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Research article

Enhancing anaerobic syntrophic propionate degradation using modified polyvinyl alcohol gel beads

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ABSTRACT

Modified polyvinyl alcohol (PVA) beads serve as effective anaerobic microbe immobilization carriers. PVA beads were mixed with different conductive materials, activated carbon, magnetite, and green tuff stone powder. In this study, modified PVA beads were used to investigate the effect of using, promote methane production, and enhance direct interspecies electron transfer (DIET) on the anaerobic syntrophic degradation of propionate, which is an essential intermediate process for generating methane in anaerobic digesters. The batch experiment showed that PVA mixed with activated carbon had the highest methane conversion rate of 72%, whereas the rates for control (sludge) was 61%. Moreover, the lag time during the second and third feedings was shorter by 5-fold than for the first feeding when modified PVA beads were added. The syntrophic propionate degrading microorganisms in the modified PVA beads was *Syntrophobacter* and *Methanobacterium*, either *Methanoculleus* or *Methanosaeta*. The modified PVA beads hold at least 10 times larger syntrophs than normal PVA. Therefore, composite PVA with conductive materials can promote methane production, accelerate propionate consumption, and enhance electron transfer in related microbial species.

1. Introduction

Propionate is an essential intermediate for anaerobic degradation under methanogenic conditions, and it is a central metabolite in the syntrophic relationship between acetogenic bacteria and methanogenic archaea. Hence, it is crucial to maintain the conjunctive consortia of propionate-oxidizing syntrophic bacteria and hydrogenotrophic methanogens in anaerobic reactors for propionate degradation. Additionally, syntrophic bacteria are often difficult to isolate because the two groups of microbes are dependent on each other. Generally, bacteria require hydrogen scavengers, and archaea require hydrogen suppliers. Both microbes consume mutual syntrophy yield energy ($\Delta G = 31$ kJ/mol) to live (Hattori, 2008). This small energy sharing directly affects the slow growth of microbes. Moreover, these cultures which rely on their limited pH, temperature, hydrogen partial pressure, toxins, and volatile fatty acids (VFAs), especially when the substrate concentration exceeds 5,000 mg COD/L (Li et al., 2020), also directly affect the biodegradation of propionate in syntrophic relation (Li et al., 2012).

Polyvinyl alcohol (PVA) gel beads are synthetic polymers that are widely used to immobilize cells during anaerobic processes, such as in up-flowing anaerobic sludge bed reactors or anaerobic digesters (El-Naas et al., 2013; Gani et al., 2016). Their prominent features include ease of separation in wastewater, non-toxicity to microorganisms, higher specific gravity than water, high mechanical strength, high durability in water, resistance to environmental fluctuations, and cheap production (Bae et al., 2015; Jeong et al., 2016; Cho et al., 2017). PVA gel beads are microporous and suitable for retaining biomass or microorganisms. They have been modified for relevant applications, such as in PVA mixtures with aluminum ions for phosphate removal (Hui et al., 2014), with maghemite and titania nanoparticles to reduce radioactivity (Majidnia, and Idris, 2015), and with chitosan/iron to enhance annamox activity (Wang et al., 2020).

Recently, direct interspecies electron transfer (DIET) has been suggested as an alternative to syntrophic metabolism with carbon substrates in anaerobic digesters to promote methane production (Lovley, 2011; Zhao et al., 2015; Dang et al., 2016). Several studies have shown the efficiency of conductive materials that may potentially promote anaerobic treatment, reduce the lag phase, enhance methane production, and serve as electron conduits to promote DIET between bacteria and methanogenic archaea for accelerating syntrophic methanogenesis

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(Barua and Dhar, 2017; Baek et al., 2018; Gahlot et al., 2020). Magnetite (MT) as iron nanoparticle possess properties for accelerating methanogenic conversion of substrates, such as propionate, as demonstrated through DIET (Cruz Viggi et al., 2014; Baek et al., 2016). Based on these findings, we hypothesized that the combined use of PVA gel beads with conductive materials could enhance the active syntrophic community biomass immobilized on modified PVA gel beads, making them potentially useful for inoculation or maintaining active syntrophic communities in reactors.

The objective of this study was to investigate the effects of using PVA beads encapsulating different conductive materials, activated carbon (AC), MT, and green tuff stone powder (GT) on the anaerobic syntrophic degradation of propionate. GT consists of several minerals, such as SiO_2 (62%), FeO or Fe_2O_3 (15%), and Al_2O_3 (9%) (Fujita et al., 2019), and it is a local material that is widely distributed along the Sea of Japan-facing side of Honshu Island. GT is a suitable material to study and to compare with AC and MT. This study will utilize the outstanding features of PVA beads and the abilities of each conductive material to improve modified PVA beads as an alternative immobilizing carrier in the anaerobic process.

