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Anaerobic Digestion of Cigarette Butts: Microbial Community Analysis and Energy Production Estimation

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Abstract: Anaerobic digestion using cigarette butts, one of most littered items, was studied not only as a waste treatment, but also as an energy production method. Methane production from cigarette butts was measured through the biochemical methane potential (BMP) test and it was evaluated whether it is possible to produce electrical energy. Intact cigarettes or individual components (filter, paper, and leaf) were supplied as the sole carbon source (substrate) for the BMP test. The tendency of methane production indicated biodegradation in the order of paper, filter, and leaves; however, the filter of cigarettes was the substrate produced the highest amount of methane per total solid. The microbial community was also analyzed in each anaerobic digestion reactor, and substrate-specific microorganisms were identified, such as *Proteiniphilum* strain (filter) and *Methanobacterium formicicum* (paper). In intact cigarettes, the related microbial community became dominant over time in the order of paper, filter, and leaf. The conversion of cigarette butts to methane, a renewable energy source, can be proposed as a sustainable route for energy demand, for example, in a smoking room.

Keywords: cigarette butts; anaerobic digestion; microbial community; methane; waste to energy

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1. Introduction

Cigarette butts (CBs) are one of the most littered items worldwide. A report published in this regard shows that on International Coastal Cleanup Day in 2017, CBs with plastic filters accounted for the highest quantity (2.4 million), among the trash items collected (Ocean Conservancy). Damage to the ecosystem through CBs has also been reported. CBs reduce the germination and shoot length reached by grass and clover by up to 25% and reduce the amount of root biomass of clover by almost 60% [1]. This study indicated that cigarette filters made with cellulose acetate, a biobased plastic filter, may contribute to plant stress [1]. Accordingly, methods for recycling tobacco waste have been proposed. Reports have also been published regarding recycling CBs into high value materials such as cellulose pulp [2] and N-doped carbon powder [3].

However, there are few studies on CBs as an energy source through anaerobic digestion (AD). The end product of anaerobic digestion is biogas, which consists of methane and carbon dioxide. The energy content of the biogas is determined by its high content of methane. Recently, research has been conducted on a method of upgrading biogas (increasing methane composition) by converting surplus power into hydrogen through water electrolysis and using that hydrogen as a reducing agent to convert CO_2 into methane [4,5]. The conversion of tobacco to biogas was previously studied using tobacco plants [6] and stalks [7]. The objective of previous studies was primarily to treat tobacco plant waste as agriculture waste. One research reported these as littered items, and the biogas yield was 0.17–0.28 m^3 kg TS^{-1} of tobacco [8]. However, it was less than the yield obtained for agricultural residues, such as 0.55–0.62 m^3 kg TS^{-1} for rice straw [9],

0.4–1.0 m^3 kg TS^{-1} for maize straw [10], and 0.6 m^3 kg TS^{-1} for mango leaves [11]. Since CB is composed of polymers such as cellulose acetate, the first step in AD, the hydrolysis step, is rate-determining. The cellulose content of CBs has been reported to be 43.4–68.4% [8]; thus, pretreatment is required to increase the biodegradability of this waste. Other recalcitrant organic matter such as rice straw [12] and pine wood [13] also increase biodegradability and biogas production after pretreatment. Compared to the untreated corn stover, NaOH-pretreated corn stover improves anaerobic biogas production by up to 48.5% [2].

Research on the anaerobic digestion of CBs has been conducted from the perspective of waste disposal, not from the perspective of energy. In this study, the possible electricity production was predicted by measuring the methane production amount for each element of cigarette butts, and lamp lighting in a smoking room was calculated as an example to see if an energy sustainable system could be constructed through this. The objective of this study was to evaluate the potential of CBs that can be used as an energy source through anaerobic digestion and to analyze the detailed degradation process by investigating the microbial community of AD fed with CBs and individual components of CBs; filter, paper, and leaf. Finally, the energy production from AD with CBs discarded in the smoking room was estimated.

2. Materials and Methods

2.1. Details of the Tobacco Sample Used

A single Esse Prime cigarette (KT&G, South Korea), the most popular cigarette in Korea, containing 4.5 mg tar and 0.45 mg nicotine (based on information provided by the manufacturer) was used. Cigarettes are made up of filters, paper, and leaves (Figure 1). Filters are made of cellulose acetate fibers, a synthetic plastic-like substance. Cellulose acetate is not easily biodegradable and can exist in the environment for more than a decade [14]. Leaves of tobacco plants contain ammonia, volatile fatty acids (VFAs), dextrose, CaO, K_2O, SO_4^{2-}, and approximately 3000 chemical constituents [15]. Nicotine, cadmium, and lead are representative chemicals classified as harmful or potentially harmful constituents in tobacco products and tobacco smoke in cigarettes by the US Food and Drug Administration in 2012. Generally, a cigarette (0.52 g) is made of 0.08 g (15.4%) of filter, 0.07 g (13.5%) of paper, and 0.37 g (71.2%) of leaves (Figure 1). To use cigarettes as feedstock in AD reactors, intact cigarettes or individual components (filters, paper, or leaves) were chopped using a commercial blender (Figure 1). The CBs were presterilized by autoclaving at 121 °C for 15 min under 15~22 psi. The sterilization process was also a heat treatment process. Thermal treatment was selected as the pretreatment method to simulate actual smoking on unused cigarettes. After thermal pretreatment, the CBs were prepared at 50 g/L in phosphate buffer (pH 7.4, 100 mM).

2.2. BMP Test

The biochemical methane production (BMP) test was applied to evaluate methane generation from CBs [16]. The BMP test was performed in a 500 mL Duran bottle at a working volume of 200 mL. Anaerobic sludge from a municipal wastewater plant (Jungnang, Seoul, South Korea) was added as inoculum (100 mL, pH 7.4 ± 0.1, VFAS 108.3 ± 25.2 mg/L, alkalinity 5.9 ± 0.8 g/L as $CaCO_3$, TS 22.6 ± 0.02%, VS 70.7 ± 1.04%) in autoclaved bottles and 100 mL of CBs solution prepared at 50 g/L in phosphate buffer (100 mM, pH 7.4) was used. Final cigarette concentration for the BMP test was 25 g/L. The VS ratio of inoculum to substrate was between 3.2 and 4.0. After confirming that there was no indigenous biogas production, CBs were injected as the sole carbon source. To achieve anaerobic conditions, all bottles were purged with argon (Ar) gas for 30 min. All bottles were prepared in duplicates and incubated at 37 °C with mild agitation at 150 rpm. The biogas production was monitored daily using a sterile syringe, to equilibrate the pressure within the bottle with atmospheric pressure. It was run as a batch system and the experiment ended when no biogas was released. Average AD period was 43 ± 7.5 day. The biogas component was

analyzed by gas chromatography–thermal conductivity detection (GC–TCD) (6500GC System, YL Instruments, Korea), and the final biogas content was determined by multiplying the volume and fractional percentage.

$$\text{Methane production (mL)} = \text{biogas volume (mL)} \times \text{Methane fraction (\%)} \quad (1)$$

The pH and the VFAs, total carbon (TC), and total inorganic carbon (IC) content were analyzed every three days. Total organic carbon (TOC) was calculated by subtracting IC from TC.

Figure 1. Cigarette composition used for anaerobic digestion. The inserted pictures show the powder after the chopping process.

