

Article

Effect of Activated Carbon as an Additive on the Anaerobic Digestion of Food Wastewater

Jae-Hyuk Lee ¹ , Hyo-Jin Shin ², Yeo-Bon Seo ², Jae-Seong Lee ², Jun-Hyeong Lee ³ and Young-Man Yoon ^{2,4,5,*} ¹ BKT Co., Ltd., Daejeon 34109, Republic of Korea; dlwogur0505@naver.com² Department of Plant Life and Environmental Science, Hankyong National University, Anseong 17579, Republic of Korea; shj41621@naver.com (H.-J.S.); syb0103@hanmail.net (Y.-B.S.); rornflqkq12@naver.com (J.-S.L.)³ Carbon Neutral Maneuver Center, Korea Testing Laboratory, Seoul 08389, Republic of Korea; lwmsgud@ktl.re.kr⁴ Biogas Research Center, Hankyong National University, Anseong 17579, Republic of Korea⁵ Agricultural Carbon Neutrality Project Team, Hankyong National University, Anseong 17579, Republic of Korea

* Correspondence: yyman@hknu.ac.kr; Tel.: +82-31-670-5086

Abstract

Activated carbon (AC) may improve methane production, but its performance can depend on substrate loading and incubation time. This study evaluated 60-day batch anaerobic digestion of food wastewater at 38 °C using substrate-to-inoculum (S/I) ratios of 0.5, 1.0 and 1.5 on a volatile-solids mass basis, three nominal AC particle sizes (4, 45 and 150 µm), and AC doses of 5 and 10 g/L. In the processed dataset, at S/I = 1.5 the highest day-5 methane yield was 49.9% above the control, whereas day-60 yields were lower than the corresponding controls in all AC treatments. Endpoint soluble Ni concentrations were lower in all AC treatments, and Co concentrations were generally lower. The metal pattern suggests a possible association between nutrient availability and final methane yield. The results highlight the need to assess early methane gains together with final yield and metal availability. AC plus trace-element supplementation should be evaluated in future studies before operational recommendations are made.

Keywords: food wastewater; activated carbon; anaerobic digestion; S/I ratio; direct interspecies electron transfer

1. Introduction

In 2022, approximately 4.996 million tons of food waste were generated annually in South Korea, resulting in the production of large volumes of food wastewater during treatment processes such as feed production and composting [1]. Anaerobic digestion is considered as an alternative to landfilling and drying for treating this high-moisture waste while recovering methane.

Anaerobic digestion is recognized as a key technology for achieving carbon neutrality, as it enables high-strength organic waste such as food wastewater to be processed while simultaneously allowing the recovery of energy in the form of methane [2]. Each stage of anaerobic digestion is driven by the biochemical activities of anaerobic microorganisms, which establish syntrophic (cross-feeding) relationships with one another to facilitate the sequential degradation of organic matter [3]. The most widely recognized form of syntrophy among anaerobic microorganisms is indirect interspecies electron transfer (IIET), in