2. Materials and methods

2.1. Immobilizing PVA gel bead preparation

In this study, three kinds of PVA and composite PVA beads (AC, MT, and GT) were produced as described by Bae et al. (2015). The steps of this procedure are as follows: 1) A solution of 10% (w/v) PVA and 0.5% (w/v) sodium alginate was dissolved in distilled water and completely dissolved by heating at 70–80 °C with stirring. The solution was stored at 50 °C. 2) To prepare the solution for forming spherical beads, solution 1) were introduced to saturated 6% (w/v) $B(OH)_3$ and 0.5% (w/v) $CaCl_2$ solution drop by drop using a syringe and then gently stirred with a magnetic stirrer until spherical beads of 3–4 mm in diameter were formed. 3) PVA beads were placed in a 1 M $NaSO_4$ solution overnight to enhance their strength. Each of the composite PVA beads' materials was added to the first solution while mixing completely and following the above procedure.

2.2. Characteristics of PVA beads and composite PVA beads

The appearance (color) of PVA was observed for each batch experiment. The settling velocities of the PVA beads and modified PVA beads were measured as described by Ghangrekar et al. (2005). The attached biomass was determined by changing the weights of the unused and used beads. Moreover, the morphology of the PVA beads was observed through scanning electron microscopy (SEM; TM3030Plus, Hitachi High-Technologies, Japan).

2.3. Batch experiment

To investigate the effects of different types of PVA and modified PVA beads on methane production, this study was conducted under two conditions. The first batch operated with both anaerobic sludge and PVA beads, and the second batch only used beads transferred from the initial state. The first batch was divided into three feeding cycles (22, 12, and 8 d) whereas the second batch utilized two cycles (26 and 34 d).

The study was conducted as an anaerobic digester batch experiment. Anaerobic seed sludge was collected from an anaerobic reactor using mushroom as a substrate (Ikeda et al., 2019). The volatile solids (VS) concentration of the sludge was 28,873 (±691.8 mg/L). For the first batch experiment, 10,000 mg VS/L of seed sludge and a culture medium (10 mM NH_4Cl, 1 mM KH_2PO_4, 1 mM $MgCl_2 \cdot 6H_2O$, 1 mM $CaCl_2 \cdot 2H_2O$, and 30 mM $NaHCO_3$) were added with a working volume of 500 mL. PVA or modified PVA beads (i.e., PVA, PVA_AC, PVA_MT, and PVA_GT) (10 g each) were placed into the bottles. Before digestion, the glass bottles were flushed with nitrogen gas and sealed with rubber stoppers and aluminum caps. The bottles were placed in a bio-shaker at 170–175 rpm and 37 °C, and propionate (1,310 mg COD/L) was fed into each bottle as a substrate. This experiment was conducted in duplicate.

For the second batch experiment, PVA beads utilized in the first batch experiment were collected and used as the microbial source. The operational processes (culture medium, substrate feeding, shaking cycle, and temperature) followed those of the first experiment.

2.4. Analysis methods

VS and total solids were analyzed following a standard method (APHA, 2012). The volume of the biogas was measured using a glass syringe. The methane concentration in the biogas was measured using a gas chromatograph equipped with a thermal conductivity detector (GC-8A, Shimadzu, Japan). The remaining substrate and substrate conversion were calculated using the theoretical equivalent relationship and the resulting coverage the modified Gompertz equation (Sedano-Núñez et al., 2018).

2.5. Calculation of methane production

The experimental data in the batch experiment were used to predict methane production using the modified Gompertz equation.

$$P = P_0 \cdot \exp\left\{ -\exp\left[\frac{R_{\max} \cdot e}{P_0} \cdot (t_0 - t_1)\right]\right\} \tag{1}$$

P: Cumulative methane production (mL)
P_0: Methane production potential (mL)
R_{max}: Maximum methane production rate (mL/d)
t_0: Lag phase period (d)
t: Cumulative time for methane production (d)
e: Mathematical constant (2.718282)

2.6. Microbial community analysis

The anaerobic sludge and PVA beads were collected at the end of the experiment. The microbial community of each sample was analyzed using high-throughput 16S rRNA sequencing. DNA was extracted using the FastDNA™ Spin Kit for Soil (MP Biomedicals, CA, USA). The V3–V4 region of 16S rRNA was amplified via PCR using the primer sets 515F and 806R. The amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany), and high throughput 16S rRNA gene sequencing was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA).