2.3. Analytical Methods

The gas composition was analyzed by GC–TCD (gas chromatography–thermal conductivity detector) with a Carboxen®-1006 PLOT capillary GC column (Supelco, L × I.D. 30 m × 0.32 mm, average thickness 15 μm) using carrier Ar (flow rate 2 mL/min) and oven, injector, and detector temperatures of 60, 230, and 230 °C, respectively. The pH was measured using a meter (HI11310, Hanna instrument, Woonsocket, RI, USA). Total organic carbon (TOC) was determined by TOC-L (total organic carbon analyzer, Shimadzu, Kyoto, Japan). Volatile fatty acid (VFAS) concentrations were measured by gas chromatography–flame ionization detection (GC–FID, 6890N, Agilent Technologies, Santa Clara, CA, USA) equipped with RESTECK Stabilwax (30 m × 0.25 μm × 0.25 μm) using carrier N_2 (flow rate 2 mL/min) with 5 m of intraguard. The temperature of the oven, injector, and detector

were 50, 250, and 250 °C. VFAS production was calculated from maximum VFAS concentration analyzed by GC–FID. Volatile solid (VS) was determined following EPA method 1684. All data are expressed as average ± standard deviation.

2.4. Modified Gompertz Model

The modified Gompertz model is one of most commonly applied kinetic models in batch tests [17].

$$V_{CH_4}(t) = P \times \exp\left\{-exp\left[\frac{R_{max}\ \exp(1)}{P}\ (\lambda - t) + 1\right]\right\} \quad (2)$$

In Equation (2), V_{CH4} (t) is the cumulative methane production (mL) at a given time t (h). R_{max} is the maximum specific methane production rate (mL/h). P is the methane production potential (mL) and λ is the lag phase time (h) [18]. Anaerobic digestion (AD) period was calculated using the modified Gompertz model equation, up to time indicating less than 10% of methane production potential. The period excluded the lag phase.

2.5. Microbial Community Analysis

DNA was extracted from 0.5 g of each sample using a Fast DNA™ Spin Kit for soil (MP Biomedicals, LLC, Solon, OH, USA), according to the manufacturer's protocol. The concentration of extracted double stranded DNA was determined using an Infinite M200 PRO microplate reader (Tecan Austria GmbH, Grödig, Austria). The DNA was then stored in a freezer at −27 °C for further analyses. Amplification of the V3-V4 region of the bacterial 16S rRNA gene was performed for each sample using 341F and 805R primers. Samples were amplified for Illumina platform using a forward and reverse fusion primer. The forward primer was constructed with the (5′–3′) Nextera consensus (TCGTCGGCAGCGTC), a sequencing adaptor (AGATGTGTATAAGAGACAG), and the appropriate forward primer selected for the bacterial diversity assay (341F: CCTACGGGNGGCWGCAG) [19]. The reverse fusion primer was constructed with the (5′–3′) Nextera consensus (GTCTCGTGGGCTCGG), a sequencing adaptor, and the appropriate reverse primer for the bacterial diversity assay (805R: GACTACHVGGGTATCTAATCC) [19]. To detect methanogen species, Arch519F (CAGCCGCCGCGGTAA) and Arch934R (GTGCTCCCCCGCCAATTC) were used as methanogen-specific primers [20]. Amplifications were performed in a reaction volume of 25 µL, containing Dr. MAX DNA polymerase (Doctor Protein Inc., Korea), 10 pmol of each primer, and 1 µL of template. Reactions were carried out using the following thermal profile: 95 °C for 3 min, then 25 cycles at 95 °C for 30 s each, 55 °C for 30 s, 72 °C for 30 s, followed by one cycle at 72 °C for 5 min. DNA sequencing was performed by ChunLab, Inc. (Seoul, Korea) using an Illumina/MiSeq platform (San Diego, CA, USA), according to the manufacturer's protocol.

Raw sequence reads from each biogas plant sample were processed, and the compositions and proportions of bacteria and methanogens in shared sets of multiple samples were analyzed using CLcommunity (Version 3.46, Chunlab Inc., Seoul, Korea). Each sequencing read was processed and taxonomically assigned using the EzTaxon e-database [21]. Typical taxonomic suffixes were "_s" for species, and "Unclassified taxons" were indicated with _uc [22]. The overall phylogenetic distance among communities was estimated using the unweighted pair group method with arithmetic mean (UPGMA) clustering [23,24]. Bacterial community distribution at the phylum and species level matrix was visualized using Circos [25]. Sequencing reads generated in this study are available in the EMBL SRA database under the accession number PRJNA664880.

3. Results and Discussion

3.1. Data for BMP Test

Methane production was normalized using the division by total solids (TS) (Figure 2). VS ratios of filter, paper, and leaves were 99.7%, 80.0%, and 86.7%, respectively (ash pictures after VS measurement are shown in Figure S1). The amount of methane was

presented as accumulated methane production from AD. Methane production with each of the cigarette constituents was as follows: filter—545.8 NmL/g TS (547.4 NmL/g VS), paper—391.7 NmL/g TS (489.6 NmL/g VS), and leaves—162.2 NmL/g TS (187.1 NmL/g VS) ($p < 0.05$, Figure 2 and Table 1). The methane production value of the whole cigarette was much less than the sum of individual methane production multiplied by the composition ratio. To simplify final energy calculations, methane production per total solids is shown. The consumption rate of VFAS can determine the residual VFAS concentration, and the measured concentration might not show actual VFAS production, but with the maximum concentration analyzed, VFAS production was the highest in leaves (820.5 mg/g TS), followed by that in paper (374.3 mg/g), and filter (132.9 mg/g). However, it refers to net production and does not represent actual total production. All VFAS produced from the total cigarette, filter and paper was consumed and finally converted to biogas, but not all VFAS produced from leaves was consumed (Figure S2). TOC removal efficiency was found to be the highest in paper (75.5%), followed by that in filters (59.4%) and leaves (50.3%) (Table 1). The time profiles of VFAS, TC, TOC, and IC are shown in Supplementary Materials as Figures S2 and S3. The VS concentration was in the order of filter (99.7%), leaves (86.7%), and paper (80.0%), and paper showed a higher organic carbon removal rate compared to the VS concentration it contained. Cellulose acetate in filters is generally regarded as a biodegradable plastic [26]. Tobacco, an agriculture and household waste, has been reported as a low methane-producing material [8]. A study on the codigestion of tobacco stalks, wheat stalks, and pig manure showed 163 L CH_4/kg VS (volatile solid) at 35 °C [7]. Another study reported 53.84 L CH_4/kg fresh tobacco, obtained for the mixture of 15% tobacco/85% water with a hydraulic retention time of 16 days [6]. Methane production from intact cigarettes was 137.8 mL/g TS, in our batch test (Table 1). The methane content was 51.4–56% (Table 1). Theoretical composition of methane in biogas is related to the mean oxidation state of carbon in the specific substrate [27]. Based on the methane content of intact cigarettes, the mean oxidation state of carbon in intact cigarettes was found to be similar to that of phenyl alanine (−0.44 carbon oxidation state, 56% CH_4) and insulin (−0.08, 51% CH_4) [27].