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which electron carriers such as hydrogen (H_2) and formate mediate electron transfer from fermentative and acetogenic bacteria to methanogenic archaea [4]. However, IIET relies on the concentration gradients and diffusion of electron carriers such as hydrogen (H_2) and formate. Therefore, the concentration of these intermediates must be maintained at sufficiently low levels to ensure that the degradation of volatile fatty acids (VFAs), key intermediates in anaerobic digestion, remains thermodynamically favorable and that enzymatic reactions and electron transfer processes proceed efficiently [5,6]. In particular, the anaerobic digestion of rapidly hydrolyzable substrates with high organic acid contents, such as food wastewater, is susceptible to IIET inhibition and VFA accumulation, both of which can ultimately cause the digestion efficiency to deteriorate [4,7]. Consequently, numerous studies have investigated the effect of adding conductive materials to stimulate direct interspecies electron transfer (DIET) as a strategy to mitigate the deterioration of the anaerobic digestion efficiency associated with IIET limitations. DIET, mediated by conductive materials, is a direct electron exchange mechanism that does not require the diffusion of electron carriers such as H_2 and formate. Consequently, DIET offers thermodynamic advantages over IIET and promotes efficient electron transfer between syntrophic microorganisms [8]. Moreover, DIET facilitates the thermodynamically favorable degradation of VFAs and supports direct methanogenesis through the utilization of H^+ , e^- , and CO_2 generated during this process, thereby preventing the accumulation of VFAs and enhancing the overall efficiency of anaerobic digestion [9]. Conductive materials that are commonly used to promote DIET can be broadly classified into iron-based materials, such as zero-valent iron, magnetite, and hematite, and carbon-based materials, such as graphite, activated carbon, and biochar. The distinct physicochemical properties of these materials, including their electrical conductivity, particle size, and specific surface area, can significantly influence anaerobic microbial communities and the overall efficiency of anaerobic digestion [10,11]. Carbon-based conductive materials have been investigated as possible mediators of DIET. At the same time, these materials provide a large surface area that facilitates microbial attachment and biofilm formation [12,13]. In particular, the porous structure, rough surface morphology, and high biocompatibility of activated carbon promote microbial attachment. Furthermore, activated carbon functions as an electrical conduit by facilitating extracellular electron transfer by partially substituting for the conductive pili (e-pili) and cytochrome c involved in microbial electron transport [14,15]. In addition, activated carbon can accept electrons from electrogenic microorganisms, such as certain species of *Geobacter*, and subsequently transfer them to methanogens, including *Methanosarcina*, *Methanothrix* (*Methanosaeta*), and *Methanolinea* species, thereby accelerating the metabolism of VFAs to enhance the production of methane [16]. In this regard, the addition of activated carbon to food wastewater, which is characterized by high organic acid content, may alleviate the susceptibility of its anaerobic digestion stability to decline as the organic loading increases due to imbalances between the acidogenesis and methanogenesis stages. However, the effectiveness of supplementation with activated carbon in enhancing anaerobic digestion reportedly varies depending on the substrate-to-inoculum (S/I) ratio and the operating conditions associated with the addition of conductive material [15,17], thus highlighting the need for further investigation under a wider range of experimental conditions. Against this background, this study aimed to investigate the effects of supplementation with activated carbon on anaerobic digestion performance at various substrate-to-inoculum (S/I; substrate VS/inoculum VS) ratios using food wastewater, a substrate susceptible to performance deterioration during anaerobic digestion. To this end, batch anaerobic digestion experiments were conducted to evaluate the effects of the organic loading, activated carbon dosage, and particle size on the anaerobic digestion efficiency, while changes in the methanogenic community were analyzed throughout the operational period. The practical aim was to

identify loading-dependent benefits and limitations of AC addition to guide subsequent continuous-reactor studies and trace-element monitoring.

2. Materials and Methods

2.1. Substrate and Inoculum

Food wastewater (FW), generated during dewatering or washing, was collected from a food waste recycling facility in Icheon, Gyeonggi-do, Republic of Korea. Food wastewater digestate (FWD), used as the inoculum, was obtained from a mesophilic digester processing 2.5 L/day of food wastewater in Anseong, Gyeonggi-do, Republic of Korea. Their physicochemical characteristics are summarized in Table 1.

Table 1. Physicochemical characteristics of the substrate and inoculum.

Parameter	pH	TS	VS	TKN	NH ₄ ⁺ -N	COD	SCOD	Alkalinity (as CaCO ₃)	TVFAs (as Acetate)
						(mg/L)			
FW	3.54–3.60	107,744	92,478	4699	577	137,468	94,690	-	6176
FWD	8.19–8.27	14,278	7233	2680	1879	10,533	5159	10,938	-

All concentrations are in mg/L; pH is unitless. Non-pH values are arithmetic means of three measurements ($n = 3$); pH is the observed minimum–maximum range ($n = 3$). Alkalinity is expressed as CaCO₃ and TVFAs as acetate. Abbreviations are defined in the Abbreviations section.

2.2. Biochemical Methane Potential Test in a Batch Anaerobic Reactor

Biochemical methane potential (BMP) tests used 250 mL serum bottles with 125 mL inoculum at 38 °C for 60 days. S/I was the mass of substrate VS added divided by inoculum VS (g VS/g VS); substrate volumes of approximately 4.89, 9.78 and 14.67 mL gave S/I ratios of 0.5, 1.0 and 1.5. AC of nominal particle sizes 4, 45 and 150 µm (4 µm: RNDKOREA, Gwangmyeong, Republic of Korea; 45 and 150 µm: Sigma-Aldrich, St. Louis, MO, USA) was added at 5 or 10 g/L of inoculum-plus-substrate liquid volume. Table 2 lists the six AC treatments and control applied at each S/I ratio. Three bottles were prepared per condition. Inoculum-only blanks corrected baseline endogenous methane, and substrate-plus-inoculum controls contained no AC. The headspace was flushed with N₂ before sealing with butyl rubber stoppers and aluminum crimps [18].