3. Results and discussion

3.1. Performance of PVA and modified PVA beads in methane production

The experimental results for the cumulative methane production and average methane production rates are shown in Figure 1 and Table 1. During feeding cycle 1 of the first batch (22 d), almost all materials exceeded ~60% methane conversion rate. This was the efficiency of the methane produced from the initial substrate feeding compared to the theoretical methane potential of propionate, apart from PVA_GT, which had the lowest conversion rate (~45%). Methane production rates of PVA, PVA_AC, and PVA_MT were higher (17.2, 16.3, and 17.2 mL/d, respectively) than those of sludge (15.5 mL/d; Table 1). However, PVA_GT only produced 12.5 mL/d of methane, which was the lowest recorded value (Figure 1a). The complicated GT composition may have affected the efficiency of the methane production rate in the first feeding. However, all batches reached the maximum methane production rate within 15 d. The lag phase was an important parameter that indicated the period during which the microbial community adjusted to the new

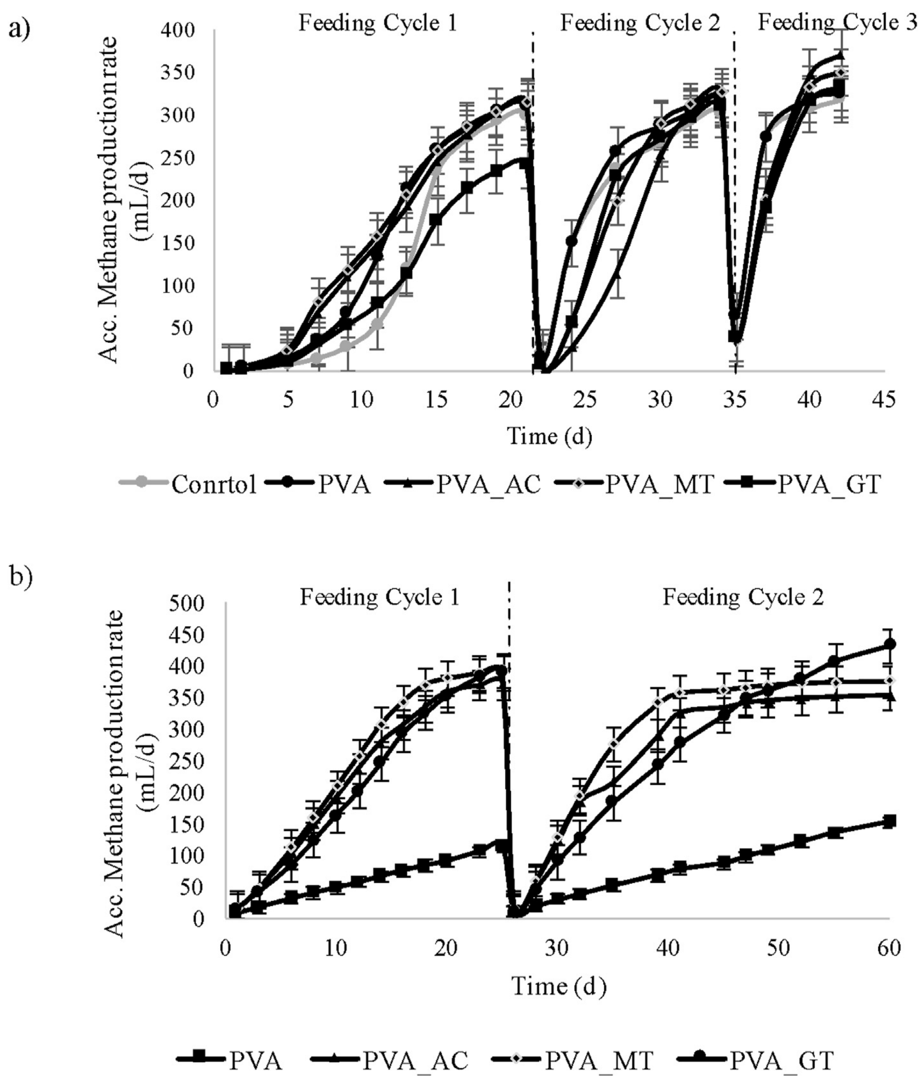

Figure 1. Accumulative methane production amount in a) combined sludge with modified PVA beads (first batch) and b) modified PVA beads only (second batch).

environment and predicted the degradation of propionate to methane. The lag phase of the PVA beads was shorter (5 d) than that of the control (9 d).

During feeding cycles 2 (12 d) and 3 (8 d) (Figure 1a), the maximum methane production rate increased for the control and PVA batches by up to 91 mL/d and rapidly reached their maximum rates within 3 d. The rates for the modified PVA beads, PVA_AC, PVA_MT, and PVA_GT were 70, 67, and 63 mL/d, respectively (Table 1). During the second and third feeding, in the initial lag phase, which depicted the adaptation of anaerobic microbial communities to the batch condition, modified PVA beads did not display a lag phase. An electron transfer might have occurred in modified PVA beads, and it may have activated microbial communities to reach the growth phase. Therefore, adding conductive materials may promote anaerobic treatment in terms of methane production and cultured microorganisms involved in syntrophic metabolism (Jung et al., 2016; Kato et al., 2012; Liu et al., 2012; Zhao et al., 2016).