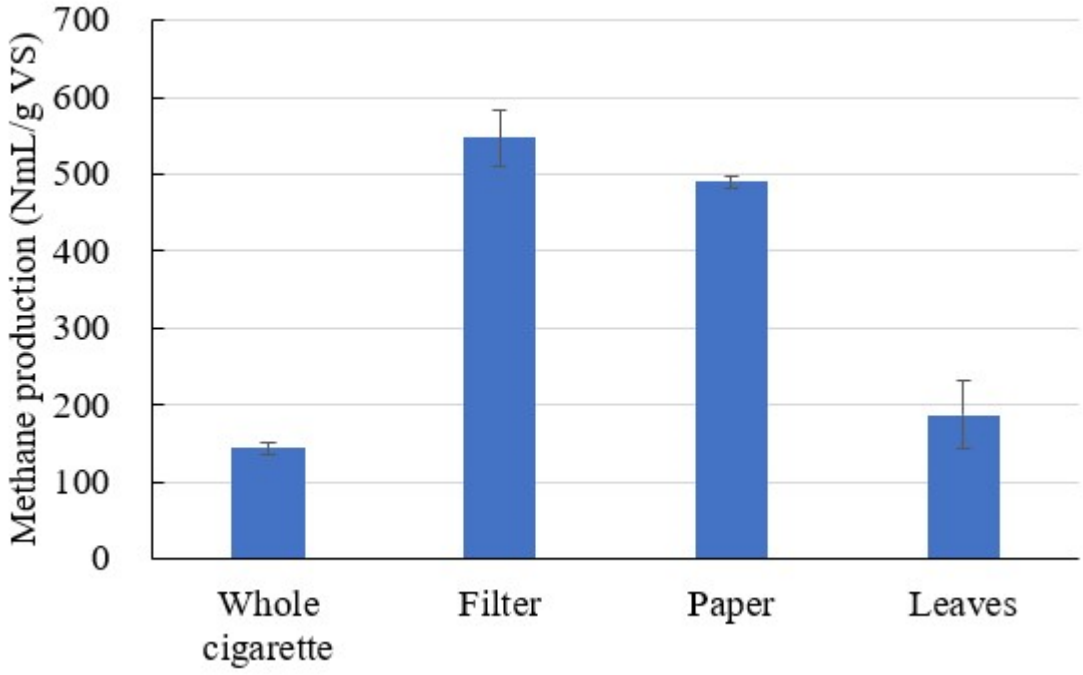


Figure 2. Amount of methane produced by each component of cigarette butts. Methane production was normalized using the division by volatile solids (VS).

Table 1. Results of anaerobic digestion using cigarette butts or individual components and kinetic parameters calculated from the modified Gompertz model. All data are expressed as mean ± standard deviation.

Results of Anaerobic Digestion	Total Methane Production		VFAS Production (mg/g TS *)	TOC Removal (%)	Methane Content (%)
	(NmL/g TS *)	(NmL/g VS)			
Whole cigarette	126.2 (±7.0)	143.8 (±7.9)	357.4 (±17.8)	53.0 (±2.6)	56.0 (±0.6)
Filter	545.8 (±36.7)	547.4 (±36.8)	132.9 (±19.3)	59.4 (±4.1)	56.6 (±1.1)
Paper	391.7 (±6.8)	489.6 (±8.5)	374.3 (±8.9)	75.5 (±3.6)	56.7 (±0.7)
Leaves	162.2 (±38.7)	187.1 (±44.6)	820.5 (±32.8)	50.3 (±6.3)	51.4 (±3.8)
Results of Modified Gompertz Model Applied	**Methane Production Potential (BMP)**		**Maximum Specific Methane Production Rate (R_{max}, mL/hr/g TS *)**	**Lag Phase Time (λ, hr)**	**AD Period ** (hr)**
	(NmL/g TS *)	**(NmL/g VS)**			
Whole cigarette	139.6	159.0	0.17	98.1	1300
Filter	405.8	407.1	0.49	225.3	875.5
Paper	237.9	297.4	0.30	50.2	864.2
Leaves	7.6	88.7	0.09	551.5	1100.8

* The results were normalized by dividing by the injected feedstock mass. The methane volume is expressed as values at room temperature (25 ± 3 °C) and atmospheric pressure. ** The period was calculated using the modified Gompertz model equation, up to the time indicating less than 10% of methane production potential (BMP) and the lag phase time was subtracted from the period. The amount of feedstock was 5 g.

To understand AD with intact cigarettes, experimental data were applied for the modified Gompertz model (see Supplementary Materials, Figure S4, for details). The methane production potential of intact cigarettes was found to be 152.4 mL/TS. The highest methane production potential of 443.0 mL/g TS was observed for the filter compartment of cigarettes. The filter of the cigarette is the part that is leftover in the highest quantity after smoking, and it contributes the most to the production of methane from CBs.

The ease of biodegradability of the substrate can be judged by the duration of the lag phase. The lag phase duration, analyzed using the modified Gompertz model, increased in the following order: paper (50.2 h), filter (225.3 h), and leaves (551.5 h). However, the lag phase shortened or disappeared with repeated batches (data not shown). The enriched microbiome could reduce the duration of the anaerobic process because an augmented microbiome could shorten a lag phase and abundant CBs-degrading microorganisms could increase the degradation rate.

3.2. Microbiome

3.2.1. Bacterial Community

Figure 3 shows the UPGMA clustering of the bacterial community in the sample fed as intact cigarettes and as individual components. The results showed that the initial bacterial community of the intact cigarette sample was similar to that of the paper-only sample, but the final community of the intact cigarette sample was similar to that of the leaf-only sample (Figure 3). Bacterial community analysis also showed that the biodegradation process occurred in the order of paper, filter, and leaf, as indicated by the methane production delay time (Table 1).

At the phylum level, *Bacteroides* dominated the bacterial community of filter and paper-fed AD, whereas *Firmicutes* dominated the leaf-fed AD (Figure 4a). *Proteiniphilum* FJ189548_s was mostly found in filter-fed AD (Figure 4b and Table 2). *Proteiniphilum* was isolated from a propionate-degrading mixture, which used yeast extract and peptone as the energy source, producing acetate and propionate [28]. *Proteiniphilum* is also found in cellulose-fed microbial fuel cells and degrades polysaccharides and xylans to simple organic compounds, such as acetate and succinate [29–32]. Bacterial community in paper-fed AD showed more diversity than in filter-fed AD (Figure 4b). Proteolytic bacteria such as *Petrimonas mucosa*, *Bacteroides graminisolvens*, and *Aminobacterium mobile* were dominant in paper-fed AD (Table 2). The isolation of *Petrimonas mucosa* and *Bacteroides graminisolvens* from biogas reactors [33,34] and *Aminobacterium mobile* from an anaerobic lagoon

has been reported [35]. *Petrimonas mucosa* has weak extracellular enzyme activity against cellulose [33]. *Bacteroides graminisolvens* utilize cellobiose, but not cellulose [34]. Proteolytic bacteria such as the *Bacillus* spp. and *Arthrobacter* spp., isolated from an agricultural waste treatment plant, are able to degrade intact feathers [36]. The protein content in coarse tobacco waste has been reported to be in the range 2.7–4.8%, whereas carbohydrates and minerals contribute 25–50% and 12–45%, respectively, to the tobacco waste [8]. However, bacterial community analysis revealed that proteolytic bacteria were dominant, irrespective of the substrate composition. The predominance of proteolytic bacteria in filter/paper-fed AD suggests that proteolytic bacteria play an important role in the AD of CBs. The abundance of proteolytic strains in a system fed with paper were reported in a metaproteomics study and suggested a possible correlation between cellulose methanation and proteolytic activity [37].

Leaf-fed AD comprised a unique bacterial community. In this sample, a variety of species were found without a largely dominant species (Figure 4b and Table 2). *Aminobacterium mobile*, *Clostridium celatum*, *Paraclostridium benzoelyticum*, *Clostridium butyricum*, *Cloacamonas acidaminovorans*, and *Escherichia coli* were found in leaf-fed AD. *Clostridium butyricum*, a fermentative bacterium, was found only in leaf-fed AD among individual leaf, paper, and filter (Table 2).