Table 2. Experimental conditions applied at each S/I ratio (0.5, 1.0 and 1.5).

Experimental Conditions	Nominal Particle Size	AC Dose
	(µm)	(g/L)
Control	-	-
PS-1	4	5
PS-2	45	5
PS-3	150	5
PS-1	4	10
PS-2	45	10
PS-3	150	10

Biogas volume was measured by water displacement [19], and methane concentration was measured using a GC-2030 gas chromatograph (Shimadzu, Republic of Korea) with a thermal conductivity detector, a HayeSep Q column (80/100 mesh, 6 ft × 2 mm I.D.) and argon carrier gas at 40 mL/min. Measurements were made daily after manual mixing, with subsequent intervals adjusted to gas production. Gas volumes were corrected to dry standard conditions (0 °C, 1 atm). Methane yield was normalized to substrate VS added

and expressed as $\text{Nm}^3/\text{kg VS}_{\text{added}}$. MY5, MY10 and MY60 denote cumulative yields on days 5, 10 and 60. Cumulative methane volume was fitted in SigmaPlot (version 12.5, Systat Software, Inc., San Jose, CA, USA) using the four-parameter logistic function in Equation (1). The fitted curve was differentiated to obtain the methane production-rate curve in Equation (2).

$$M(t) = \frac{a-d}{1+(t/c)^b} + d \quad (1)$$

$$M'(t) = \frac{(d-a)b(t/c)^{b-1}}{c[1+(t/c)^b]^2} \quad (2)$$

Here, $M(t)$ is cumulative methane volume (mL), t is time (days), a and d are volume plateaus, b is the shape parameter and c is a time-scale parameter. R_m (mL/day) is the maximum first derivative of the fitted cumulative methane-volume curve. R_m -day (days) is the time at this maximum; the second derivative was used to locate candidate peak times.

2.3. Analytical Methods

AC specific surface area was measured by BET gas adsorption (3Flex version 3.02, Micromeritics, Norcross, GA, USA). Electrical conductivity was measured with an HPRM-FA2 powder resistivity system (HANTECH, Hwaseong, Republic of Korea) at 1000 kgf/cm^2 . Particle-size distributions were measured by laser diffraction (Malvern Panalytical, Malvern, Worcestershire, UK). The area-weighted $D_{[2,3]}$ and volume-weighted $D_{[3,4]}$ diameters and other measured properties are listed in Table 3.

Table 3. Physical properties of the conductive materials.

Conductive Material (-)	Particle Size (-)	Nominal Particle Size (μm)	Specific Surface Area ¹ (m^2/g)	Average Diameter $D_{[2,3]}$ ² (μm)	Average Diameter $D_{[3,4]}$ ³ (μm)	Conductivity (at 1000 kgf/cm^2) (S/cm)
AC ⁴	PS-1	4	910.6	3.9	5.1	0.6
	PS-2	45	1110.2	9.9	20.7	3.5
	PS-3	150	1300.4	45.3	101.6	— ⁵

All values are means of three replicates ($n = 3$). ¹ Specific surface area by BET analysis, ² Area-weighted (Sauter) mean diameter, ³ Volume-weighted (De Brouckere) mean diameter, ⁴ Activated carbon, ⁵ Out of range for resistance measurement ($10^{-4} \sim 10^7 \Omega$).

