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Discovery of a Microbial Carrier with High Adsorption Affinity for Syntrophic Long-Chain Fatty Acid-Degrading Microorganisms

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ABSTRACT: Long-chain fatty acid (LCFA) degradation primarily involves several species of *Syntrophomonas* and hydrogenotrophic methanogens, constituting the rate-limiting step in anaerobic digestion. It is crucial to augment their abundance to enhance LCFA degradation. Utilizing microbial carriers presents an effective strategy to maintain the microorganisms on the surface and prevent their washout from the digester. In this study, we aimed to identify a suitable microbial carrier with a superior adsorption capacity for LCFA-degrading microorganisms. We tested various polymers, poly(vinyl alcohol) (PVA), polypropylene (PP), polyethylene glycol (PEG), and polyvinylidene chloride (PVDC), adding them to the sludge at the concentration of 28.25 g L^{-1} and incubating with olive oil. The amplicon sequencing analysis revealed that PVDC retained *Syntrophomonas* more abundantly than the other polymers. Remarkably, PVDC predominantly adsorbed LCFA-degrading *S. sapovorans* and *S. zehnderi*, whereas medium- to short-chain fatty acid-degrading *S. wolfei* was abundant in the sludge. Moreover, hydrogenotrophic *Methanospirillum hungatei* was detected at 2.3−9.5 times higher abundance on PVDC compared to the sludge. Further analysis indicated that not only these LCFA-degrading syntrophic microbial communities but also *Propionispira* and *Anaerosinus*, which are capable of lipid hydrolysis and glycerol degradation, became dominant on PVDC. Actually, chemical analysis confirmed that adding PVDC promoted the olive oil degradation. These results underscore the potential of PVDC in promoting anaerobic LCFA degradation.
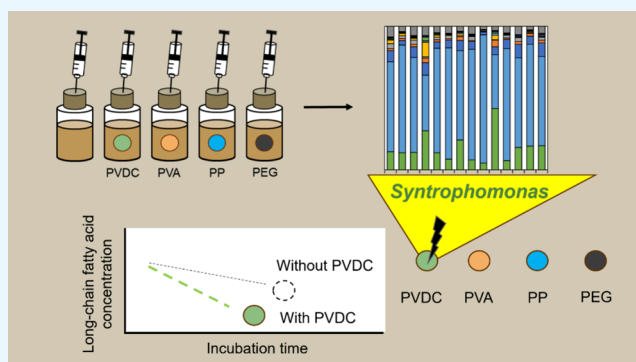
INTRODUCTION

In recent years, the exacerbation of environmental issues, such as global warming and the depletion of fossil fuels, has highlighted the limitations of a societal structure rooted in mass production and consumption. In 2015, the 21st Conference of the Parties to the United Nations Framework Convention on Climate Change (COP21) established long-term goals for mitigating global warming. Archiving a suitable and better society requires the construction of a resource-efficient circular system. Anaerobic digestion is a sequential bioprocess that is carried out by a series of functional microbes involved in hydrolysis, acidogenesis, acetogenesis, and methanogenesis.[1] Anaerobic digestion offers a viable technology as it can recover methane gas from various organic wastes, contributing to the realization of a sustainable society.

Fat, oil, and grease (FOG) are present in wastewater from edible oil producers, food processing industries, slaughterhouses, and food wastes.[2] FOG can generate a substantial amount of biogas, approximately 1.01 m^3 kg^{-1}, surpassing the potential energy of proteins (0.74 m^3 kg^{-1}) and carbohydrates (0.37 m^3 kg^{-1}).[3] However, the anaerobic digestion of FOG is considered challenging. In the anaerobic digestion process,

FOG is first hydrolyzed into long-chain fatty acid (LCFA) and glycerol.[4] LCFA tends to adhere onto microbial cell membranes, restricting their metabolic activities.[5] This metabolic inhibition affects methanogenic archaea more severely than bacteria.[6] Additionally, LCFA accumulation causes a barrier to substrate diffusion and accession.[7,8] It also leads to the sludge flotation and biomass washout, resulting in a significant reduction in biogas recovery rates.[7,8] Therefore, promoting LCFA degradation is strongly needed to accomplish stable and efficient methanogenesis from FOG.[2]
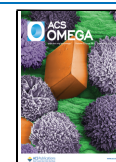
LCFA degradation occurs through β-oxidation reactions primarily by a group of bacteria belonging to the genus *Syntrophomonas*.[9] This reaction requires low hydrogen partial pressure for favorable thermodynamics.[10] Therefore, the presence of hydrogenotrophic methanogens as syntrophic

partners alongside β-oxidizing bacteria is crucial for LCFA degradation.[11] Both *Syntrophomonas* species and hydrogenotrophic methanogens exhibit very slow growth rates, with reported doubling times of 40 h for *S. sapovorans*[12] and 9.4 h for *M. bryantii*.[13] Thus, it is important to prevent the slow-growing syntrophic LCFA-degrading microorganisms (*Syntrophomonas* species and hydrogen-utilizing methanogens) from being washed out from the digester.

Utilizing microbial carriers presents an effective strategy to maintain microorganisms on the surface and prevent them from being washed out. Aivasidis (1989) used a porous glass sponge to retain anaerobic microorganisms, and it improved the fermentation efficiency.[14] The microbial selectivity of the carrier is dependent on its characteristics. For instance, Verrier et al. (1987) conducted experiments on the adsorption of methanogens to various polymers, demonstrating the ease of attachment of the hydrogenotrophic methanogen *M. hungatei* to hydrophilic surfaces.[15] Activated carbon has also been used as the microbial carrier, which enriched hydrolytic and fermentative bacteria and promoted volatile fatty acid (VFA) degradation.[16] However, as far as we know, carriers that adsorb syntrophic LCFA-degrading microorganisms and promote LCFA degradation have not been identified. This study aims to identify microbial carriers with superior adsorption capacities for syntrophic LCFA-degrading microorganisms. We remarked on polymers because the microbial adsorption capacity of them has not been explored except for that of methanogens.[15] First, we performed batch anaerobic digestion of lipids with the addition of various polymers and evaluated their adsorption capacity for syntrophic LCFA-degrading microorganisms. Second, using a polymer that most abundantly adsorbs syntrophic LCFA-degrading microorganisms, we further assessed its effect on LCFA degradation and the microbial community transitions during anaerobic digestion at the species level.