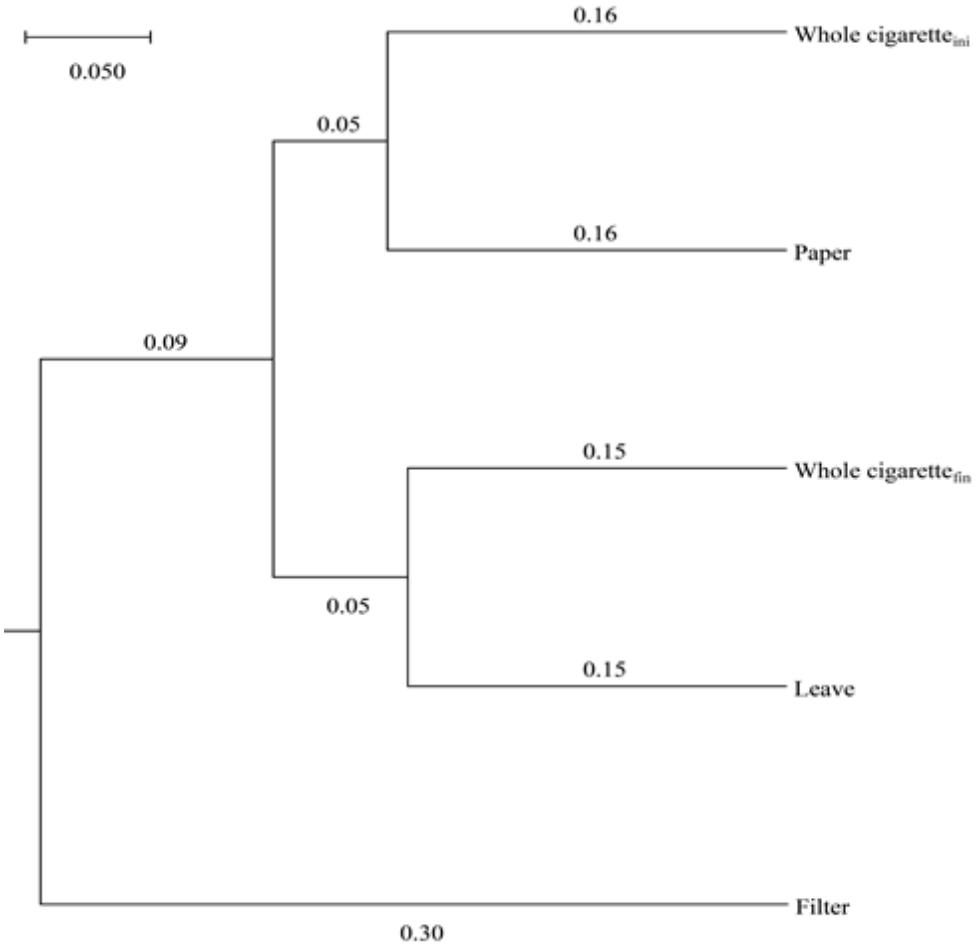
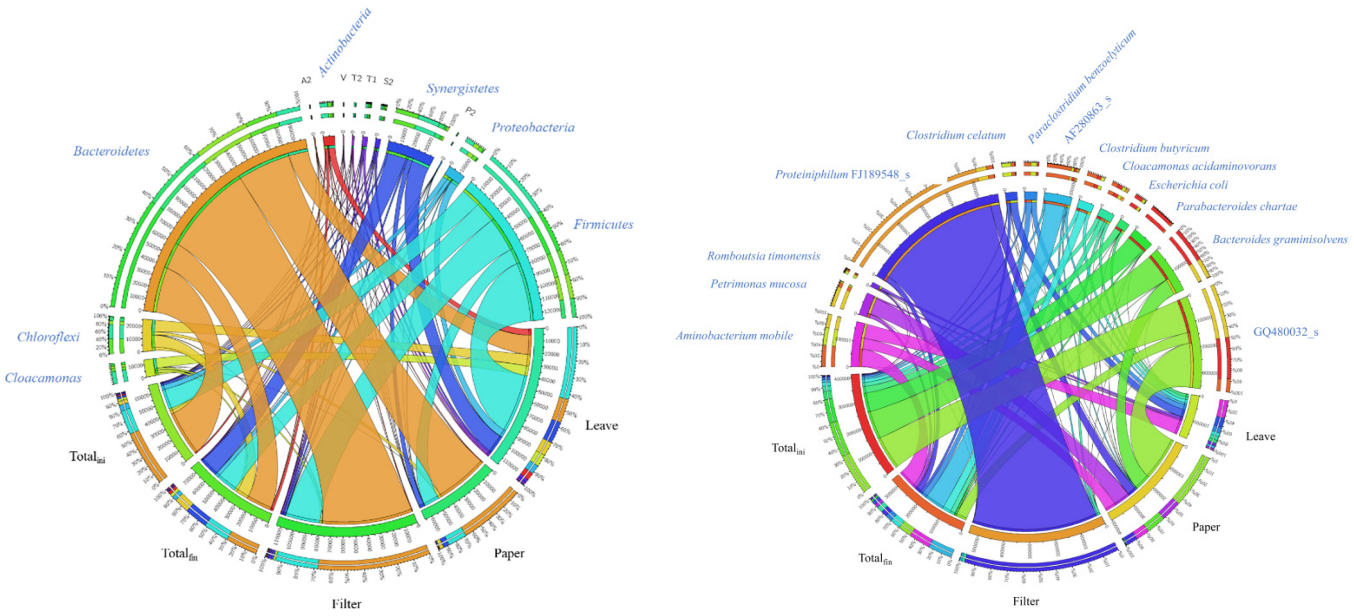


Figure 3. UPGMA clustering of bacterial microbes fed intact cigarettes or individual components for anaerobic digestion.



(**a**) (**b**)

Figure 4. Circos representation of bacterial communities from the anaerobic digestion of intact cigarettes or individual components as: (**a**) phylum level; (**b**) species level.

Table 2. Bacterial microbes (species level) in an AD reactor fed cigarette butts or individual components. Numbers represent the distribution as percentage at the species level, and the column colors indicate high distribution in the three components: pink (filter), paper (yellow), and leaf (green).

Taxon Name	Whole Cigarette$_{ini}$	Whole Cigarette$_{fin}$	Filter	Paper	Leaf	Isolation Source/Identification	Supposed Substrate
GQ480032_s	13.72	4.38	0.00	18.00	0.02	Only reported gene sequence (analysis of bacterial community changes during sewage treated process, unpublished, Genbank: GQ480032.1)	Paper
Bacteroides graminisol-vens	11.69	0.12	0.00	6.64	0.04	Xylanolytic anaerobe isolated from a methanogenic reactor treating cattle waste [34]	Paper
Parabacteroides chartae	7.86	0.68	0.00	0.06	0.02	Isolated from wastewater of a paper mill [38]	
Escherichia coli	2.72	0.11	0.00	0.11	1.08		Leaf
Cloacamonas acidaminovo-rans	1.70	1.95	1.03	0.02	1.21	Proteolytic anaerobes [39]	Filter, Leaf
Clostridium butyricum	1.32	3.42	0.00	0.00	1.30	Fermentative H_2 production [40]	Leaf
AF280863_s	0.81	8.10	0.65	0.41	0.84	Isolated from a bioreactor treating pharmaceutical wastewater [41]	

