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Hydrogen and Methane Production from Food Waste in a Two-stage Mesophilic Anaerobic Digestion System Supplemented by Peanut Shell Biochar

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ABSTRACT

This paper investigated the effect of supplementing different concentrations of peanut shell biochar (PSBC) on hydrogen (H_2) and methane (CH_4) yield carried out on two-stage mesophilic anaerobic digestion system from food waste (FW). A working volume of 300 mL was incubated at 35°C for hydrogen production, while a working volume of 400 mL, supplemented with 100 mL of additional inoculum, was incubated at the same temperature for methane production. The Gompertz model was employed to analyze the changes in H_2 and CH_4 potential, production rate, and their lag time before and after supplementing peanut shell biochar (PSBC). The results showed that the reactor with biochar supplements increased the yields of H_2 by 40.99% and CH_4 by 41.36%, compared to those without biochar. Additionally, different PSBC concentrations of peanut shell biochar (3 g/L, 6 g/L and 9 g/L) were supplemented to identify optimal biochar concentration. The reactor supplemented with 3 g/L of PSBC exhibited a maximum hydrogen production rate of 27.07 mL/g VS per day and a maximum methane production rate of 40.51 mL g VS per day. Similarly, for same reactors, the average cumulative H_2 and CH_4 yield was 37.84 mL/g VS and 452.79 mL/g VS, respectively.

1. INTRODUCTION

Anaerobic digestion (AD) of food waste (FW) involves microorganisms breaking down organic matter without oxygen, resulting in biogas primarily composed of methane, carbon dioxide, and hydrogen. Kitchen waste mostly comprises FW, which is rich in organic and easily biodegradable fractions. Thus, one of the promising solutions for managing kitchen waste or FW is biogas production via anaerobic digestion [1]. A two-stage anaerobic digestion (2S-AD) system consists of two separate reactors, where, in the first stage, the complex organic compounds undergo the process of hydrolysis facilitated by hydrolytic microorganisms and enzymes such as cellulase, lipases and proteases [2],[3], followed by acidogenesis facilitated by acidogenic bacteria, mostly from *Clostridium, Bacillus* and *Enterobacter* genera, [2] to ferment and produce volatile fatty acids (VFAs), short amino acid chains and bio hydrogen (H_2) [2]. The digestate from the first stage, containing abundant VFAs and few undigested organic matters, is used in the second stage reactor where active methanogens utilize these intermediates to produce methane (CH_4) and carbon dioxide [2], [4], [5]. The hydrogen produced during acidogenesis is a transient intermediate, meaning that it is eventually utilized by other microorganisms in subsequent stages if a conventional one-stage AD system is applied, particularly during methanogenesis, where methane is the primary end-product [4]. Therefore, the conventional one-stage anaerobic digestion realizes only a fraction of the potential energy of the substrate, while it has been recognized that additional energy source can be realized in the form of hydrogen [2]. On the other hand, a 2S-AD system separates acidogenesis and methanogenesis, such that the methanogens are inactivated by preheating the inoculum in the first stage reactor [6], leading to the collection of the transient hydrogen gas while maintaining appropriate conditions [2], [4], [5], and in the second stage, reactor is enhanced for the microbial activity of methanogens, thereby the collection of methane gas from second stage reactor [2], [4], [5], [7]. In this way, two-stage anaerobic digestion systems have demonstrated better performance in terms of biomethane yield and system stability than single-stage digesters. Additionally, the efficiency of two-stage anaerobic digestion systems can be further enhanced by the addition of biochar in both reactors. Biochar is a highly porous carbonaceous material derived through pyrolysis

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of organic biomass in an oxygen-limited environment. Recent studies show biochar is being produced using agricultural residues [8], [9], solid waste [10], [11], [12], organic matter [13], [14], wood chips [15], straw, rice husks, and walnut shells [16]. Biochar has good conductivity, increased surface area, and high absorptivity. Because of these properties, biochar has been widely utilized as a low-cost adsorbent [17], [18], [19], [20] as a soil ameliorant [16], or as a catalyst carrier [21]. Recently, the increased availability of peanut shells has made them prominent candidates for biochar production [22]. Additionally, after controlled pyrolysis, peanut shell biochar (PSBC) has shown higher porosity, re-orientation of vessels, and a greater number of aromatic compounds than similar organic biomass [23]. Peanut shells, like pyrolyzed walnut shells, have a high solid and energy output, which increases their calorific value by reducing moisture and volatile components [16]. Additionally, the incorporation of biochar in anaerobic digestion has been highlighted, capitalizing on its multifaceted properties, primarily aimed at improving the efficiency and performance of the digestion process. The high porosity of PSBC can serve as a suitable habitat for microbes involved in AD [23], thereby enhancing microbial colonization and activity [23], assisting in improved breakdown of organic matter, leading to increased biogas production [24]. Inhibitory compounds present in kitchen waste, such as detergents, heavy metals, and high salt concentrations, can hinder microbial activity in an anaerobic digestion system [4], [5]. However, PSBC are adsorbents, allowing them to adsorb inhibitory compounds, thereby mitigating the negative effects of substances that could inhibit microbial activity during anaerobic digestion [25]. Similarly, the organic components in kitchen waste are easily degraded and often cause the accumulation of volatile fatty acids (VFAs) [4]. Due to VFA accumulation, the pH value drops drastically, inhibiting methanogen activity [4]. Biochar can address this issue as it can act as a buffer and stabilize pH levels in AD systems [26], maintaining an optimal pH range for the activity of methanogens [26]. As such, this research aims to study the effect of supplementing biochar derived from peanut shells on the 2S-AD process and understand how the presence of PSBC influences the overall efficiency and outcomes of the 2S-AD process for producing H_2 and CH_4.