■ MATERIALS AND METHODS

Assessing Microbial Adsorption onto Polymers during Lipid Degradation. Anaerobic digester sludge was collected from a mesophilic food waste treatment plant (Tokyo, Japan) and incubated with 1 mL L^{-1} olive oil (NACALAI TESQUE, Inc., Kyoto, Japan) in a 2 L screwcap bottle at 35 °C. After the consumption of substrate was confirmed by the cessation of biogas generation, a batch experiment was carried out by adding 20 mL of sludge and medium into 50 mL vials. The medium consisted of 1 mL L^{-1} of trace elements and vitamin solution (NITE Biological Resource Center medium no. 398), 0.14 g L^{-1} of KH$_2$PO$_4$, 0.54 g L^{-1} of NH$_4$Cl, 0.20 g L^{-1} of MgCl$_2$·6H$_2$O, 0.15 g L^{-1} of CaCl$_2$·2H$_2$O, 2.5 g L^{-1} of NaHCO$_3$, and 0.20 g L^{-1} of yeast extract (Difco, MI, USA). Besides, 1.13 g of the following polymers was added to each vial in triplicate: poly(vinyl alcohol) (PVA, Yukigaya Chemical Industry Co., Ltd., Tokyo, Japan), polypropylene (PP, Kansai Industrial Co., Ltd., Hiroshima, Japan), polyethylene glycol (PEG, Kansai Paint Co., Ltd., Osaka, Japan), and polyvinylidene chloride (PVDC, Asahi Kasei Home Products Co., Ltd., Tokyo, Japan). The conductivity of each polymer was measured using a Loresta-AX MCP-T370 (Nittoseiko Analytech Co., Ltd., Kanagawa, Japan), and all of them exhibited no conductivity. Then 1 mL L^{-1} olive oil was added to each vial and capped with a butyl rubber stopper. The vials were purged with nitrogen gas to remove oxygen and incubated at 35 °C. Biogas produced

during incubation was collected in a sterile syringe stabbed into the butyl rubber stopper.

Chemical Analysis. Gas concentration (CH$_4$ and CO$_2$), chemical oxygen demand (COD), and VFAs were determined as in the previous report.[17] LCFA concentrations were determined using the method presented in our previous study.[18]

DNA Extraction and Amplicon Sequencing. On day 39, sludge samples and polymers were collected from each vial. Polymers were washed with sterile water to remove the sludge attached. DNA extraction was performed using a FastDNA SPIN Kit for Soil (MP Biomedicals, CA, USA) according to the manufacturer's protocol. The prokaryotic 16S rRNA gene V4 region was amplified using a two-step tailed-polymerase chain reaction (PCR) method with the primer set of 515f (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806r (5′-GGAC-TACHVGGGTWTCTAAT-3′) according to a previous study.[19] The first and second PCR products were purified using an AMPure XP (Beckman Coulter, CA, USA). The quality of the library was confirmed using a Fragment Analyzer and a dsDNA 915 Reagent kit (Agilent Technologies, CA, USA). Subsequently, the products were pooled and sequenced using a 2 × 300 bp Illumina MiSeq system.

Sequence processing and taxonomic assignments were performed using Qiime2 ver. 2021.11.[20] The primer sequences were removed using the cutadapt plugin[21] with --p-error-rate and --p-discard-untrimmed options. Then sequence processing, such as denoising, quality filtering, dereplication, chimera removal, and merging the paired-end reads, was conducted using the DADA2 plugin[22] with the parameter of --p-trim-left-f 25, --p-trim-left-r 25, --p-trunc-len-f 240, and --p-trunc-len-r 200. The obtained amplicon sequence variants (ASVs) were classified using the silva-138-99-515-806-nb-classifier.qza database using the feature-classifier plugin.[23] The Shannon index was calculated using the Qiime2 diversity plugin with a sampling depth of 62,010, which was determined by constructing rarefaction curves. The principal coordinate analysis (PCoA) was performed using the Qiime2 diversity plugin and visualized by R software package version 4. 3. 2.[24]

Further Assessment of PVDC Microbial Adsorption Capability and the Additive Effect on Lipid Degradation Efficiency. To assess the potential of PVDC as a microbial carrier retaining LCFA-degrading microorganisms in more detail, another batch experiment was performed. This experiment was performed using the same time frame with the same sludge as the one described in the previous study.[18] Briefly, 30 mL of sludge and medium were mixed and placed into a 100 mL vial. Then 3 mL L^{-1} olive oil and 2.26 g of PVDC were added. After purging with nitrogen gas, the vials were incubated at 35 °C. Thirty-six vials were prepared (18 with PVDC and 18 without PVDC) to collect the samples for LCFA analysis and DNA extraction at preset intervals (on days 0, 2, 4, 7, 12, and 31) in triplicate.