Table 2. *Cont.*

Taxon Name	Whole Cigarette$_{ini}$	Whole Cigarette$_{fin}$	Filter	Paper	Leaf	Isolation Source/Identification	Supposed Substrate
Paraclostridium benzoelyticum	0.69	2.23	0.00	0.00	2.00	Isolated from marine sediment [42]	Leaf
Clostridium celatum	0.31	1.08	0.03	0.06	3.14	Isolated from normal human feces [43]	Leaf
Proteiniphilum FJ189548_s	0.22	2.06	51.89	3.58	0.03	Belonging to *Proteiniphilum* proteolytic genus [28]	Filter, paper
Romboutsia timonensis	0.12	0.48	0.04	0.13	1.01	Isolated from human gut [44]	Leaf
Petrimonas mucosa	0.09	1.41	0.04	6.78	0.57	Isolated from anaerobic reactor fed with maize silage and pig and cattle manure [33]	Paper
Aminobacterium mobile	0.06	7.23	0.00	4.43	5.87	Amino acid-degrading bacteria [35]	Paper, leaf

3.2.2. Archaeal Community

Strains of methanogens were highly dependent on the substrate (Figure 5). Hydrogenotrophic methanogen was mostly dominant; however, in filter-fed AD, *Methanosaeta concilii*, an acetoclastic methanogen, was dominant (Figure 5 and Table 3). *Methanosaeta* spp., a potential indicator for anaerobic conversion of long-chain fatty acids to methane [45], and *Methanosaeta concilii* have been identified as the main methanogens present in a full-scale anaerobic bioreactor treating paper mill wastewater [46]. Interestingly, *Methanobacterium formicicum* was highly dominant (77.72%) in paper-fed AD (Table 3). *M. formicicum* has been found during AD in the presence of long chain fatty acids [47] even though it can utilize the low carbon such as CO_2 and formate [48] and has been described as the H_2-utilizing partner of the VFAS-degrading microbiome [49]. There was no significant difference in the types of volatile fatty acids among filter, paper, leaf-fed AD (Figure S2). The reason for the dominance of *M. formicicum* in paper-fed AD remains unknown, but the coculture of *M. formicicum* with anaerobic fungi shows high production of cellulolytic and xylanolytic enzymes [50]. In this study, *Methanobacterium congolense* was present at 22.68% in leaf-fed AD (Table 3). *Methanobacterium congolense* was earlier isolated from the AD reactor of cassava peel [51] and also found in a biogas plant with low VFAS concentration (<500 ppm) [52]. It has also been used to study the conversion of low-concentration CO_2 to CH_4 [53]. Methanogens acting on intact cigarettes were predominantly uncultured *Methanosarcina* species (denoted as *Methanosarcina_uc* in Figure 5 and Table 3). These species are capable of using various compounds (H_2/CO_2, acetate, formate/methanol) as the energy source [54,55]; hence, it is difficult to determine the main substrate in intact cigarettes.

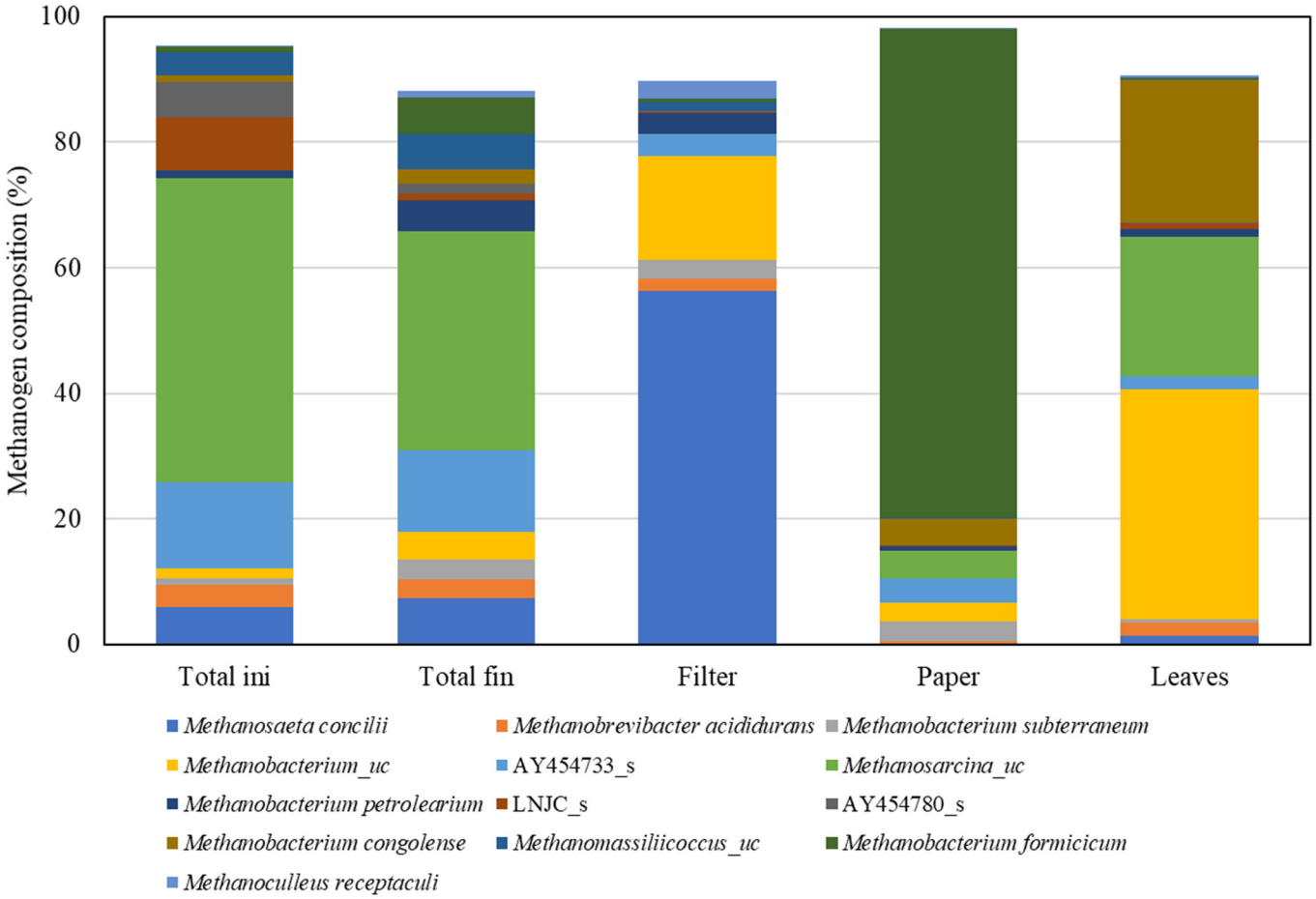


Figure 5. Methanogen distribution at the species level in anaerobic digestion reactor fed intact cigarettes or individual components.

Table 3. Methanogens in an AD reactor fed cigarette butts or individual components.

