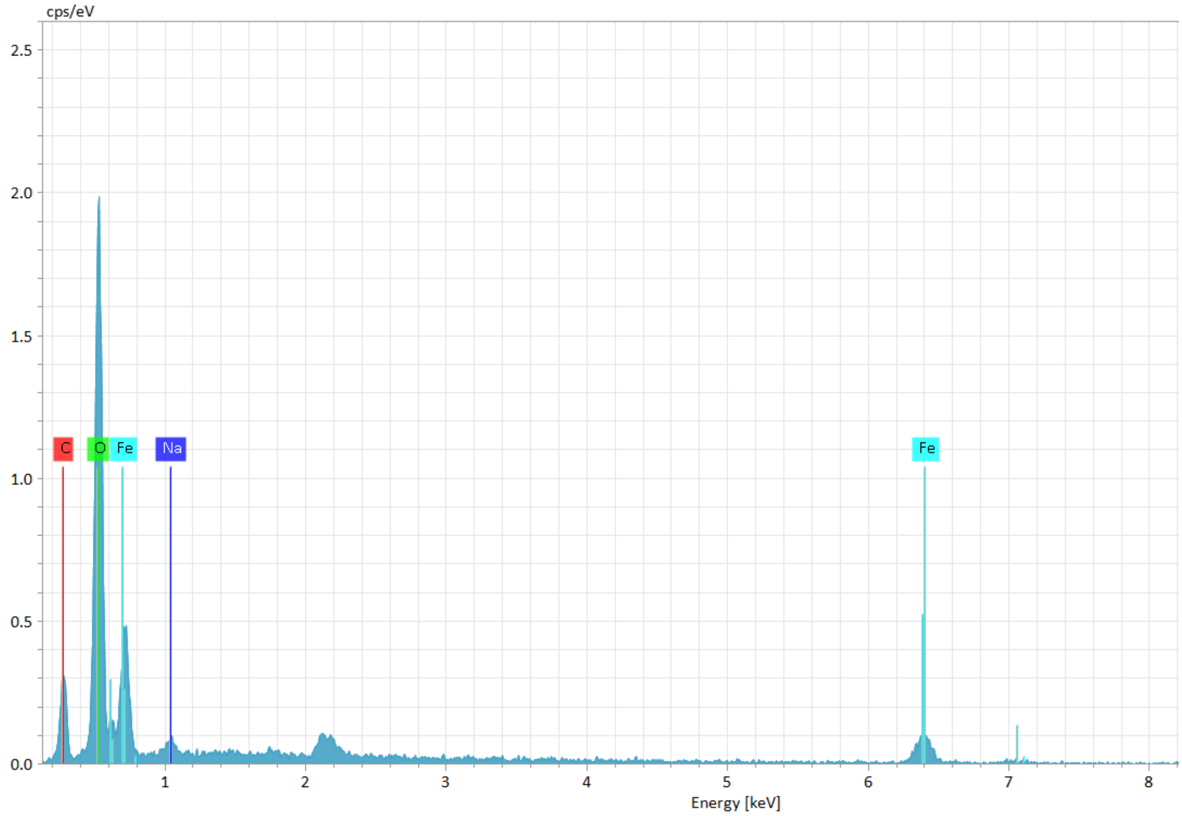
a)


cps/eV
2.5
2.0
1.5
1.0
0.5
0.0
Fe
Fe
1
2
3
4
5
6
7
8
Energy [keV]

b)


SEM HV: 10.0 kV
WD: 15.21 mm
MIRA3 TESCAN
View field: 34.6 µm
Det: SE
10 µm
SEM MAG: 6.00 kx
Date(m/d/y): 02/27/20
Performance in nanospace

c)


cps/eV
2.5
2.0
1.5
1.0
0.5
0.0
C
O
Fe
Na
Fe
1
2
3
4
5
6
7
8
Energy [keV]

d)
Fig 6

Table 1: Content of EPS in anaerobic sludge

Sample	Polysaccharides (mg/g SS)		Proteins (mg/g SS)	
	Loosely bound EPS	Tightly bound EPS	Loosely bound EPS	Tightly bound EPS
original sludge	1.68	0.62	32.39	16.27
CO_2	5.86	2.28	55.69	29.83
CZ 16	7.06	2.12	62.13	32.39
CZ 32	4.74	1.41	52.53	21.78
CZ 64	6.10	1.76	57.97	28.22
CZ 96	2.93	0.79	40.31	19.90
CZ 224	5.78	2.48	49.31	23.52
CO_2/H_2	7.08	4.20	63.94	34.00

Table 2: Methanogenic activities test and concentration of ferrous and ferric.