Nearly Full-Length 16S rRNA Amplicon Sequencing. DNA extraction was performed as previously mentioned above. DNA libraries were constructed based on a two-step tailed PCR with the following primer sets: Bacterial 16S rRNA gene was amplified using 27F (5′-GAGTTTGATCCTGGCT-CAG-3′) and 1492R (5′-GGCTACCTTGTTACGACTT-3′).[25] Archaeal 16S rRNA gene was amplified using A1F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGY-TACCTTGTTACGACTT-3′).[26] The first PCR amplification was performed using a KOD One PCR Master Mix
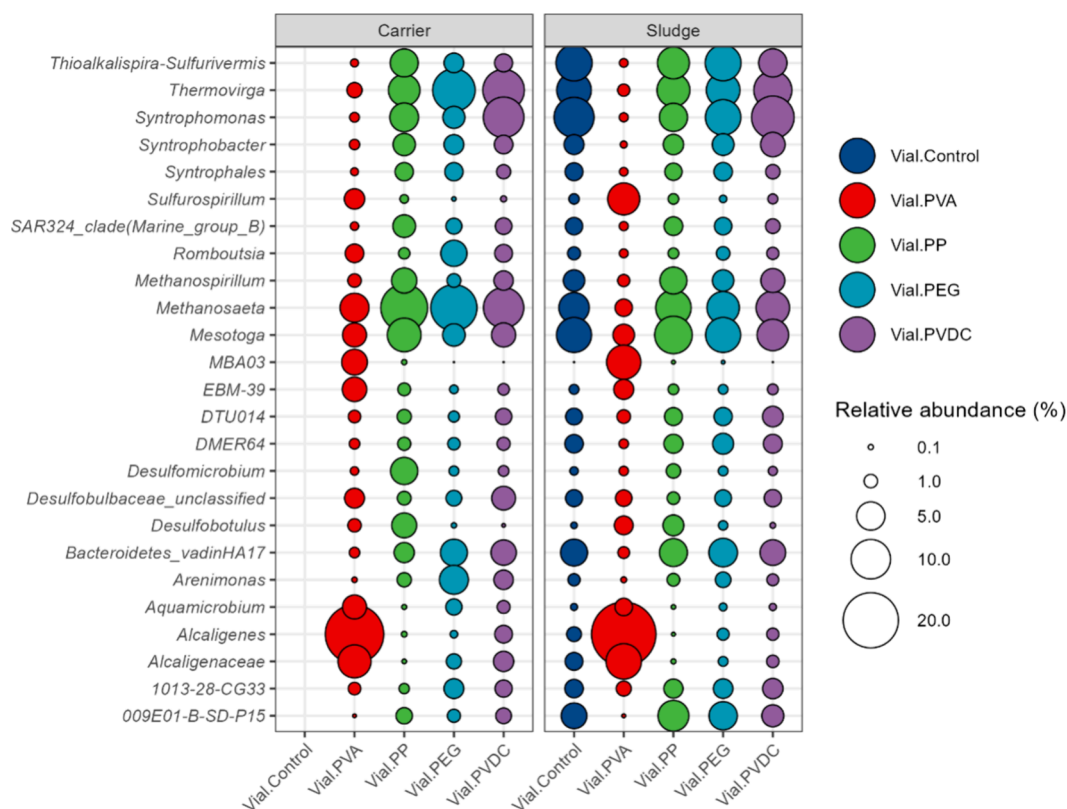
Figure 1. Relative abundances of predominant bacteria (level-6 on Qiime2 platform) in the sludge and on polymers from each vial on day 39. The circle size corresponds to the abundance of the taxa.

(TOYOBO Co., Ltd., Osaka, Japan) with the condition as follows: initial denaturation at 98 °C 1 min; 28 cycles of denaturation at 98 °C 10 s, annealing at 55 °C 5 s, and extension at 68 °C 10 s; and a final extension at 68 °C 5 min. The second PCR was performed using a KAPA HiFi DNA polymerase (NIPPON Genetics Co., Ltd., Tokyo, Japan) with the condition as follows: initial denaturation at 95 °C 3 min; eight cycles of denaturation at 95 °C 30 s, annealing at 55 °C 30 s, and extension at 72 °C 1 min; and a final extension at 72 °C 5 min. Each PCR product was purified using AMpure XP. Library was prepared using a Ligation Sequencing Kit (SQK-LSK110, Oxford Nanopore Technologies, Oxford, UK). Sequencing was carried out using GridIONx5 equipped with a MinION flow cell R10.3.

The base-calling was performed using Guppy, and then the adaptor and barcode sequences were removed using Porechop v 0.2.3 (https://github.com/rrwick/Porechop). Subsequently, 75 bp from the 5′ end of each read was trimmed, and then the reads with lengths shorter than 1300 bp or longer than 1950 bp were removed by Nanofit v 2.8.0.[27] The read quality was visualized by Nanoplot v 1.39.0.[27] Taxonomic analysis was performed by mapping the reads against the National Library of Medicine (NCBI) Refseq 16S rRNA database using Centrifuge v 1.0.4.[28] The result was visualized using Pavian.[29] The raw sequencing data sets in this study are available under the DDBJ BioProject ID of PRJDB17851.

Quantitative PCR. TaqMan-based quantitative PCR was carried out with the primer and probe sets targeting genus *Syntrophomonas*[30] and order Methanomicrobiales[31] as mentioned in our previous study.[17] The DNA extracts were assayed in triplicate in a 25 µL reaction mixture, which consisted of 12.5 µL of Probe qPCR Mix; 0.25 µL of 20 µmol L^{-1} forward

primer, reverse primer, and TaqMan probe; 2 µL of template; and 9.75 µL of sterile water. The PCR conditions for Methanomicrobiales were as follows: initial denaturation at 95 °C for 30 s followed by 40 cycles of denaturation at 95 °C for 10 s and annealing and extension at 63 °C for 30 s. The PCR conditions for *Syntrophomonas* were as follows: initial denaturation at 94 °C for 10 min followed by 35 cycles of denaturation at 94 °C 10 s, annealing at 51 °C for 5 s, and extension at 72 °C 10 s. All real-time PCR amplification and detection were carried out using a Thermal Cycler Dice real-time system (TaKaRa Bio).