For the second batch, the microbial community immobilization efficiency of the PVA carrier was evaluated for its ability to promote methane production; only PVA beads with attached biomass from the first batch were inoculated (Figure 1b). The cumulative amounts of methane produced using PVA_MT, PVA_GT, and PVA_AC were 392, 390, and 372 mL, respectively (Figure 1b), which were higher than the methane production (114 mL) using conventional PVA beads. Methane production rates per day were 19.0, 16.4, and 17.7 mL/d for

Table 1. Average methane production rate (mL/d) and highest production rate in the experiments.

Sample	1st Batch (Sludge with PVA beads)			2nd Batch (PVA beads with attached biomass)	
	Feeding cycle 1 (Day 0–22)	Feeding cycle 2 (Day 23–34)	Feeding cycle 3 (Day 35–43)	Feeding cycle 1 (Day 0–26)	Feeding cycle 2 (Day 27–60)
Sludge	7.5 ± 6.7 (15.5)	31.3 ± 11.4 (49.8)	50.6 ± 22.0 (91.1)	-	-
PVA	10.1 ± 6.6 (17.2)	31.9 ± 12.5 (49.6)	51.7 ± 21.7 (91.3)	5.3 ± 1.2 (8.8)	4.4 ± 2.0 (10.9)
PVA_AC	10.9 ± 5.9 (16.3)	18.9 ± 9.7 (69.0)	44.7 ± 13.6 (69.0)	17.7 ± 1.9 (20.0)	13.6 ± 2.8 (17.2)
PVA_MT	11.6 ± 6.0 (17.2)	23.6 ± 10.7 (32.9)	42.3 ± 14.1 (66.6)	19.0 ± 2.5 (21.9)	15.1 ± 3.7 (21.2)
PVA_GT	7.1 ± 4.6 (12.5)	24.2 ± 10.3 (37.8)	40.3 ± 11.5 (62.9)	16.4 ± 1.4 (18.4)	12.4 ± 2.0 (14.6)

Note: (#) represents the highest methane production rate in these experiments.

Table 2. Methane production on PVA beads using the modified Gompertz equation.

Sample	1^{st} Batch (Sludge with PVA beads)				2^{nd} Batch (PVA beads with attached biomass)			
	T_0 (d)	R_{max} (mL/d)	P_0 (mL)	R^2	T_0 (d)	R_{max} (mL/d)	P_0 (mL)	R^2
PVA	*(3)	8.8	114	0.98	*(3)	10.9	153	0.96
PVA_AC	*(12)	20.0	372	0.97	*(19)	17.2	354	0.94
PVA_MT	*(14)	21.9	392	0.97	*(13)	21.2	376	0.94
PVA_GT	*(16)	18.4	391	0.98	*(19)	14.6	433	0.96

Note: * Lag time phase was not detected in the batch time of the experiment, and (#) is the maximum rate detected per day.

PVA_MT, PVA_GT, and PVA_AC, respectively, whereas the rate for conventional PVA was 3 to 4-fold lower at 5.3 mL/d. Moreover, the results clearly showed that the methane conversion efficiency of the modified PVA beads exceeded 70%, whereas that for PVA was only 22% during cycle 1. The methane production rate reached a maximum within 14 d for PVA_MT and PVA_AC and within 16 d for PVA_GT. In the case of PVA without conductive material, the amount of methane production in the first batch was 2.0–2.5-fold higher than that in the second batch. The methane gas detected in the first batch was mainly produced from bulk sludge only. The reason for the low methane production was the small amount of sludge retention in PVA caused by the difference in the porous structure of the PVA beads with and without conductive materials. These are discussed in the following sections.

During feeding cycle 2 of the second batch, methane production rates were slightly lower than those for feeding cycle 1 (Table 1). However, the methane conversion rate exceeded 83% or more for PVA_GT (as seen in Figure 1b) and 70% for PVA_MT and PVA_ AC within two weeks, whereas only a 29% methane conversion rate was recorded for the conventional PVA control. The addition of AC and MT might be the key to better propionate degradation performance and a strategy to boost the methane production (Yang et al., 2020; Xing et al., 2020). Based on the performance of the modified PVA beads with attached biomass for similar periods (25 d), no significant differences were found. In the second cycle, PVA_GT showed a considerably different curve from those of the other conductive material cultures. The reaction of PVA_GT was slower than the others at one period after feeding, but it increased steadily, and it took several days to reach the maximum point. GT consists of several minerals that can be used as conductive materials, whereas AC and MT have only one composition that is limited to electron transfers. Thus, PVA_GT is a suitable and cost-effective candidate for real-world applications, as GT is the byproduct of stone processing.