The physicochemical characteristics, including the pH, total solids (TS), volatile solids (VS), chemical oxygen demand (COD), soluble chemical oxygen demand (SCOD), total Kjeldahl nitrogen (TKN), ammonium nitrogen ($\text{NH}_4^+\text{-N}$), and alkalinity, were analyzed according to Standard Methods [20]. Total volatile fatty acids (TVFAs) were analyzed using a gas chromatograph (GC-2010, Shimadzu Scientific Korea Corp., Seoul, Republic of Korea) equipped with a flame ionization detector (FID). A BP21 column ($25 \text{ m} \times 0.32 \text{ mm} \times 0.25 \mu\text{m}$) was used for chromatographic separation, and high-purity nitrogen (N_2) was employed as the carrier gas at a total flow rate of 30 mL/min . TVFA concentrations were expressed as acetate-equivalent concentrations based on the molar mass of acetate. Trace element concentrations were determined in accordance with the Standard Methods for Water Pollution Analysis (ICP-MS method for metals) using an inductively coupled plasma mass spectrometer (ICP-MS; PlasmaQuant MS Elite, Analytik Jena GmbH+Co., Jena, Germany) [21]. After the 60-day BMP test, samples from the three BMP reactors in each condition were pooled and syringe filtered to remove suspended solids (TSS). The filtrate was analyzed three times, and the mean concentration is reported in Section 3.2.1.

2.4. Microbial Community Analysis

DNA was extracted using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) and quantified with Quant-IT PicoGreen (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Libraries were prepared following the Illumina 16 S Metagenomic Sequencing Library protocol using Arc 787F/1059R primers. PCR used input gDNA (5 ng/10 ng), 5× reaction buffer, 1 mM dNTP mix, 500 nM each primer and Herculanase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA, USA). The primer sequences, including Illumina adapter overhangs, were:

Arc 787F (5′–3′) :

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATTAGATACCCSBGTAGTCC

Arc 1059R (5′–3′) :

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCATGCACCWCCTCT

The first PCR comprised 95 °C for 3 min; 25 cycles of 95 °C, 63 °C and 72 °C for 30 s each; and 72 °C for 5 min. After AMPure bead purification (Agencourt Bioscience, Beverly, MA, USA), 10 µL of the product underwent indexing PCR with Nextera XT primers under the same conditions, but with 10 cycles. Libraries were again purified with AMPure beads, quantified by qPCR using the KAPA Library Quantification kit for Illumina platforms, assessed using TapeStation D1000 ScreenTape (Agilent Technologies, Santa Clara, CA, USA) and sequenced on an Illumina MiSeq.

2.5. Statistical Analysis

Statistical differences in methane yield between the control test and the test involving amendment with activated carbon were evaluated using analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons. All statistical analyses were performed using RStudio (R version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria). No inferential statistical tests were performed for metal concentrations. ND values were not replaced by zero.

3. Results and Discussion

3.1. Effect of Activated Carbon on Methane Production and Reaction Kinetics

Figures 1–3 show cumulative methane yields and relative changes in R_m and R_{m-day} at S/I ratios of 0.5, 1.0 and 1.5. Table 4 summarizes the yields on days 5, 10 and 60 and the kinetic indices.

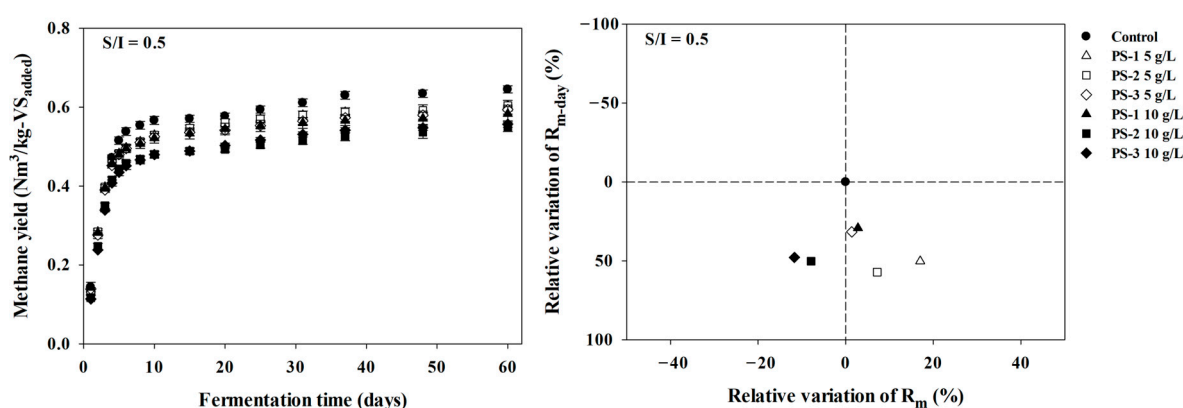


Figure 1. Methane yield and relative kinetic changes at S/I = 0.5 (Dashed lines in the right panel indicate zero change relative to the control).