■ RESULTS

The Effect of Polymer Addition on the Anaerobic LCFA Degradation. For the sake of simplicity, we mentioned the vials without a microbial carrier as Vial$_{Control}$ and the ones with PVA, PP, PEG, and PVDC as Vial$_{PVA}$, Vial$_{PP}$, Vial$_{PEG}$, and Vial$_{PVDC}$, respectively. During the 39 day incubation, $95 \pm 2\%$ of LCFAs were degraded in Vial$_{Control}$ (Table S1). The LCFA degradation rates in Vial$_{PVA}$, Vial$_{PP}$, Vial$_{PEG}$, and Vial$_{PVDC}$ were 68 ± 9, 90 ± 1, 92 ± 2, and $94 \pm 1\%$, respectively. Vial$_{PEG}$ showed a significantly lower LCFA degradation rate than other vials (Tukey HSD, $p < 0.001$). Actually, free LCFAs were detected only from Vial$_{PVA}$ on day 39. There were no significant differences in LCFA degradation rates among Vial$_{control}$, Vial$_{PP}$, Vial$_{PEG}$, and Vial$_{PVDC}$ (Tukey HSD, $p > 0.8$). The result indicated that the addition of PVA inhibited the anaerobic degradation of LCFAs.

The cumulative methane production from each vial is shown in Figure S1. During the 39 day incubation, 94 ± 8 mL of gCOD L^{-1} of methane was produced in Vial$_{Control}$. On the other hand, the methane productions in Vial$_{PVA}$, Vial$_{PP}$,
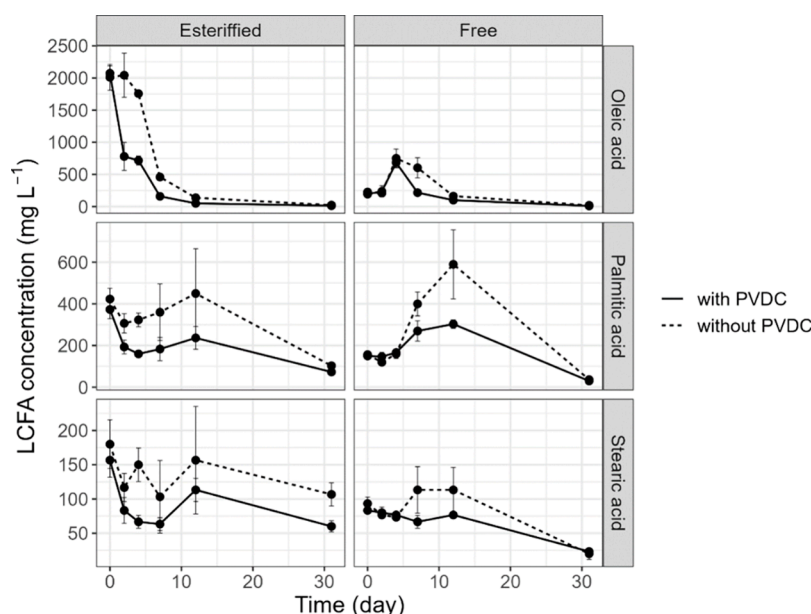
Figure 2. LCFA concentrations in the vials with and without PVDC during olive oil degradation.

Vial$_{PEG}$, and Vial$_{PVDC}$ were 4 ± 5, 42 ± 15, 102 ± 5, and 114 ± 5 mL gCOD L^{-1}, respectively. Vial$_{PVA}$ and Vial$_{PP}$ exhibited significantly lower methane productions, which were 4.6 and 45% of Vial$_{control}$ (Tukey HSD, $p < 0.005$). There were no significant differences between Vial$_{control}$ and Vial$_{PEG}$ nor Vial$_{PVDC}$ (Tukey HSD, $p > 0.2$). At least, the addition of PEG and PVDC did not inhibit the methane production.

Microbial Community Analysis. To evaluate the microbial adsorption of polymers, 16S rRNA gene amplicon sequences were analyzed from samples collected on day 39. Each sample yielded 62,010−79,881 nonchimeric reads, (average 70,201) and clustered into 280 ± 39 ASVs (Tables S2 and S3). PCoA analysis indicated the differences in the microbial community structure in the sludges from Vial$_{PVA}$, Vial$_{PP}$, and Vial$_{PEG}$ compared to those from Vial$_{Control}$ and Vial$_{PVDC}$ (Figure S2). The microbial community structures of PP, PEG, and PVDC were relatively similar. The microbial structures of the sludge from Vial$_{PVA}$ and on PVA were distinct compared to other samples. We also analyzed the alpha diversity for each sample based on the Shannon index, which ranged from 5.0 to 6.2. No significant differences were observed between the groups (Kruskal−Wallis, $p = 0.2$).

Figure 1 illustrates the detailed microbial community structures in the sludge and on the polymers from each vial. In the sludge from Vial$_{control}$, *Syntrophomonas* was the most abundant (10%) followed by Thioalkalispira-Sulfurivermis (8.3%) and *Mesotoga* (7.8%). In the sludge from Vial$_{PVA}$, *Alcaligenes* was the most abundant (27.4%) followed by unclassified Alcaligenaceae (8.0%) and *Methanosaeta* (7.4%). Similarly, on PVA, *Alcaligenes* was the most abundant (22.5%) followed by unclassified Alcaligenaceae (6.9%) and *Methanosaeta* (5.3%). The relative abundance of *Syntrophomonas* was only 0.4% in Vial$_{PVA}$ sludge and 0.5% in PVA. In the sludge from Vial$_{PP}$, *Mesotoga* was the most abundant (9.0%) followed by *Methanosaeta* (8.0%), and *Thermovirga* (6.9%). On PP, *Mathanosaeta* was the most abundant (14.2%) followed by *Mesotoga* (7.2%), *Thermovirga* (6.3%), and *Syntrophomonas* (5.2%). In Vial$_{PEG}$ sludge, Thioalkalispira-Sulfurivermis was the most abundant (8.2%) followed by *Syntrophomonas* (8.0%)