2. METHODS AND MATERIALS

2.1 Substrate, Inoculum, and Biochar

Food Waste (FW): Food waste used as the substrate in this study was generated from Kathmandu University's (Nepal) canteen. The major component of this food was waste, rice, green vegetables, meat, oil, onion, and tomato. The collected FW was blended with a kitchen blender to homogenize the waste and was stored in sealed plastic bags at -4ºC for further use. The pH of homogenized food waste was measured using a pH probe. Total solids and volatile solids in the food waste were determined by drying the sample in an oven at 105±1°C and then incinerating it in a muffle furnace at 550°C for 1 hour.

Inoculum: Digestate from the anaerobic digestion unit of the Guheshwori municipal wastewater treatment plant (Kathmandu, Nepal) fed with primary and secondary sludge was used as inoculum. For the first stage of 2S-AD, the inoculum was thermally pretreated at 105±1℃ for 60 minutes to eliminate methanogens and create a suitable environment for hydrogen-producing bacteria [27][28]. The pretreated inoculum was kept at 25±2ºC for 30 minutes before being used. For the second stage of 2S-AD, the inoculum was used directly, without any heat treatment.

Biochar: Peanut shells were used as raw material for biochar preparation. These shells were collected from a local market in Kathmandu, Nepal. The collected shells were crushed using the ceramic grinding bowl and rod. After being crushed, the shells were passed through a 2 mm sieve to remove fines and separate shells larger than 2 mm. Only the shells greater than 2mm were used for pyrolysis. Next, the shells were air-dried in an oven at 105℃ for 24 hours. Then, the shells were filled in ceramic crucibles covered with fitting ceramic lids that act as a muffle furnace. Finally, the pyrolysis was carried out at 700 ℃ for a 60 minute residence duration [29], [30]. Thus, the obtained peanut shell biochar (PSBC) samples were stored in plastic-sealed bags until further experiments.

2.2 Two-stage Anaerobic Digestion Tests Setup

Two-stage anaerobic digestion was carried out in batch mode using 500 mL borosilicate glass reagent bottles fitted with gas-releasing outlet pipes and a flow valve. The bottles were sealed airtight to maintain anaerobic conditions. For the first stage of 2S-AD, food waste and heat-treated inoculum were fed at a ratio of 1:1 (VS basis). The working volume of 300 mL was maintained using distilled water. To examine the effect of supplementing PSBC, , batch reactors were introduced with PSBC at three different concentrations: 3, 6, and 9 g/L. The reactors were placed in a water bath maintained at 35±2℃ for 7 days until the hydrogen gas production was stopped. For the second stage of 2S-AD, 100 mL of untreated anaerobic digestate was introduced after 7 days in the same reactors. The reactors were resealed and placed in a water bath maintained at 35±2℃ for 58 days. Control groups (containing FW and inoculum, without biochar) and blank groups (containing inoculum only) were tested for reference. The experiments were carried out in triplicate. The biogas was collected for volume, and gas composition analysis was performed every day. The digestion mixtures in bottles were mixed before sampling the biogas.

2.3 Analytical Methods and Data Analysis

The water displacement method was used to measure daily volumetric hydrogen and methane production [27]. The composition of the biogases obtained from the 2S-AD tests was analyzed using a portable gas analyzer (Gasboard-3100P, Hubei Cubic-Ruiyi Instrument Co., Ltd, China). The gas produced by the blanks was subtracted, and the average values of the hydrogen and

methane were presented with error bars that showed the standard deviations.

To obtain the lag phase, maximum production potential and rate of both H_2 and CH_4 production in each treatment, the following modified Gompertz model was implemented [28].

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

Where P(t) is the cumulative gas production (mL/g VS), t is the time [hour], P_{max} is the maximum hydrogen or methane production potential [mL/g VS], R_{max} is the maximum hydrogen or methane production rate [mL/g VS per day] and λ is the lag phase [day], also referred to as the lag phase of culture in adapting to a new environment and beginning to produce hydrogen or methane [29].