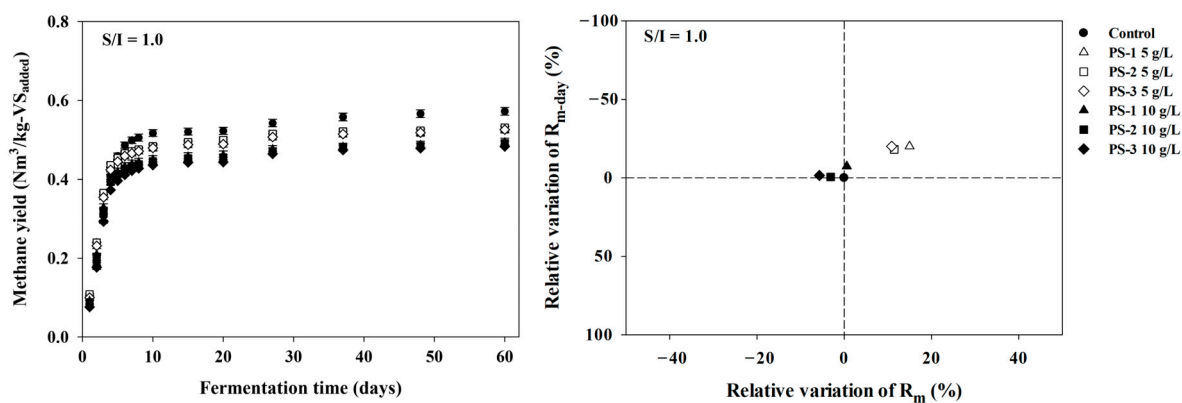


Figure 2. Methane yield and relative kinetic changes at $S/I = 1.0$ (Dashed lines in the right panel indicate zero change relative to the control).

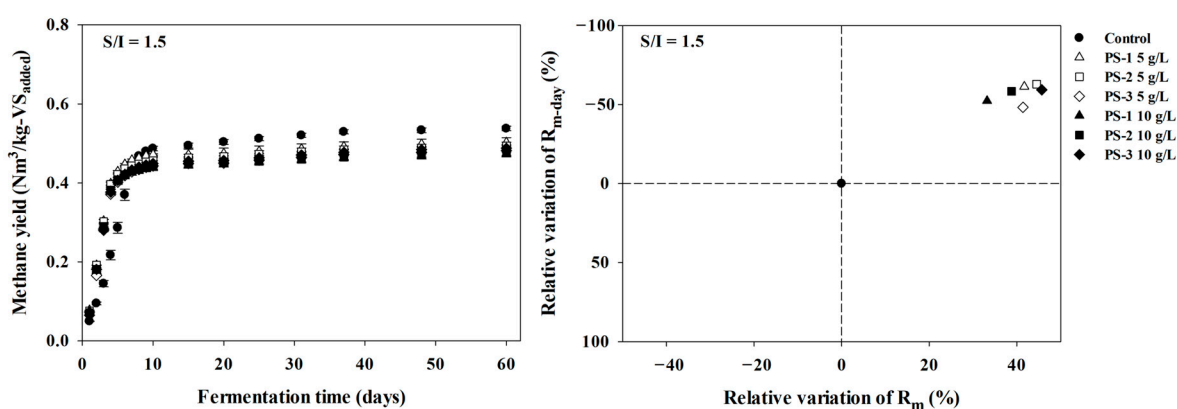


Figure 3. Methane yield and relative kinetic changes at $S/I = 1.5$ (Dashed lines in the right panel indicate zero change relative to the control).

At $S/I = 0.5$ and 1.0 , all AC treatments had lower MY5, MY10 and MY60 than their corresponding controls (Table 4). The reported R_{m-day} values were higher than the control at $S/I = 0.5$ but lower at $S/I = 1.0$. Thus, a change in peak timing should not be equated with an increase in cumulative yield. The blackwater study of Florentino et al. [22] provides a qualitative comparison, although its substrate and operating conditions differ from those used here.