A modified Gompertz equation was applied in this study to simulate the results from the second batch experiment. The important parameters are shown in Table 2. The lag phases for all the digesters were not detected on days 26 and 34 during the second feeding. Thus, the addition of conductive materials to PVA beads did not affect the lag time. Moreover, PVA had the lowest maximum methane production rate and potential. Therefore, AC, MT, and GT may have been responsible for increasing R_{max}.

Biomass from each digester was collected at the end of the first batch (day 42). The startup sludge in each vial was 10,000 mg VS/L. The sludge increase in the control (without PVA gel beads), PVA, and PVA_GT was ~13%, whereas PVA_AC and PVA_MT produced more sludge (43% and 27%, respectively). Because conductive AC or MT are inert materials, they can improve propionate degradation and increase methane production rates by providing microbial attachment sites (Aziz et al., 2011; Xu et al., 2020).

3.2. Characterization of PVA gel beads

The PVA beads were characterized on days 0 and 42 (the end of the first batch) to determine the concentrations of the attached biomass. The four types of unused PVA beads had different colors, but after 42 d of incubation, all four types turned blackish, whereas the sludge retained its original color after the start of the experiment (Figure 2). The biomass attachment on the PVA beads was measured by weighing the PVA before and after use. PVA_MT had the largest amount of attached biomass, at 0.030 g sludge (wet weight)/g-beads. PVA_GT and PVA_AC had 0.023 and 0.019 g sludge/g-beads, respectively, whereas PVA had the smallest amount of attached biomass at 0.002 g sludge/g-beads.

SEM was used to observe the colonization of microorganisms on the surface and interior (cross-section) of PVA beads as well as the variation in overall morphology (Figure 3). The SEM images of the unused PVA beads show their porous internal structure. The used-modified PVA beads

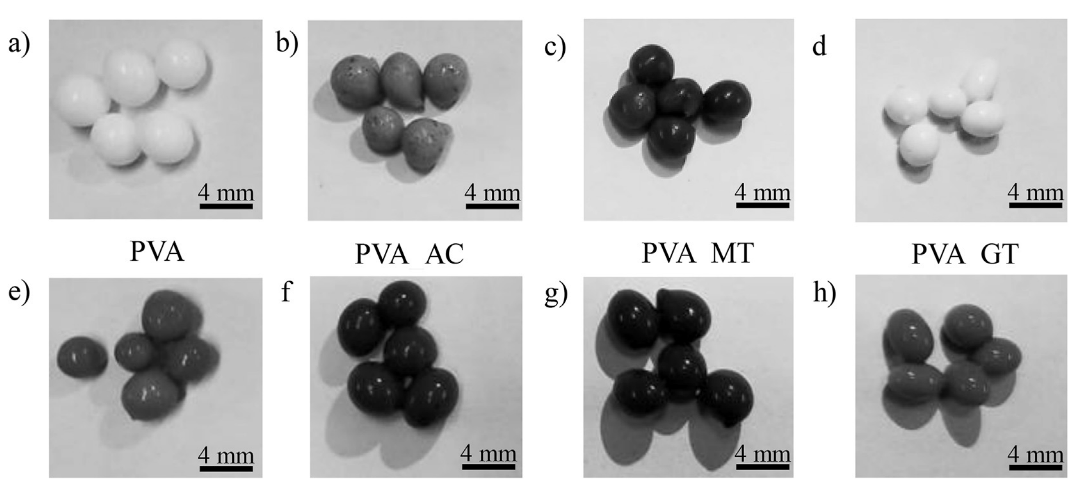

Figure 2. Appearance of unused a) PVA, b) PVA_AC, c) PVA_MT, and d) PVA_GT. Post-use: e) PVA, f) PVA_AC, g) PVA_MT, and h) PVA_GT.