Taxon Name	Whole Cigarette$_{ini}$	Whole Cigarette$_{fin}$	Filter	Paper	Leaf	Methanogen Type
*Methanosarcina*_uc	48.35	34.92	0.80	4.40	22.08	
Methanosaeta concilii	6.02	7.40	56.30	0.10	1.45	Acetoclastic [56]
Methanobrevibacter acididurans	3.43	3.01	1.90	0.34	1.96	Hydrogenotrophic [56]
Methanobacterium_uc	1.63	4.38	16.46	2.91	36.56	
Methanobacterium petrolearium	1.27	4.86	3.33	0.76	1.29	Hydrogenotrophic [57]
Methanobacterium congolense	1.15	2.35	0.04	4.35	22.68	Hydrogenotrophic [51]
Methanobacterium subterraneum	1.06	3.18	3.06	3.31	0.60	Hydrogenotrophic [58]
Methanobacterium formicicum	0.87	5.68	0.55	77.72	0.12	Hydrogenotrophic [59]
Methanobacterium palustre	0.76	1.85	0.68	0.54	0.27	Hydrogenotrophic [60]
Methanobacterium beijingense	0.76	3.90	1.64	0.39	0.54	Hydrogenotrophic [61]
Methanoculleus receptaculi	0.23	1.11	2.81	0.00	0.32	Hydrogenotrophic [62]
Methanobrevibacter boviskoreani	0.00	0.01	0.00	0.00	2.25	Hydrogenotrophic [63]
Methanosphaera stadtmanae	0.23	0.14	0.33	0.02	2.16	Hydrogenotrophic [64]

3.3. Estimation of Energy Conversion in Methane Production from CBs

The methane production from intact cigarettes or individual components was analyzed, and the modified Gompertz model was applied to determine the methane production potential (Table 1). The values obtained were used to calculate the energy produced from the AD of CBs. (Table 4). Methane production was calculated after considering the weight of 500 CBs after smoking. The weight of each part of the cigarette thrown away after smoking was assumed as 1/10 for the leaf, 3/10 for the paper, and the filter part as it was. The methane production potential is the result of the modified Gompertz model applied to AD using individual cigarette components (Table 4). The coefficient for converting methane to energy was 9.4 Wh/L CH_4, which was used to convert methane production energy into electrical energy [65]. The median value of the power of light bulbs (32–40 W) in a smoking room; 36 W was used as the power of the lighting bulb. Based on the calculations, electrical energy produced was sufficient to light the bulb for 5.7 h (Table 4, see Supplementary Materials for detailed calculation). This result shows the possibility of waste being recycled as energy through the process of AD, together with disposing CBs, which are the most littered item.

Table 4. Energy calculation estimated from methane production from cigarette butts leftover in a smoking room.

Cigarette Butt after Smoking (g)		Assumed Waste Weight, If 500 Cigarette Butts per Day	Methane Production Potential, (mL CH_4/g TS)	Methane Production (mL CH_4)
Filter	0.08	40	443	17720
Paper	0.02	10	259.7	2597
Leaf	0.04	20	83.9	1678
Daily Methane Production (L/day)				22.0
Energy Conversion to Electricity			9.4	Wh/L methane *
			206.8	Wh
			36	W bulb
			5.7	hr

* The value was referred from IRENA report [65].

4. Conclusions

The production of methane from CBs by AD is a promising method to dispose of the most wasted items worldwide and convert this waste into energy. Particularly, it is a component discarded after smoking, and the filter made of cellulose acetate showed the most methane production per TS. Proteolytic bacteria were dominant in the AD of CBs, and the enriched microbiome rapidly produced methane after the lag period. Thus, using a local feedstock such as CBs in a smoking room, we envision a sustainable system by gaining energy from methane production through wastes.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/en14248290/s1, Figure S1: The pictures of paper, leaves, and filter after VS measurement. The photo shows the ash remaining after burning 6 g TS each at 550 °C., Figure S2: Time profile of volatile fatty acid concentrations with total CB or each component as a substrate for biochemical methane potential test., Figure S3: Time profile of total carbon (TC), total organic carbon (TOC), and inorganic carbon (IC) with total CB or each component as a substrate for biochemical methane potential test., Figure S4: The modified Gompertz model depending on the kinds of substrates as: (a) ground tobacco, (b) ground filter, (c) ground paper, (d) ground leaf. Spots show experimental values and lines indicate modeling predictions.

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References

1. Green, D.S.; Boots, B.; Da Silva Carvalho, J.; Starkey, T. Cigarette butts have adverse effects on initial growth of perennial ryegrass (gramineae: *Lolium perenne* L.) and white clover (leguminosae: *Trifolium repens* L.). *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109418. [CrossRef]
2. D'heni Teixeira, M.B.; Duarte, M.A.B.; Raposo Garcez, L.; Camargo Rubim, J.; Hofmann Gatti, T.; Suarez, P.A.Z. Process development for cigarette butts recycling into cellulose pulp. *Waste Manag.* **2017**, *60*, 140–150. [CrossRef]
3. Yu, C.; Hou, H.; Liu, X.; Han, L.; Yao, Y.; Dai, Z.; Li, D. The recovery of the waste cigarette butts for N-doped carbon anode in lithium ion battery. *Front. Mater.* **2018**, *5*. [CrossRef]
4. Baena-Moreno, F.M.; Zhang, Z.; Zhang, X.P.; Reina, T.R. Profitability analysis of a novel configuration to synergize biogas upgrading and Power-to-Gas. *Energy Convers. Manag.* **2020**, *224*, 113369. [CrossRef]
5. Collet, P.; Flottes, E.; Favre, A.; Raynal, L.; Pierre, H.; Capela, S.; Peregrina, C. Techno-economic and Life Cycle Assessment of methane production via biogas upgrading and power to gas technology. *Appl. Energy* **2017**, *192*, 282–295. [CrossRef]
6. González-González, A.; Cuadros, F.; Ruiz-Celma, A.; López-Rodríguez, F. Potential application of anaerobic digestion to tobacco plant. *Fuel* **2013**, *113*, 415–419. [CrossRef]
7. Liu, Y.; Dong, J.; Liu, G.; Yang, H.; Liu, W.; Wang, L.; Kong, C.; Zheng, D.; Yang, J.; Deng, L.; et al. Co-digestion of tobacco waste with different agricultural biomass feedstocks and the inhibition of tobacco viruses by anaerobic digestion. *Biores. Technol.* **2015**, *189*, 210–216. [CrossRef]
8. Meher, K.K.; Panchwagh, A.M.; Rangrass, S.; Gollakota, K.G. Biomethanation of tobacco waste. *Environ. Pollut.* **1995**, *90*, 199–202. [CrossRef]
9. Polprasert, C.; Edwards, P.; Rajput, V.; Pacharaprakiti, C. Integrated biogas technology in the tropics 1. Performance of small-scale digesters. *Waste Manag. Res.* **1986**, *4*, 197–213. [CrossRef]
10. Deublein, D.; Steinhauser, A. *Biogas from Waste and Renewable Resources: An Introduction*; John Wiley & Sons: Hoboken, NJ, USA, 2011.
11. Shyam, M.; Sharma, P.K. Solid-state anaerobic digestion of cattle dung and agro-residues in small-capacity field digesters. *Biores. Technol.* **1994**, *48*, 203–207. [CrossRef]
12. He, Y.; Pang, Y.; Liu, Y.; Li, X.; Wang, K. Physicochemical characterization of rice straw pretreated with sodium hydroxide in the solid state for enhancing biogas production. *Energy Fuels* **2008**, *22*, 2775–2781. [CrossRef]
13. Salehian, P.; Karimi, K.; Zilouei, H.; Jeihanipour, A.J.F. Improvement of biogas production from pine wood by alkali pretreatment. *Fuel* **2013**, *106*, 484–489. [CrossRef]
14. Benavente, M.; Arevalo Caballero, M.J.; Silvero, G.; López-Coca, I.; Escobar, V. Cellulose acetate recovery from cigarette butts. *Proceedings* **2019**, *2*, 1447. [CrossRef]
15. Leffingwell, J.C. *Chemical Constituents of Tobacco Leaf and Differences among Tobacco Types*; Elsevier: Amsterdam, The Netherlands, 2001; pp. 173–232.
16. Filer, J.; Ding, H.; Chang, S. Biochemical Methane Potential (BMP) assay method for anaerobic digestion research. *Water* **2019**, *11*, 921. [CrossRef]
17. Etuwe, C.N.; Momoh, Y.O.L.; Iyagba, E.T. Development of mathematical models and application of the modified gompertz model for designing batch biogas reactors. *Waste Biomass Valori.* **2016**, *7*, 543–550. [CrossRef]
18. Nguyen, D.D.; Chang, S.W.; Jeong, S.Y.; Jeung, J.; Kim, S.; Guo, W.; Ngo, H.H. Dry thermophilic semi-continuous anaerobic digestion of food waste: Performance evaluation, modified Gompertz model analysis, and energy balance. *Energ. Convers. Manag.* **2016**, *128*, 203–210. [CrossRef]
19. Fadrosh, D.W.; Ma, B.; Gajer, P.; Sengamalay, N.; Ott, S.; Brotman, R.M.; Ravel, J. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome* **2014**, *2*, 6. [CrossRef]
20. Song, H.; Choi, O.; Pandey, A.; Kim, Y.G.; Joo, J.S.; Sang, B.-I. Simultaneous production of methane and acetate by thermophilic mixed culture from carbon dioxide in bioelectrochemical system. *Biores. Technol.* **2019**, *281*, 474–479. [CrossRef] [PubMed]
21. Chun, J.; Lee, J.-H.; Jung, Y.; Kim, M.; Kim, S.; Kim, B.K.; Lim, Y.-W. EzTaxon: A web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2259–2261. [CrossRef]