At $S/I = 1.5$, AC increased MY5 from $0.286 \text{ Nm}^3/\text{kg VSadded}$ in the control to $0.402\text{--}0.429 \text{ Nm}^3/\text{kg VSadded}$. PS-1 and PS-2 at 5 g/L yielded 0.429 and $0.422 \text{ Nm}^3/\text{kg VSadded}$, corresponding to increases of 49.9% and 47.4% , respectively, calculated from unrounded yields. All AC treatments also showed higher R_m and shorter R_{m-day} than the control. For PS-1 and PS-2 at 5 g/L , R_m increased from 107.9 to 153.3 and 156.3 mL/day , respectively, while R_{m-day} decreased from 5.43 to 2.09 and 2.01 days. However, MY10 and MY60 were lower than the control for every AC treatment. The improvement therefore concerned early methane production and did not extend to the final yield.

No consistent monotonic relationship between nominal AC particle size and methane yield was observed across all S/I ratios and doses. Particle-size groups also differed in specific surface area, conductivity and, for PS-1, supplier (Table 3). The effects therefore cannot be assigned to particle size alone.

Table 4. Methane yield and reaction kinetics at S/I ratios of 0.5, 1.0, and 1.5.

S/I Ratio (-)	Particle Size (-)	AC Dose (g/L)	5 Days	Fermentation Time		Reaction Kinetics	
				10 Days (Nm ³ /kg-VS _{added})	60 Days	R _m ¹ (mL/Day)	R _{m-day} ² (Day)
0.5	Control	-	0.515 ^a	0.567 ^a	0.645 ^a	63.7	1.18
	PS-1	5	0.508 ^a	0.556 ^a	0.621 ^b	74.7	1.77
	PS-2	5	0.483 ^b	0.530 ^b	0.604 ^{bc}	68.3	1.85
	PS-3	5	0.480 ^b	0.526 ^b	0.594 ^c	64.6	1.55
	PS-1	10	0.480 ^b	0.521 ^b	0.583 ^c	65.5	1.52
	PS-2	10	0.444 ^c	0.481 ^c	0.551 ^d	58.6	1.77
1.0	PS-3	10	0.435 ^c	0.480 ^c	0.557 ^d	56.1	1.74
	Control	-	0.459 ^a	0.517 ^a	0.572 ^a	109.2	2.79
	PS-1	5	0.453 ^{ab}	0.483 ^b	0.527 ^b	125.7	2.23
	PS-2	5	0.454 ^{ab}	0.484 ^b	0.531 ^b	121.9	2.29
	PS-3	5	0.445 ^b	0.479 ^b	0.526 ^b	121.2	2.23
	PS-1	10	0.421 ^c	0.453 ^c	0.496 ^c	109.9	2.59
1.5	PS-2	10	0.414 ^c	0.445 ^{cd}	0.491 ^c	105.7	2.78
	PS-3	10	0.396 ^d	0.436 ^d	0.483 ^c	102.9	2.75
	Control	-	0.286 ^c	0.487 ^a	0.538 ^a	107.9	5.43
	PS-1	5	0.429 ^a	0.472 ^b	0.502 ^b	153.3	2.09
	PS-2	5	0.422 ^a	0.456 ^c	0.493 ^{bc}	156.3	2.01
	PS-3	5	0.402 ^b	0.443 ^d	0.482 ^{cde}	153.0	2.80
	PS-1	10	0.403 ^b	0.438 ^d	0.473 ^e	144.1	2.59
	PS-2	10	0.408 ^b	0.441 ^d	0.476 ^{de}	150.1	2.26
	PS-3	10	0.407 ^b	0.449 ^{cd}	0.488 ^{cd}	157.7	2.20

MY values are means (Nm³/kg substrate VS added). Groups with different letters indicate a significant difference ($p < 0.05$). ¹ R_m: maximum methane production rate; ² R_{m-day}: time at R_m.

3.2. Effect of Activated Carbon on Anaerobic Digestate

3.2.1. Soluble Trace Elements

Table 5 reports soluble-metal concentrations measured after the 60-day BMP test. Ni was lower in every AC treatment than in the corresponding control. Co was generally lower, with one exception: at S/I = 1.5, PS-2 at 5 g/L had 0.007 mg/L Co compared with 0.006 mg/L in the control. These endpoint differences accompanied lower MY₆₀.

Table 5. Soluble trace elements in the anaerobic digestate after 60 days at S/I ratios of 0.5, 1.0, and 1.5.