and *Mesotoga* (7.8%). On PEG, *Methanosaeta* was the most abundant (13.9%) followed by *Thermovirga* (11.7%) and *Arenimonas* (5.2%). *Syntrophomonas* occupied 2.5% of the community. In the sludge from Vial$_{PVDC}$, *Syntrophomonas* was the most abundant (11.7%) followed by *Thermovirga* (7.2%) and *Methanosaeta* (7.1%). On PVDC, *Thermovirga* was the most abundant followed by *Syntrophomonas* (10.6%) and *Methanosaeta* (10.5%). Overall, compared to other carriers, PVDC adsorbed *Syntrophomonas* at 2.1−22.2 times higher abundance. The results revealed that PVDC is the most superior microbial carrier to retain LCFA-degrading microorganisms.

Further Assessment of the Effect of PVDC Addition on Lipid Degradation. Because *Syntrophomonas* attached most abundantly on PVDC among the polymers, it was inferred that the addition of PVDC promotes the degradation of LCFAs. From this perspective, we analyzed the transitions of LCFA concentration during olive oil degradation, increasing the amount of olive oil added. Figure 2 shows the transitions of LCFA concentrations in the vials with and without PVDC. Esterified oleic acid was the main component of olive oil. On day 0, the esterified oleic acids were detected at the concentrations of 2073 ± 111 and 2010 ± 200 mg L^{-1} in the vials with PVDC and without PVDC, respectively. On day 2, their concentrations were 780 ± 216 and 2043 ± 342 mg L^{-1} in the vials with PVDC and without PVDC, respectively. A rapid reduction of approximately 1200 mg L^{-1} was observed in the vials with PVDC.

The hydrolysis of esterified LCFA produces free LCFAs as the hydrolysates. At the beginning of incubation, the concentration of oleic acid was 193 ± 31 mg L^{-1} in the PVDC-added vials and 227 ± 25 mg L^{-1} in the vials without PVDC addition. By day 4, the concentration increased to 683 ± 83 mg L^{-1} in the vials with PVDC and 750 ± 144 mg L^{-1} in the vials without PVDC. On day 7, the concentration of oleic acid was 217 ± 19 mg L^{-1} in the vials with PVDC and 603 ± 156 mg L^{-1} in the vials without PVDC. A significant decrease in oleic acid concentration was observed in the PVDC-added vials from day 4 to day 7 of incubation (t test, $p < 0.05$).
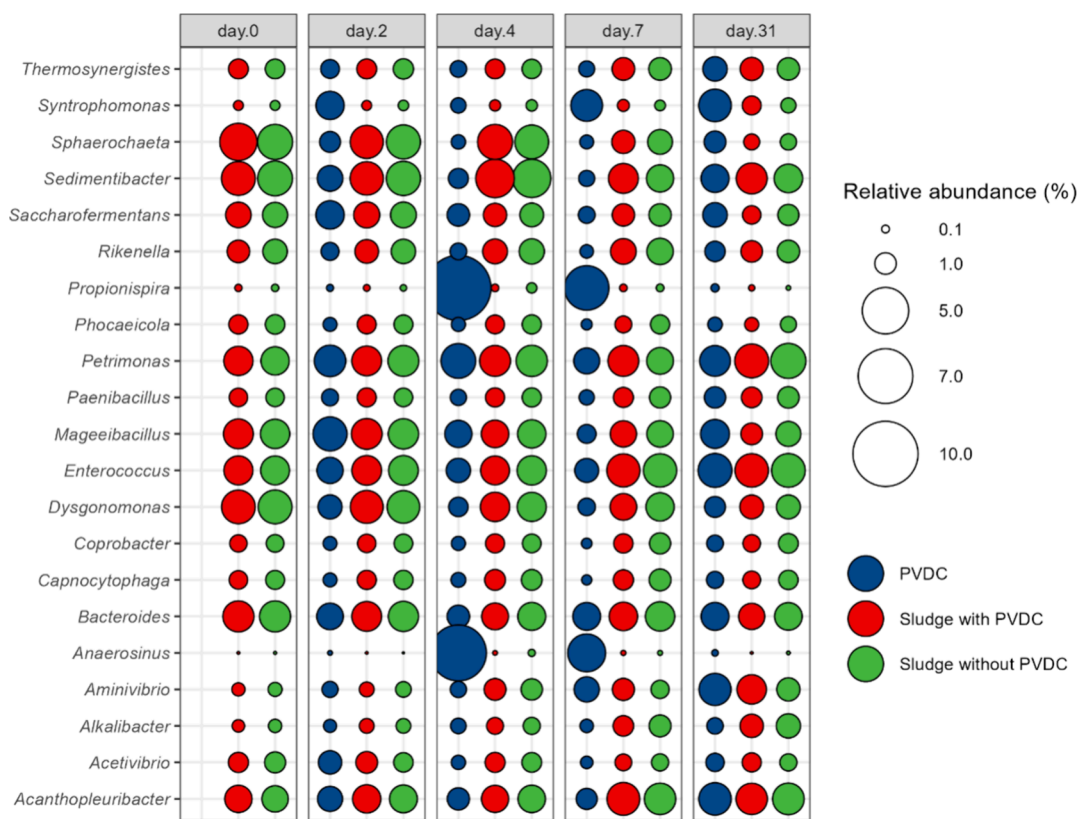
Figure 3. Relative abundances of predominant bacterial genera in the sludges with or without PVDC addition and on PVDC. The circle size corresponds to the abundances of the taxa.
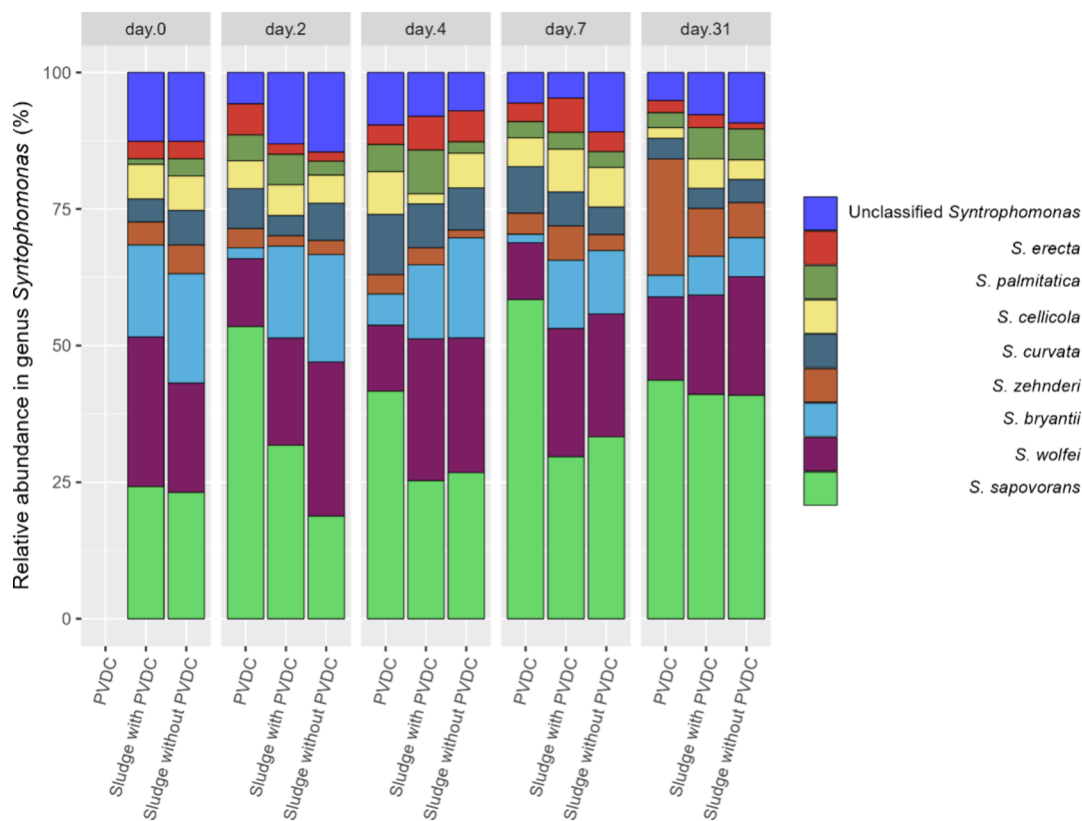