22. Kim, O.-S.; Cho, Y.-J.; Lee, K.; Yoon, S.-H.; Kim, M.; Na, H.; Park, S.-C.; Jeon, Y.S.; Lee, J.-H.; Yi, H.; et al. Introducing EzTaxon-e: A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 716–721. [CrossRef]
23. Sneath, P.H.; Sokal, R.R. *Numerical Taxonomy. The Principles and Practice of Numerical Classification*; CABI: Wallingford, UK, 1973.
24. Hur, I.; Chun, J. A method for comparing multiple bacterial community structures from 16S rDNA clone library sequences. *J. Microbiol.* **2004**, *42*, 9–13.
25. Krzywinski, M.; Schein, J.; Birol, I.; Connors, J.; Gascoyne, R.; Horsman, D.; Jones, S.J.; Marra, M.A. Circos: An information aesthetic for comparative genomics. *Genome Res.* **2009**, *19*, 1639–1645. [CrossRef]
26. Ach, A. Biodegradable plastics based on cellulose acetate. *J. Macromol. Sci. A* **1993**, *30*, 733–740. [CrossRef]
27. Van Lier, J.B.; Mahmoud, N.; Zeeman, G. Anaerobic wastewater treatment. In *Biological Wastewater Treatment: Principles, Modelling Design*; IWA Publishing: London, UK, 2008; pp. 415–456.
28. Chen, S.; Dong, X. *Proteiniphilum acetatigenes* gen. nov., sp. nov., from a UASB reactor treating brewery wastewater. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 2257–2261. [CrossRef]
29. Hodgson, D.M.; Smith, A.; Dahale, S.; Stratford, J.P.; Li, J.V.; Grüning, A.; Bushell, M.E.; Marchesi, J.R.; Avignone Rossa, C. Segregation of the anodic microbial communities in a microbial fuel cell cascade. *Front. Microbiol.* **2016**, *7*, 699. [CrossRef] [PubMed]
30. Li, W.; Quan, X.; Chen, L.; Zheng, Y. Application of slow-release carbon sources embedded in polymer for stable and extended power generation in microbial fuel cells. *Chemosphere* **2020**, *244*, 125515. [CrossRef]
31. Toczyłowska-Mamińska, R.; Szymona, K.; Król, P.; Gliniewicz, K.; Pielech-Przybylska, K.; Kloch, M.; Logan, B.E. Evolving microbial communities in cellulose-fed microbial fuel cell. *Energies* **2018**, *11*, 124. [CrossRef]
32. Tomazetto, G.; Hahnke, S.; Wibberg, D.; Pühler, A.; Klocke, M.; Schlüter, A. *Proteiniphilum saccharofermentans* str. M3/6T isolated from a laboratory biogas reactor is versatile in polysaccharide and oligopeptide utilization as deduced from genome-based metabolic reconstructions. *Biotechnol. Rep.* **2018**, *18*, e00254. [CrossRef] [PubMed]
33. Hahnke, S.; Langer, T.; Koeck, D.E.; Klocke, M. Description of *Proteiniphilum saccharofermentans* sp. nov., *Petrimonas mucosa* sp. nov. and *Fermentimonas caenicola* gen. nov., sp. nov., isolated from mesophilic laboratory-scale biogas reactors, and emended description of the genus *Proteiniphilum*. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 1466–1475. [CrossRef]
34. Nishiyama, T.; Ueki, A.; Kaku, N.; Watanabe, K.; Ueki, K. Bacteroides graminisolvens sp. nov., a xylanolytic anaerobe isolated from a methanogenic reactor treating cattle waste. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 1901–1907. [CrossRef]
35. Baena, S.; Fardeau, M.-L.; Labat, M.; Ollivier, B.; Garcia, J.-L.; Patel, B. *Aminobacterium mobile* sp. nov., a new anaerobic amino-acid-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* **2000**, *50*, 259–264. [CrossRef]
36. Nnolim, N.E.; Okoh, A.I.; Nwodo, U.U. Proteolytic bacteria isolated from agro-waste dumpsites produced keratinolytic enzymes. *Biotechnol. Rep.* **2020**, *27*, e00483. [CrossRef] [PubMed]
37. Lü, F.; Bize, A.; Guillot, A.; Monnet, V.; Madigou, C.; Chapleur, O.; Mazéas, L.; He, P.; Bouchez, T. Metaproteomics of cellulose methanisation under thermophilic conditions reveals a surprisingly high proteolytic activity. *ISME J.* **2014**, *8*, 88–102. [CrossRef]
38. Tan, H.-Q.; Li, T.-T.; Zhu, C.; Zhang, X.-Q.; Wu, M.; Zhu, X.-F. *Parabacteroides chartae* sp. nov., an obligately anaerobic species from wastewater of a paper mill. *Int. J. Syst. Evol. Microbiol.* **2012**, *62*, 2613–2617. [CrossRef] [PubMed]
39. Pelletier, E.; Kreimeyer, A.; Bocs, S.; Rouy, Z.; Gyapay, G.; Chouari, R.; Rivière, D.; Ganesan, A.; Daegelen, P.; Sghir, A.; et al. "*Candidatus* Cloacamonas acidaminovorans": Genome sequence reconstruction provides a first glimpse of a new bacterial division. *J. Bacteriol.* **2008**, *190*, 2572–2579. [CrossRef]
40. Chen, W.-M.; Tseng, Z.-J.; Lee, K.-S.; Chang, J.-S. Fermentative hydrogen production with *Clostridium butyricum* CGS5 isolated from anaerobic sewage sludge. *Int. J. Hydrogen Energy* **2005**, *30*, 1063–1070. [CrossRef]
41. LaPara, T.M.; Nakatsu, C.H.; Pantea, L.; Alleman, J.E. Phylogenetic analysis of bacterial communities in mesophilic and thermophilic bioreactors treating pharmaceutical wastewater. *Appl. Environ. Microbiol.* **2000**, *66*, 3951–3959. [CrossRef] [PubMed]
42. Sasi Jyothsna, T.S.; Tushar, L.; Sasikala, C.; Ramana, C.V. *Paraclostridium benzoelyticum* gen. nov., sp. nov., isolated from marine sediment and reclassification of *Clostridium bifermentans* as *Paraclostridium bifermentans* comb. nov. Proposal of a new genus *Paeniclostridium* gen. nov. to accommodate *Clostridium sordellii* and *Clostridium ghonii*. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 1268–1274.
43. Hauschild, A.H.W.; Holdeman, L.V. *Clostridium celatum* sp.nov., isolated from normal human feces. *Int. J. Syst. Evol. Microbiol.* **1974**, *24*, 478–481. [CrossRef]
44. Ricaboni, D.; Mailhe, M.; Khelaifia, S.; Raoult, D.; Million, M. *Romboutsia timonensis*, a new species isolated from human gut. *New Microbes New Infect.* **2016**, *12*, 6–7. [CrossRef]
45. Silva, S.A.; Salvador, A.F.; Cavaleiro, A.J.; Pereira, M.A.; Stams, A.J.M.; Alves, M.M.; Sousa, D.Z. Toxicity of long chain fatty acids towards acetate conversion by *Methanosaeta concilii* and *Methanosarcina mazei*. *Microb. Biotechnol.* **2016**, *9*, 514–518. [CrossRef]
46. Roest, K.; Heilig, H.G.H.J.; Smidt, H.; de Vos, W.M.; Stams, A.J.M.; Akkermans, A.D.L. Community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater. *Syst. Appl. Microbiol.* **2005**, *28*, 175–185. [CrossRef]
47. Sousa, D.Z.; Salvador, A.F.; Ramos, J.; Guedes, A.P.; Barbosa, S.; Stams, A.J.M.; Alves, M.M.; Pereira, M.A. Activity and viability of methanogens in anaerobic digestion of unsaturated and saturated long-chain fatty acids. *Appl. Environ. Microbiol.* **2013**, *79*, 4239–4245. [CrossRef] [PubMed]