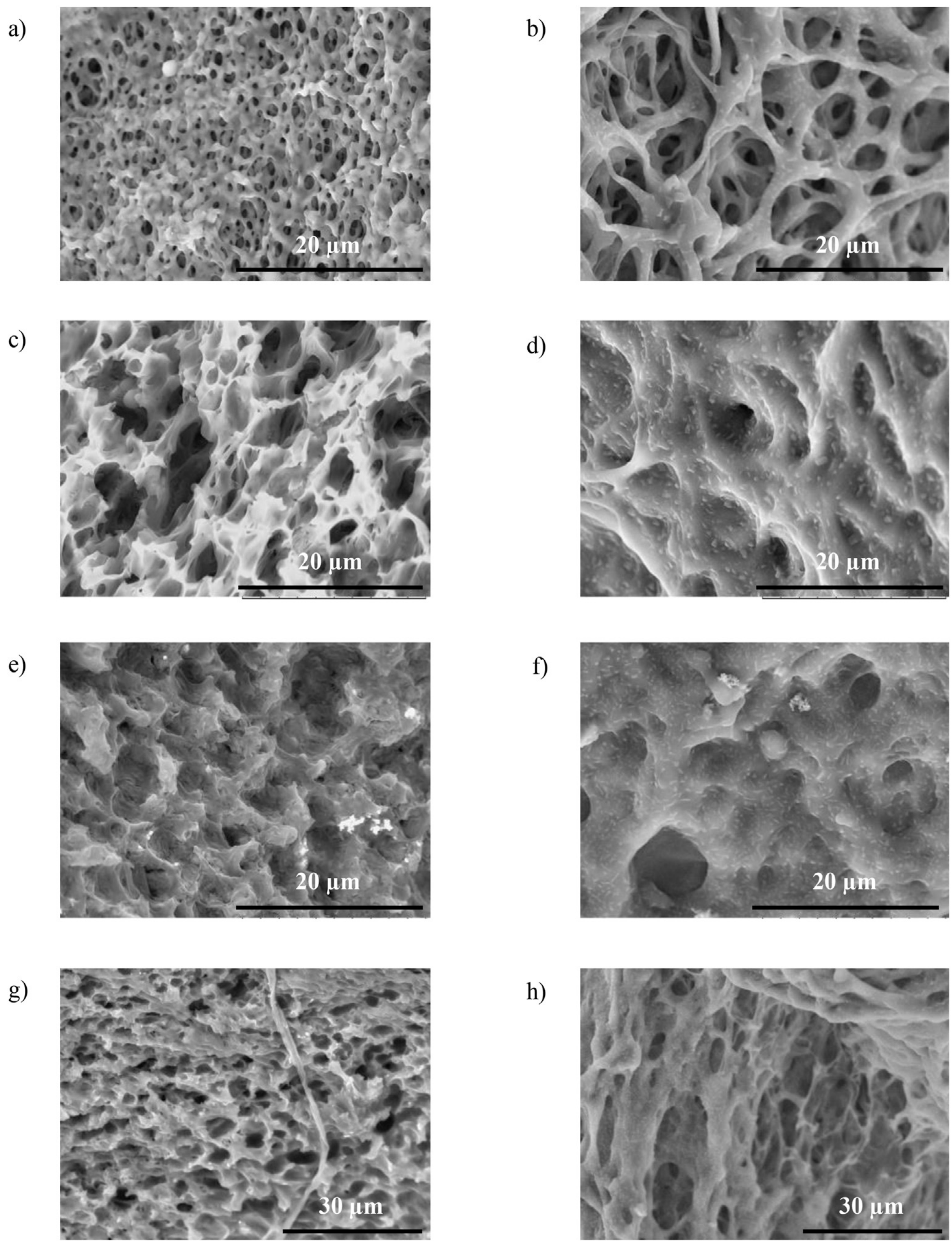

Figure 3. SEM images of cross sections for a) unused PVA bead, b) PVA bead at day 42, c) unused PVA_AC, d) PVA_AC on day 42, e) unused PVA_MT, f) PVA_MT at day 42, g) unused PVA_GT, and d) PVA_GT at day 42.

had a porous network structure and were covered with a polymer that might be composed of microorganisms (Figure 3d, f, and h), whereas conventional PVA showed a clear structure. PVA seems to have a porous structure with a sufficiently large pore size for the attachment of microbial cells. When considering the structure of the unused PVA beads, a tiny porous and tight layer was observed. This was one reason for microbes not entering the beads. Only certain microbes could progress toward from surface and did not inhabit the interior of the beads. Although biomass was also attached to the surface area of the beads, it was easily washed out because of the sheer force of the shaking cycle, which could be the main reason for the low sludge retention of the PVA beads without conductive material. The surfaces of the PVA beads were covered in the suspended sludge (Figure 2), thereby suggesting that the microorganisms progressed from the surface to the interior of the beads with high porosity as facilitated by substrate and metabolite diffusion (Zhang et al., 2009). Thus, the addition of conductive materials resulted in a porous structure with a sufficiently large pore size for the attachment of microbial cells. Moreover, electron transfer between cells to cell microbes via conductive materials occurred. The conductive materials added to PVA beads strongly contribute to the strength and compactness, including effectively improving biomass retention (Wang et al., 2020). Therefore, the modified PVA beads held more sludge than conventional PVA beads, which was likely responsible for the higher methane production rate in the second batch (Figure 1b).