Figure 4. Compositions of genus *Syntrophomonas* in the sludges with or without PVDC addition and on PVDC.
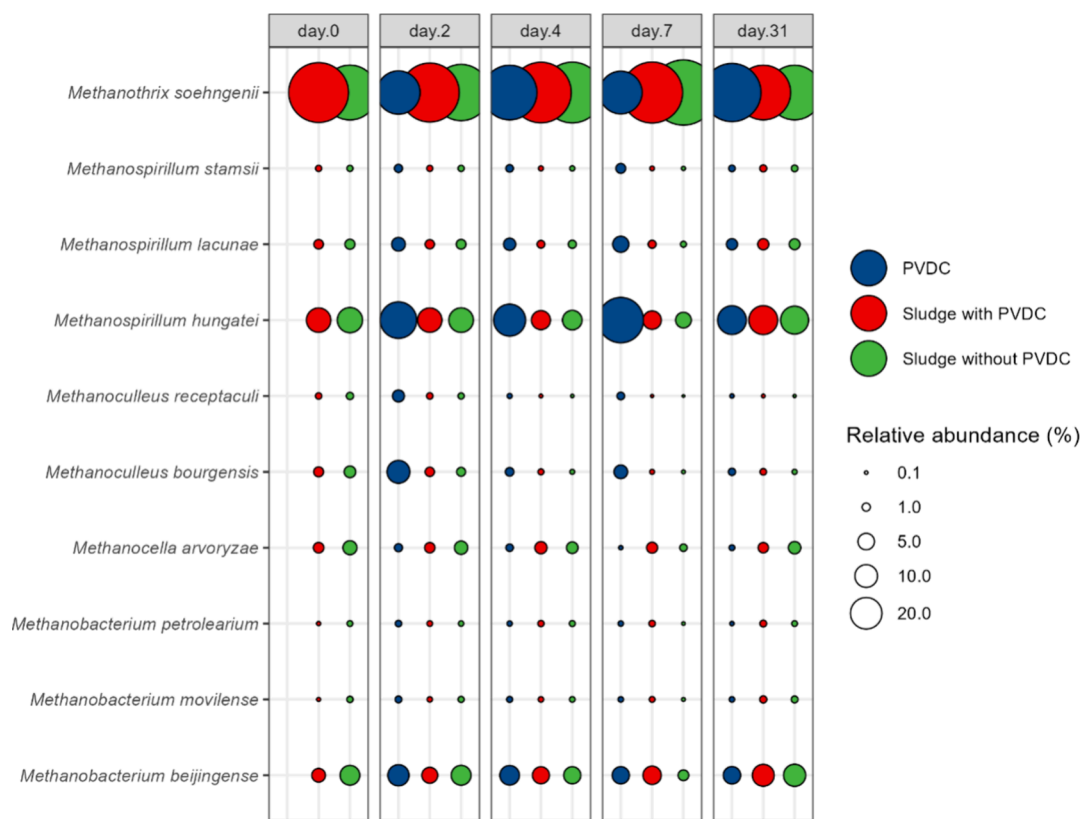
Figure 5. Relative abundances of predominant archaeal species in the sludges with or without PVDC addition and on PVDC.