48. Chellapandi, P.; Bharathi, M.; Sangavai, C.; Prathiviraj, R. *Methanobacterium formicicum* as a target rumen methanogen for the development of new methane mitigation interventions: A review. *Vet. Anim. Sci.* **2018**, *6*, 86–94. [CrossRef] [PubMed]
49. Stantscheff, R.; Kuever, J.; Rabenstein, A.; Seyfarth, K.; Dröge, S.; König, H. Isolation and differentiation of methanogenic Archaea from mesophilic corn-fed on-farm biogas plants with special emphasis on the genus *Methanobacterium*. *Appl. Microbiol. Biot.* **2014**, *98*, 5719–5735. [CrossRef]
50. Teunissen, M.J.; Kets, E.P.; Op den Camp, H.J.; Huis in't Veld, J.H.; Vogels, G.D. Effect of coculture of anaerobic fungi isolated from ruminants and non-ruminants with methanogenic bacteria on cellulolytic and xylanolytic enzyme activities. *Arch. Microbiol.* **1992**, *157*, 176–182. [CrossRef] [PubMed]
51. Cuzin, N.; Ouattara, A.S.; Labat, M.; Garcia, J.L. *Methanobacterium congolense* sp. nov., from a methanogenic fermentation of cassava peel. *Int. J. Syst. Evol. Microbiol.* **2001**, *51*, 489–493. [CrossRef]
52. Tejerizo, G.T.; Kim, Y.S.; Maus, I.; Wibberg, D.; Winkler, A.; Off, S.; Pühler, A.; Scherer, P.; Schlüter, A. Genome sequence of Methanobacterium congolense strain Buetzberg, a hydrogenotrophic, methanogenic archaeon, isolated from a mesophilic industrial-scale biogas plant utilizing bio-waste. *J. Biotechnol.* **2017**, *247*, 1–5. [CrossRef]
53. Chen, X.; Ottosen, L.D.M.; Kofoed, M.V.W. How low can you go: Methane production of *Methanobacterium congolense* at low CO_2 concentrations. *Front. Bioeng. Biotechnol.* **2019**, *7*. [CrossRef]
54. Mah, R.A.; Smith, M.R.; Baresi, L. Studies on an acetate-fermenting strain of Methanosarcina. *Appl. Environ. Microbiol.* **1978**, *35*, 1174–1184. [CrossRef]
55. Mah, R.A.; Kuenen, J.G.; Quayle, J.R. Methanogenesis and Methanogenic Partnerships. *Biol. Sci.* **1982**, *297*, 599–616.
56. Patel, G.B.; Sprott, G.D. *Methanosaeta concilii* gen. nov., sp. nov. ("*Methanothrix concilii*") and *Methanosaeta thermoacetophila* nom. rev., comb. nov. *Int. J. Syst. Evol. Microbiol.* **1990**, *40*, 79–82. [CrossRef]
57. Mori, K.; Harayama, S. *Methanobacterium petrolearium* sp. nov. and *Methanobacterium ferruginis* sp. nov., mesophilic methanogens isolated from salty environments. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 138–143. [CrossRef]
58. Kotelnikova, S.; Macario, A.J.; Pedersen, K. *Methanobacterium subterraneum* sp. nov., a new alkaliphilic, eurythermic and halotolerant methanogen isolated from deep granitic groundwater. *Int. J. Syst. Bacteriol.* **1998**, *48*, 357–367. [CrossRef]
59. Battumur, U.; Yoon, Y.-M.; Kim, C.-H. Isolation and characterization of a new *Methanobacterium formicicum* KOR-1 from an anaerobic digester using pig slurry. *Asian-Aust. J. Anim. Sci.* **2016**, *29*, 586–593. [CrossRef] [PubMed]
60. Zellner, G.; Bleicher, K.; Braun, E.; Kneifel, H.; Tindall, B.J.; de Macario, E.C.; Winter, J. Characterization of a new mesophilic, secondary alcohol-utilizing methanogen, *Methanobacterium palustre* spec. nov. from a peat bog. *Arch. Microbiol.* **1988**, *151*, 1–9. [CrossRef]
61. Ma, K.; Liu, X.; Dong, X. *Methanobacterium beijingense* sp. nov., a novel methanogen isolated from anaerobic digesters. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 325–329. [CrossRef]
62. Cheng, L.; Qiu, T.L.; Li, X.; Wang, W.D.; Deng, Y.; Yin, X.B.; Zhang, H. Isolation and characterization of *Methanoculleus receptaculi* sp. nov. from Shengli oil field, China. *FEMS Microbiol. Lett.* **2008**, *285*, 65–71. [CrossRef]
63. Lee, J.H.; Kumar, S.; Lee, G.H.; Chang, D.H.; Rhee, M.S.; Yoon, M.H.; Kim, B.C. *Methanobrevibacter boviskoreani* sp. nov., isolated from the rumen of Korean native cattle. *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 4196–4201. [CrossRef] [PubMed]
64. Miller, T.L.; Wolin, M.J. *Methanosphaera stadtmaniae* gen. nov., sp. nov.: A species that forms methane by reducing methanol with hydrogen. *Arch. Microbiol.* **1985**, *141*, 116–122. [CrossRef]
65. IRENA. *Measuring Small-Scale Biogas Capacity and Production*; IRENA: Abu Dhabi, United Arab Emirates, 2016.