Sample	Original anaerobic sludge before experiment	Anaerobic sludge incubated with ZVI 96 g/l	Acclimated sludge before experiment	Acclimated sludge incubated with ZVI 96 g/l
Acetoclastic methanogenic activity	0.022 g COD/g VSS.d	0.024 g COD/g VSS.d	0.031 g COD/g VSS.d	0.038 g COD/g VSS.d
Hydrogenotrophic methanogenic activity	0.004 g COD/g VSS.d	0.004 g COD/g VSS.d	0.007 g COD/g VSS.d	0.008 g COD/g VSS.d
Fe^{2+} concentration		10.8412 mg/l		7.7586 mg/l
Fe^{3+} concentration		1.5874 mg/l		1.4890 mg/l

ENHANCEMENT OF ANAEROBIC PALM OIL MILL EFFLUENT DIGESTION BY ZERO VALENT IRON ADDITION AS ELECTRON DONOR

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Extended Abstract

The conversion of the organic matter of palm oil mill effluent (POME) into methane is expected to high potential method of treatment due to its high organic content and methane is an important renewable energy source. This conversion is carried out via anaerobic processes. However, POME contains high concentrations of suspended solids (SS), oil and biofibers, which may cause problems in the operations of anaerobic processes when POME was fed without prior removals of biofibers and oil. Zero valent iron (ZVI), a reducing material, has been widely applied in wastewater treatment, groundwater purification and soil remediation [1]. ZVI was found to promote a more favorable environment for anaerobic biological processes by rapid lowering the oxidation-reduction potential (ORP) when ZVI added [2] and buffering acid, while H_2 produced by iron corrosion could serve as electron-donor for hydrogen-consuming microorganisms, such as methanogenic archaea and denitrifying bacteria [3-4].

Batch experiment was conducted in a 120 ml of serum bottle. POME was used as substrate (3 gCOD/l) and anaerobic mesophilic digested sludge was used as inoculum (12 gVSS/l) corresponding an F/M ratio of 0.25. A ZVI at concentration of 16, 32 and 64 g/l was added into serum bottle. No any pH adjustment was needed. All bottles were flushed with the N_2 and incubated at 37 ºC. All the anaerobic batch experiments were carried out in duplicate.

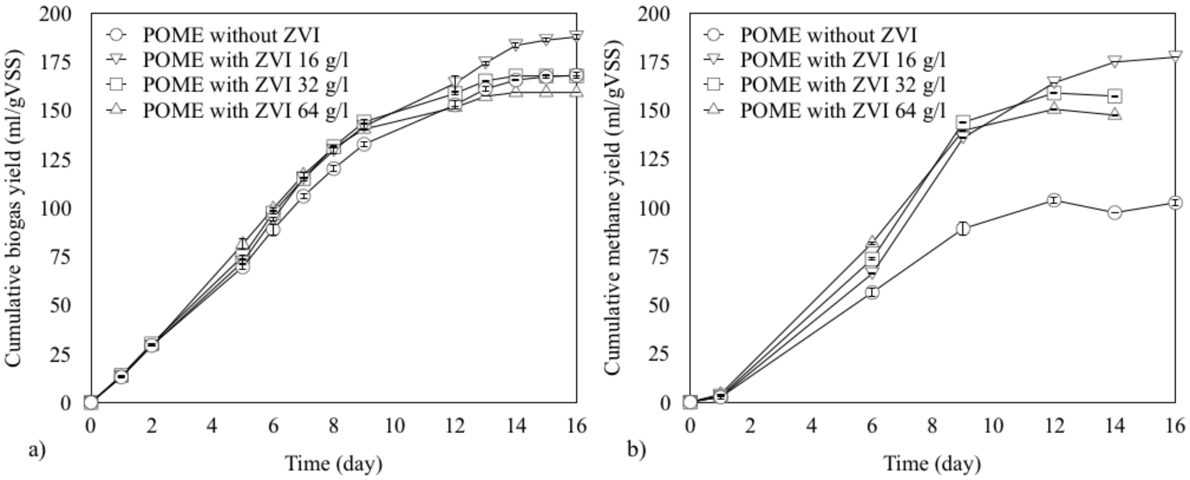

Fig. 1: (a) Cumulative biogas and (b) methane yields produced by anaerobic mesophilic digested sludge at 16, 32 and 64 g/l of ZVI.

The enhancement of various ZVI dosages on anaerobic digestion of POME was study in batch experiment. Fig. 1 presents the cumulative amounts of biogas and methane yields after 12 d. For biogas yield, any ZVI supplement showed biogas yield slightly higher than that of the control. In the result of methane yield, a supplement with ZVI at 16 g/l yielded the highest methane volume followed by 32 and 64 g/l. It indicated that the percentage of methane in the biogas was affected by ZVI dosage. At ZVI concentration of 16 and 32 g/l, the methane content was reached up to 100% on day 12 while the methane content of the supplement with ZVI 64 g/l was found 92.51%. At the end of batch experiment, pH value of any ZVI supplement was increased up to 9 whereas pH value of a control was 6.76 ± 0.02. The reason was that a release of H^+ led to pH value increase when ZVI was added.