S/I Ratio (-)	Particle Size (-)	AC Dose (g/L)	Fe	Ni (mg/L)	Co	Mo
0.5	Control	-	6.940	0.362	0.013	0.027
	PS-1	5	6.823	0.188	0.003	0.022
	PS-2	5	7.041	0.223	0.004	0.023
	PS-3	5	3.847	0.059	0.004	0.040
	PS-1	10	6.230	0.082	0.004	0.023
	PS-2	10	5.552	0.122	0.004	0.027
1.0	PS-3	10	2.436	0.045	ND	0.062
	Control	-	6.869	0.354	0.012	0.021
	PS-1	5	4.592	0.183	ND	0.011
	PS-2	5	6.143	0.213	0.003	0.017
	PS-3	5	2.033	0.059	ND	0.024
	PS-1	10	3.724	0.073	ND	0.006
1.5	PS-2	10	3.687	0.174	ND	0.002
	PS-3	10	1.715	0.033	ND	0.038
	Control	-	4.477	0.272	0.006	0.005
	PS-1	5	3.869	0.206	0.003	0.010
	PS-2	5	4.943	0.245	0.007	0.009
	PS-3	5	1.471	0.060	ND	0.031
	PS-1	10	2.926	0.116	ND	0.005
	PS-2	10	1.934	0.051	ND	0.016
	PS-3	10	1.379	0.048	ND	0.013

Values are means of three analyses of a pooled sample from three BMP reactors per condition. ND: not detected.

The lower filtered Ni and generally lower Co concentrations may be associated with reduced nutrient availability and lower final methane yield. Adsorption-related nutrient limitation is proposed as a hypothesis, since filtered concentrations do not directly measure biological availability [23].

Combined AC and trace-element supplementation has shown benefits in other food-waste digestion studies [24,25]. A future comparison of AC alone and AC with Ni/Co supplementation could test the proposed nutrient-limitation hypothesis.

3.2.2. Methanogenic Community

Table 6 presents the relative abundances of five reported methanogenic taxa on days 10 and 60. On day 10, the abundance assigned to *Methanosarcina flavescens* was higher in all AC treatments than in the corresponding controls. At S/I = 0.5, it increased from 49.8% in the control to 80.5% for PS-1 at 10 g/L. This pattern did not persist in every treatment on day 60: the corresponding values were 43.6% and 28.8%. Although *Methanosarcina* has been associated with DIET [26], relative-abundance changes alone do not demonstrate absolute microbial growth or electron-transfer activity.

The relative abundance assigned to *Methanoxrithrix soehngenii* generally increased between days 10 and 60, except for PS-2 at 5 g/L and S/I = 0.5 (11.4% to 7.7%). For PS-1 at 10 g/L and S/I = 1.5, it increased from 12.0% to 34.2%. Previous AC studies have discussed mechanisms other than DIET, including syntrophic acetate oxidation and changes in hydrolysis or acetogenesis [10,27]. The observed changes may involve these alternative syntrophic mechanisms, biofilm-associated effects or hydrogen/formate-mediated interactions [28]. The integrated metagenomic and metabolomic approach reported by Sudiartha et al. [29] illustrates how functional information can complement community profiles when examining syntrophic interactions.

Table 6. Relative abundance of methanogens at S/I ratios of 0.5, 1.0, and 1.5.