3.3. Microbial communities of suspended sludge and PVA bead-attached biomass

The microbial community structure of the bulk sludge at the end of the first batch experiment, which contained sludge with PVA beads, is

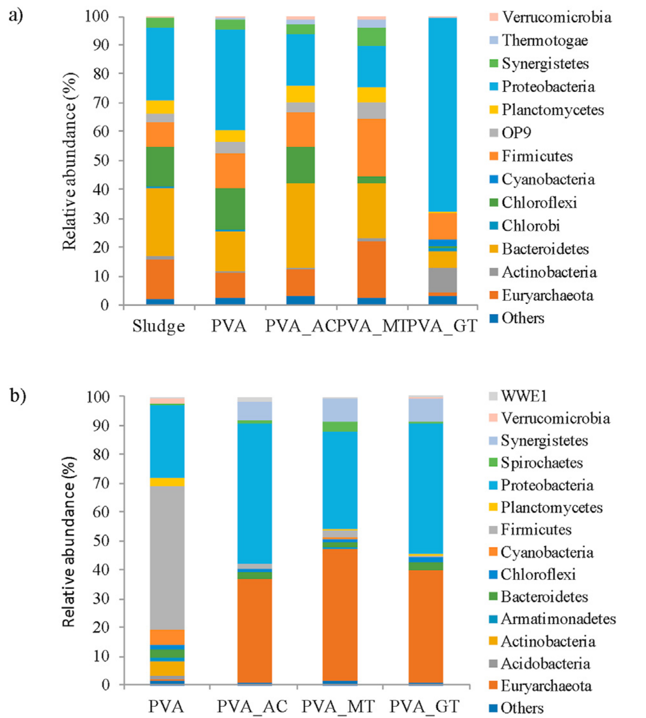

Figure 4. Phylogenetic microbial community distributions in phyla for a) bulk sludge of the first batch b) PVA beads attached to the biomass of the first batch. Sequences that accounted for less than 1% of the population were classified as "Others".

shown in Figure 4a. The dominant bacterial phyla were *Proteobacteria* and *Bacteroidetes*. Both bacterial phyla showed the highest relative abundances for all batch digesters. The highest abundance of *Proteobacteria* was detected in PVA_GT (approximately 67%), and was considerably higher than in sludge (without PVA) (25%), PVA (35%), PVA_AC (18%), and PVA_MT (14%). *Syntrophus* and *Syntrophobacter*, belonging to the *Proteobacteria* phylum, were the predominant bacterial genera in all digesters, which were especially suggested in PVA_AC to inspire syntrophic partners' growth and help form a healthy relationship in AD (Zhang et al., 2020), except PVA_GT. Both bacterial genera converted propionate to acetate and H_2. Meanwhile, H_2 was produced as an inhibitor that could directly affect other microorganisms in the digester. However, some methanogens were capable of absorbing H_2. The symbiosis of acetogenic bacteria and methanogens using hydrogen was directly affected by biogas production (Schink, 1997). *Bacteroidetes* was another dominant phylum in all batches, and the abundance of *Bacteroidetes* in PVA_AC (29%) was higher than in sludge (24%), PVA (13%), PVA_MT (19%), and PAV_GT (6%). "*Uncultured Bacteroidetes*" belonging to the *Bacteroidales* order was the predominant genera in all batch digesters except PVA_GT. *Porphyromonadaceae* and *Pseudomonadaceae* were predominated in PVA_GT. *Pseudomonas* could not directly utilize propionate and this might be counteracted to slower methane production rate in GT digester (Yuan et al., 2020). Another critical bacterial phylum that also had a high population ratio was *Firmicutes*. *Firmicutes* were detected in sludge (8% abundance) at a rate lower than in PVA (12%), PVA_AC (12%), PVA_MT (20%), and PVA_GT (9%). *Clostridium* was the predominant genus in this phylum, representing syntrophic acetate-oxidizing bacteria under mesophilic and thermophilic conditions (Shah et al., 2014). The abundance of *Clostridium* was frequently reported in previous literatures with the short period operation (Baek et al., 2016), especially in AC and MT supplementations (Peng et al., 2018; Xu et al., 2020). Notably, three phyla, *Synergistetes*, *OP9*, and *Planctomycetes*, could not be detected in PVA_GT. The genus *HA73*, belonging to the *Synergistetes* phylum, was found in anaerobic digesters. *Synergistetes* bacteria can utilize acetate through syntrophic acetate oxidation coupled with hydrogenotrophic methanogens (Ito et al., 2011). In addition, one bacterial phylum was detected only in PVA_GT. *Actinobacteria* were detected at a 9% abundance, and *Mycobacterium* was the predominant bacteria. *Mycobacterium* can inhibit environmental diversity (Ranjani et al., 2016; Gupta et al., 2018). *Mycobacterium* may affect the growth and number of microbial species in PVA_GT.