An upscale of the ZVI to enhance anaerobic process was performed in a 5.8-l working volume modified CSTR reactor. A 10 g VSS/l of anaerobic sludge was added to a reactor. The POME was diluted with tap water at a ratio of 1:1 corresponding a rage of COD 38.24-53.05 g/l and a feed flowrate of 0.2 l/d. A temperature was maintained at 37 ºC by circulating hot water through the reactor jackets. The reactor was operated until both COD removal and biogas production were steady. After that, the ZVI was added at a dosage of 16 g per litre of the reactor.

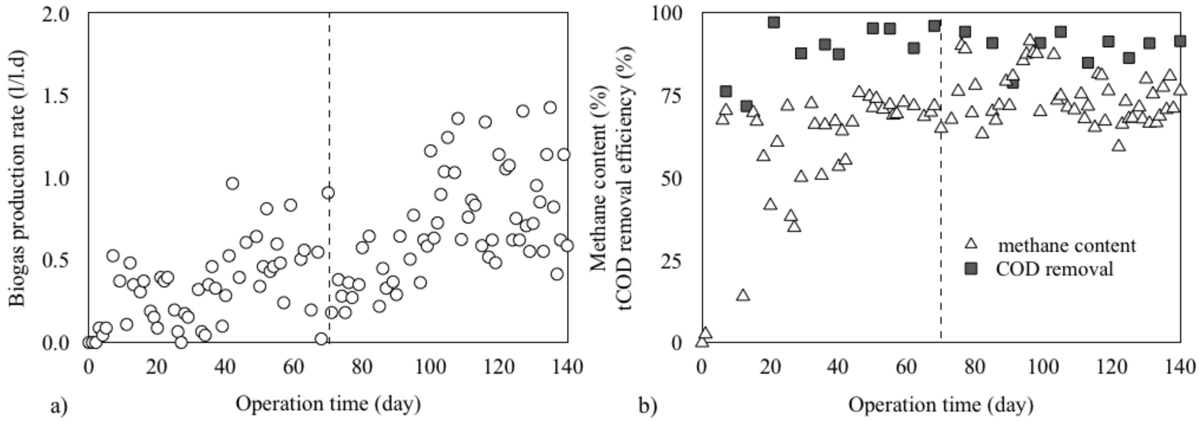

Fig. 2: Changes in reactor performances during operation process. (a) rate of biogas production; (b) percentage of methane and tCOD removal efficiency.

Fig. 2 shows the reactor performances before and after ZVI addition in a modified CSTR. In contrast with batch experimental result, the rate of biogas production of ZVI addition was significantly different compared to the no-ZVI dosage while a trend of methane content and tCOD removal efficiency in ZVI addition were similar with the no-ZVI dosage. For the VFA variation in the effluent, acetate and butyrate were the predominant VFAs. The values of acetate and butyrate of 1.71-2.30 and 1.20-1.23 g/l, respectively, were detected in the first 10 d. After that, they were stabilized in the range of 0.03-0.42 and 0.01-0.46 g/l for acetate and butyrate, respectively. The remaining of low butyrate (0.01-0.03 g/l) suggested that ZVI could accelerate the oxidization of butyrate to acetate. This result was consistent with acetogenesis activity results. Effects of ZVI addition on microbial activity, it was found that ZVI could accelerate among the hydrolysis, acidification and acetogenesis of sludge. The rate of cellulose and glucose utilizations in the ZVI dosage was 1.12 and 2.15 times higher than that of the no-ZVI dosage, respectively. Similar to hydrolysis and acidogenesis results, the rate of methane production from propionate and butyrate in the ZVI dosage was 2.43 and 2.70 times higher than that of the no-ZVI dosage, respectively.

The results could be assumed that the ZVI addition has the positive effects on the anaerobic digestion of POME and the activities of the microbial community. The result was explained with two reasons given by Anti [5] and Feng [6]. That is, the activities of major enzymes relating with hydrolysis and acidification were enhanced through the conversion of complex organics to VFAs upon ZVI addition [5-6]. Secondly, ZVI could enhance the growth of H_2-utilizing microorganisms including homoacetogens and hydrogenotrophic methanogens to consume H_2 and then drive the anaerobic digestion [6].

Keywords: modified CSTR; Microbial activity; Palm oil mill effluent; POME, zero valent iron; ZVI

Acknowledgment

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