S/I Ratio	Particle Size	AC Dose	Fermentation Time (Days) ¹									
			10	60	10	60	10	60	10	60	10	60
			<i>M. Flavescens</i> ²	<i>M. Soehngenii</i> ³	<i>M. Petrolearium</i> ⁴	<i>M. Stamsii</i> ⁵	<i>M. Luminyensis</i> ⁶					
(-)	(-)	(g/L)	(MM)	(AM)	(HM)	(HM)	(HM)	(HM)	(HM)	(HM)	(HM)	
0.5	Control	-	49.8	43.6	23.7	28.8	1.6	1.6	0.3	0.4	0.1	1.1
	PS-1	5	65.5	58.4	12.3	18.6	2.2	2.0	0.5	2.3	0.5	1.2
	PS-2	5	69.3	53.9	11.4	7.7	2.1	2.2	0.4	5.8	0.4	2.3
	PS-3	5	74.5	37.7	12.1	21.2	1.7	0.9	0.5	0.8	0.2	1.5
	PS-1	10	80.5	28.8	8.6	20.5	1.4	0.9	0.2	4.1	0.2	0.6
	PS-2	10	77.7	31.1	9.8	22.0	1.3	1.0	0.2	4.0	0.2	0.4
1.0	PS-3	10	65.9	28.7	10.6	23.0	1.4	1.1	0.5	2.9	0.5	0.7
	Control	-	66.0	27.3	19.7	24.3	1.3	1.3	0.5	4.8	0.1	0.8
	PS-1	5	75.4	47.1	15.4	27.7	0.9	1.9	0.4	1.3	0.1	0.5
	PS-2	5	74.4	42.4	14.3	27.7	1.1	1.6	0.4	0.6	0.3	0.5
	PS-3	5	77.8	41.7	10.9	31.7	1.1	1.5	0.9	0.9	0.4	0.4
	PS-1	10	71.2	42.0	10.3	30.9	1.1	2.2	1.2	1.0	0.8	0.4
1.5	PS-2	10	74.5	51.0	11.9	27.5	1.2	1.7	0.7	0.5	0.6	0.4
	PS-3	10	71.7	45.7	10.8	15.3	1.5	4.1	1.5	1.1	0.8	0.7
	Control	-	53.5	46.6	25.5	30.5	1.4	1.7	0.5	0.8	0.1	0.3
	PS-1	5	68.5	42.0	12.4	31.9	1.5	2.0	0.9	0.3	0.5	0.4
	PS-2	5	75.8	40.3	10.2	29.1	1.4	1.9	0.7	0.6	0.6	0.6
	PS-3	5	75.7	38.7	13.0	30.1	1.2	1.8	0.7	0.8	0.4	0.4
	PS-1	10	80.6	33.0	12.0	34.2	1.0	1.6	0.5	0.7	0.2	0.4
	PS-2	10	73.2	36.5	11.7	30.6	1.0	2.2	0.5	0.9	0.7	0.6
	PS-3	10	76.1	44.1	9.2	31.0	1.1	1.8	1.5	0.7	0.9	0.4

¹ Methanogens measured on major days (10 days and 60 days), ² *Methanosarcina flavescens* (Mixotrophic methanogen), ³ *Methanoxrithrix soehngenii* (Acetoclastic methanogen), ⁴ *Methanobacterium petrolearium* (Hydrogenotrophic methanogen), ⁵ *Methanospirillum stamsii* (Hydrogenotrophic methanogen), ⁶ *Methanomassiliicoccus luminyensis* (hydrogen-dependent methylotrophic methanogen [30]).

4. Conclusions

Descriptive comparisons of the processed data showed higher day-5 methane yield with AC at S/I = 1.5, whereas all AC treatments had lower day-60 yields than their corresponding controls. Early methane gains therefore did not translate into higher final yields. Lower filtered Ni and generally lower Co concentrations accompanied the lower final yields, suggesting a possible relationship with nutrient availability. These findings support considering early performance, final methane recovery and trace-element availability together when screening AC doses. Continuous-reactor validation is needed before full-scale application.

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Abbreviations

AC	activated carbon
AD	anaerobic digestion
BMP	biochemical methane potential
FW	food wastewater
FWD	food wastewater digestate
S/I	substrate-to-inoculum VS mass ratio
TS	total solids
VS	volatile solids
TKN	total Kjeldahl nitrogen
COD	chemical oxygen demand
SCOD	soluble COD
VFA	volatile fatty acid
TVFAs	total volatile fatty acids
DIET	direct interspecies electron transfer
IIET	indirect interspecies electron transfer
SAO	syntrophic acetate oxidation
GC	gas chromatography
TCD	thermal conductivity detector
FID	flame ionization detector
BET	Brunauer–Emmett–Teller
PSA	particle size analysis
ICP-MS	inductively coupled plasma mass spectrometry
DNA	deoxyribonucleic acid
PCR	polymerase chain reaction
qPCR	quantitative PCR

ANOVA	analysis of variance
MY5/MY10/MY60	cumulative methane yields on days 5/10/60
R _m	maximum methane production rate
R _m -day	time at R _m
ND	not detected
PS	nominal particle-size group
TSS	total suspended solids
MM	mixotrophic methanogen
AM	acetoclastic methanogen
HM	hydrogenotrophic methanogen

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