From the microbial community analysis, archaea communities were detected with abundances of 14%, 9%, 9%, and 19% in sludge, PVA, PVA_AC, and PVA_MT, respectively, whereas PVA_GT had an abundance of less than 1%. *Methanobacteria* and *Methanomicrobia*, belonging to Euryarchaeota, were the dominant microbial classes in all digesters. *Methanobacterium* can participate in DIET through CO_2 reduction and CH_4 production. *Methanobacterium beijingense* accounted for the largest abundances of PVA_MT and PVA_AC, respectively, and it used H_2/CO_2 and formate as substrates for growth and producing methane. MT and AC acted as electron acceptors, thereby facilitating a rapid oxidation of propionate (Ma et al., 2005). We noticed the abundances of *Methanoculleus*, which plays a role in increasing VFA concentrations (Dang et al., 2017) and *Methanosaeta, which* facilitates DIET (Zhao et al., 2017). Both archaea belong to the class *Methanomicrobia* and are hydrogenotrophic and acetolactic methanogens, respectively. They also might directly affect the performance of digesters in the same manner as *Methanobacterium* (Cruz Viggi et al., 2014; Lei et al., 2018).

The microbial community of the PVA beads attached to the biomass is shown in Figure 4b. *Proteobacteria* were dominant in all PVA beads as in the sludge microbial communities, but at different ratios. The abundances of *Proteobacteria* in the modified PVA beads were 49%, 34%, and 45% in PVA_AC, PVA_MT, and PVA_GT, respectively, which were higher than the abundance in conventional PVA (25%). The *Syntrophobacter* genera, which had a syntrophic propionate-oxidizing bacterial interaction with methanogens such as *Methanobacterium* sp. through interspecies electron transfer using H_2 and formate (Sedano-Núñez et al., 2018), was predominant in modified PVA beads with conductive materials. In addition, *Synergistetes* was detected on modified PVA beads and was significantly enriched in conductive materials (Peng et al., 2018). The phylum consists of iron-reducing microorganisms that transfer electrons through substrate oxidization during Fe reduction on MT or GT. Meanwhile, *Firmicutes* accounted for 50% of the abundance as a dominant phylum in conventional PVA beads, including *Bacillus* (12%) and *Weissella* (10%). Moreover, *Cyanobacteria* and *Actinobacteria* were detected in conventional PVA beads. The order *Acidimicrobiales*, belonging to *Actinobacteria,* are usually found in rich acidic environments (Itoh et al., 2010). Therefore, conventional PVA beads do not contain various species of bacteria that can utilize the substrate as propionate and are not converted to H_2 or formate for methanogens.

Methanomicrobia and *Methanobacteria* were the dominant archaea attached to the modified PVA beads. *Methanomicrobia* accounted for 46%, 39%, and 36% of the microbial communities in PVA_MT, PVA_GT, and PVA_AC, respectively. However, the highest abundances of *Methanoculleus* and *Methanosaeta* were detected in PVA_AC beads (approximately 87% archeal abundance). In addition to PVA_MT and PVA_GT, the predominant archaea were "*Uncultured Methanobacterium*" and *Methanobacterium beijingense*. The archaeal abundance in the conventional PVA beads was less than 1% of the total population of the archaeal community. Cell-to-cell electron transfer between syntrophic microorganisms occurred in the modified PVA beads via conductive materials and DIET. In contrast, the conventional PVA beads did not accumulate or enrich the methanogens, which directly affected the efficiency of propionate degradation. Consequently, the Euryarchaeota ratios were completely different between the conventional PVA and modified PVA beads. Thus, studying these microbial communities contributed to understanding the relationship between the bacteria and archaea that facilitate DIET via conductive materials.

4. Conclusions

The addition of conductive materials, such as AC, MT, and mineral stone such as GT as modified PVA beads can promote methane production and accelerate propionate consumption at rates 2.0–2.5-fold higher than those by PVA beads alone or sludge. Furthermore, microbial community analysis described the relationship between bacteria and archaea, which confirmed the important role of DIET. Microbial syntrophy was enhanced by using modified PVA beads, which has been demonstrated to efficiently enrich or accumulate syntrophic microbial species such as *Syntrophobacter, Methanobacterium*, *Methanoculleus,* and *Methanosaeta*. According to these modified PVA beads demonstrated satisfactory results, they suitable serve as alternative materials as immobilizing carrier in anaerobic process.

Declarations

Author contribution statement

Sitthakarn SITTHI: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Masashi HATAMOTO: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Takahiro WATARI: Contributed reagents, materials, analysis tools or data.

Takashi YAMAGUCHI: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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