Palmitic acid has been reported to be the most accumulative LCFA during anaerobic digestion,[17,32] which is generated by the hydrolysis of esterified palmitic acid or beta-oxidation. On day 0 of incubation, the concentration of palmitic acid was 150 $\pm$ 8 mg L^{-1} in the PVDC-added vials and 157 $\pm$ 12 mg L^{-1} in the vials without PVDC. Subsequently, from day 4 to day 12, an increase in palmitic acid concentration was observed in both areas. On day 4 of incubation, the concentration of palmitic acid was 167 $\pm$ 12 mg L^{-1} in the vials with PVDC and 160 $\pm$ 21 mg L^{-1} in the vials without PVDC. By day 7 of incubation, the concentrations increased to 270 $\pm$ 49 and 400 $\pm$ 57 mg L^{-1} in the vials with PVDC and without PVDC, respectively. On day 12, the concentrations further increased to 303 $\pm$ 21 mg L^{-1} in the PVDC-added vials and 590 $\pm$ 166 mg L^{-1} in the vials without PVDC. The accumulation of palmitic acid on day 12 was mitigated in the PVDC-added vials (t test, $p < 0.1$). VFA concentration monitoring revealed that acetic acid accumulation was also observed in both areas at the same period as palmitic acid accumulation (Figure S3). This acetate accumulation was also mitigated in the vials with PVDC (t test, $p < 0.05$). The cumulative methane productions in the vials with or without PVDC during incubation are shown in Figure S4. Over the 31 day incubation period, methane production in the vials with PVDC and without PVDC was 247 $\pm$ 2 and 233 $\pm$ 6 mL gCOD L^{-1}, respectively. A significantly higher methane production was observed in the PVDC-added vials (t test, $p < 0.05$). Remarkably, during days 4−12 of incubation when acetic acid accumulation was observed, methane production rates tended to improve by 35% in the PVDC-added vials (t test, $p < 0.1$). Overall, PVDC addition promoted LCFA degradation and methane production, mitigating the acetic acid accumulation.

The Transition of Microbial Community during Incubation. By the amplicon analysis using the bacterial universal primer sets, a total of 23,469−68,833 reads (average 55,299 reads) were obtained after read processing (Table S4). In all samples, 99.9% of the obtained reads were classified into the bacterial domain. The top 20 abundant bacterial genera on PVDC, the sludge with PVDC, and the sludge without PVDC are shown in Figure 3. Compared with the sludge from PVDC-added vial, the following genera were abundant on PVDC: *Anaerosinus* (4.9−101.4 times), *Propionispira* (1.2−51.3 times), and *Syntrophomonas* (1.9−10.7 times). Interestingly, both *Propionispira* and *Anaerosinus* exhibited 40 times higher relative abundances on days 4 and 7. *Syntrophomonas* is known to utilize a wide range fatty acids depending on the species.[10] To evaluate whether the dominant *Syntrophomonas* species attached to PVDC are capable of degrading LCFA, the species-level *Syntrophomonas* community structure was analyzed (Figure 4). Throughout the incubation period, *S. sapovorans* was predominant on PVDC, whereas *S. wolfei* was predominant in the sludge. Remarkably, on days 2, 4, and 7, the relative abundances of *S. sapovorans* on PVDC ranged from 41 to 58%, which was 2−3 times higher than those in the sludge. Additionally, the relative abundance of *S. zehnderi* on PVDC increased from 3.5 to 21.3% on day 31. Both *S. sapovorans* and *S. zehnderi* can degrade both unsaturated and saturated LCFAs.[12,33]

Up to here, the microbial adsorption capacity of PVDC was assessed based on a relative concept. To evaluate the absolute abundance of LCFA-degrading bacteria on PVDC, we performed qPCR targeting the 16S rRNA gene of genus *Syntrphomonas* (Figure S5). The concentrations of genus *Syntrophomonas* 16S rRNA gene in the sludge with or without

PVDC were similar throughout the incubation (t test, $p > 0.1$), suggesting that PVDC addition did not affect the absolute abundance of *Syntrophomonas* in the sludge. On day 0, the concentration of genus *Syntrophomonas* 16S rRNA gene in the sludge from PVDC-added vial was $5.78 \pm 0.50.E + 08$ copies mL^{-1}. Then on days 4, 7, 12, and 31, reduced concentrations of genus *Syntrophomonas* 16S rRNA genes were detected (Tukey HSD, $p < 0.01$). In contrast, the concentrations of genus *Syntrophomonas* 16S rRNA gene on PVDC was $1.51 \pm 0.41.E + 08$ copies mL^{-1} on day 2, and then the reduction of *Syntrophomonas* absolute abundances was not observed (Figure S5). These results suggested that PVDC effectively retained the genus *Syntrophomonas* during the lipid degradation.

As mentioned above, LCFAs are only degraded under low hydrogen partial pressure, making the presence of hydrogenotrophic methanogens crucial for LCFA degradation. Using the archaeal universal primer sets, we obtained a total of 17,833−74,604 reads (average 45,379 reads) (Table S4). Taxonomic assignment revealed that 86.2−99.3% of the obtained reads were classified into the archaeal domain. *M. soehngenii* accounted for 63−78% of the archaeal community in the sludges with or without PVDC throughout the incubation period (Figure 5). Although *M. soehngenii* was also present abundantly on PVDC, accounting for 39, 62, and 38% on days 2, 4, and 7, respectively, *M. hungatei* was also abundantly detected on PVDC. The relative abundance of *M. hungatei* on PVDC was 27, 21, and 43% on days 2, 4, and 7, respectively, which were 2.3−9.5 times higher than those in the sludge. Particularly on day 7, *M. hungatei* became the most dominant archaeal species on PVDC.

We also carried out the qPCR analysis targeting the order Methanomicrobiales that includes *M. hungatei* (Figure S6). The results indicated that the concentrations of Methanomicrobiales 16S rRNA gene in the sludge with or without PVDC were similar throughout the incubation (t test, $p > 0.1$), suggesting that PVDC addition did not affect the absolute abundance of Methanomicrobiales in the sludge. On day 2, the 16S rRNA gene of Methanomicrobiales on PVDC was $3.41 \pm 1.43.E + 09$ copies mL^{-1}, and it increased to $1.26 \pm 0.43.E + 10$, $6.53 \pm 1.41.E + 09$, and $1.05 \pm 0.27.E + 10$ copies mL^{-1} on days 4, 7, and 12, respectively. During this period, the 16S rRNA gene of Methanomicrobiales on PVDC was significantly higher than that in the sludge (t test, $p < 0.05$).

DISCUSSION

The first experiment revealed that PVDC adsorbed *Syntrophomonas* most abundantly during the 39 day incubation (Figure 1), indicating that PVDC is suitable for retaining LCFA degrading microorganisms. Vial$_{PVDC}$ exhibited one of the highest methane productions (Figure S1). Conversely, the lowest methane production in Vial$_{PVA}$ was observed. The microbial community in Vial$_{PVA}$ was distinct from the others specifically regarding the predominance of *Alcaligenes*. *Alcaligenes* is reported to oxidize methane.[34] The predominance of *Alcaligenes* in the sludge and on PVA from Vial$_{PVA}$ may have led to the inhibition of methane recovery (Figure 1). Besides, the relative abundance of *Syntrophomonas* in Vial$_{PVA}$ was extremely low (only 0.4% in the sludge and 0.5% on PVA), which possibly caused LCFA accumulation on day 39 (Table S1).

In the second experiment, the PVDC potential in terms of promoting lipid degradation and adhering to syntrophic LCFA degrading microorganisms was assessed in detail. We found

that adding PVDC promoted LCFA degradation (Figure 2). In the vial with PVDC, the esterified oleic acid concentration decreased dramatically on day 2. Meanwhile, the free oleic acid, the hydrolysate of esterified oleic acid, did not increase during this period. These results suggested that PVDC may have adsorbed olive oil during days 0−2. In addition, on PVDC, *Propionispira* and *Anaerosinus* were predominantly detected on days 4 and 7 (Figure 3). These bacteria possess capabilities of degrading glycerol, which is produced from the hydrolysis of lipids.[35,36] Besides, all detected *Anaerosinus* on PVDC was classified as *A. glycerini*, which hydrolyzes diolein[35] (Table S5). It was inferred that lipid (esterified LCFA) and glycerol degrading microorganisms such as *Propionispira* and *Anaerosinus* effectively grew on PVDC utilizing the olive oil adsorbed on PVDC. Free oleic acid and palmitic concentrations increased from day 2 to day 12 in both vials with PVDC and without PVDC. Interestingly, the rise of those concentrations was lower in the vials with PVDC, indicating that PVDC addition enabled active degradation of free LCFAs. This can be attributed to the fact that *Syntrophomonas* and hydrogenotrophic *Methanospirillum* were abundantly detected on PVDC throughout the incubation. Remarkably, the abundantly detected *Syntrophomonas* species on PVDC was *S. sapovorans*, which degrades both saturated and unsaturated LCFAs (Figure 4). The results indicated that PVDC adsorbed bacteria that are involved in lipid (esterified LCFA), glycerol, and free LCFA degradation abundantly. As mentioned above, the LCFA analysis suggested that PVDC adsorbed olive oil. That may have enhanced the localization of lipid-degrading microorganisms and their growth on PVDC by facilitating access to the substrates. PVDC addition also mitigated the acetic acid accumulation (Figure S3). Acetoclastic methanogens are reported to be susceptible to LCFA inhibition. A previous study indicated that acetate conversion to methane by acetoclastic methanogens was inhibited by 50% at 514 mg L^{-1} of palmitic acid.[37] It was suggested that the promoted LCFA degradation by the microbial community formed on PVDC mitigated the LCFA inhibition toward acetoclastic methanogens. Further studies on the mechanisms of the superior adherence of PVDC for lipid-degrading microorganisms would be required, remarking on the surface properties and chemical characteristics of both PVDC and lipid-degrading microorganisms. Overall, this study indicated that PVDC adsorbed the microorganisms that were involved in each stage of the lipid degradation process abundantly and promoted anaerobic lipid degradation significantly.

ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03998.

Cumulative methane production from each vial (Figure S1); principal coordinate analysis (PCoA) based on the ASVs from each sample; the circle size indicates the Shannon index (Figure S2); acetic acid concentrations in the vials with and without PVDC (Figure S3); cumulative methane production from the vials with and without PVDC (Figure S4); the qPCR analysis targeting genus *Syntrophomonas* (Figure S5); and the qPCR analysis targeting order Methanomicrobiales (Figure S6) (PDF)

LCFA concentrations and the degradation rates in each vial on day 39 (Table S1); summary of the statistics for DADA2 analysis (Table S2); summary of ASVs obtained in this study (Table S3); summary of statistics of gained reads after processing (Table S4); the number of reads assigned to the specific bacterial species (Table S5); and the number of reads assigned to the specific archaeal species (Table S6) (XLSX)

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Notes

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