The cumulative hydrogen and methane production results were fitted using the model. An analysis of variance (ANOVA) test was performed to compare the mean hydrogen and methane yields from each test using MS Excel®. In addition least squares difference (LSD) and Post hoc tests were carried out.

3. RESULTS

3.1 Properties of Substrate, Inoculum, and Biochar

The physicochemical characteristics of the blended Food Waste mixture were as follows: pH 5.7, total solids (TS) 18.72% w/w, and volatile solids (VS) 16.58% w/w.

Similarly, the physicochemical characteristics of the inoculum were as follows: pH 6.94, total solids (TS) 1.72%w/w, and volatile solids (VS) 1.21% w/w.

An ash content of 0.41% was observed for peanut shell biochar following the pyrolysis. The biochar yield was 20.49%.

3.2 Effect of PSBC Addition on Hydrogen Production in the First Stage

The potential hydrogen production from 2S-AD of FW using different concentrations of PSBC was examined. Here, Figure 1 illustrates the cumulative hydrogen production in the first-phase batch reactors of the 2S-AD system with and without PSBC addition over seven days. Figure 2 shows the daily hydrogen yield with and without PSBC addition over the seven days of operation.

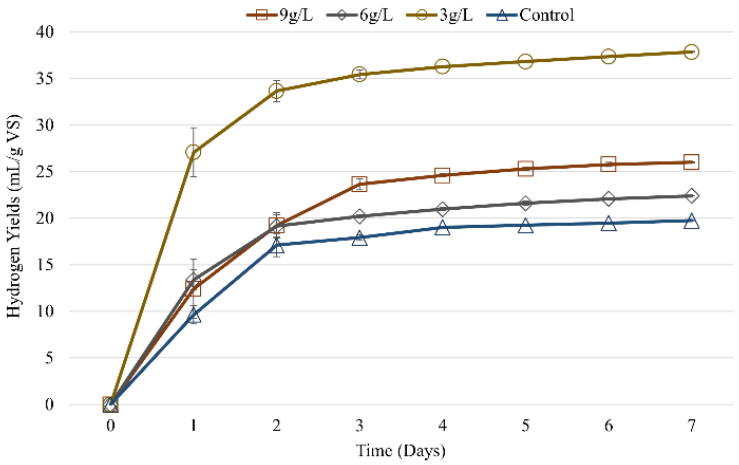

Fig. 1. Cumulative production of hydrogen during the batch tests at concentrations of PSBC 3 g/L, 6 g/L and 9 g/L, and control, respectively.

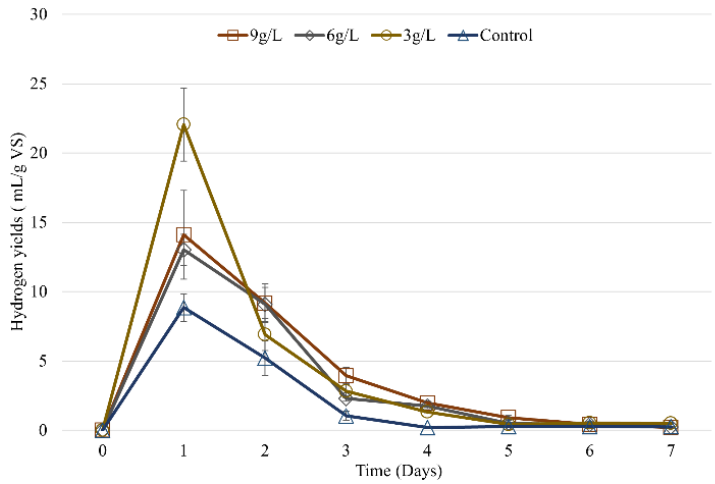

Fig. 2. Daily H_2 production during the batch tests at concentrations of PSBC 3 g/L, 6 g/L and 9 g/L, and control, respectively.

According to Figure 2, the hydrogen production started on Day 1, peaked at 27.07mL/g VS per day, and completely stopped by the 7th day. The marked increase in production rate in bottles with PSBC addition, as shown in Figure 2, suggests that the addition of PSBC to the batch reactors significantly enhanced cumulative hydrogen production compared to the reactors without PSBC addition. However, when the concentration of PSBC exceeded 3 g/L, a decline in hydrogen yield was observed. The highest hydrogen production of 37.84mL/g VS was achieved with the addition of 3 g/L PSBC.

The hydrogen composition ranged from 3% to 22% (volume basis) for 7 days of production, with a maximum cumulative hydrogen yield of 37.84 mL H_2/g VS. The remaining gas composition is mainly comprised of CO_2 and N_2. On the other hand, 0.4 - 5% of methane was detected in the gas collected from the first phase reactor, indicating that the pretreatment of inoculum by heating seemed less effective in eliminating methanogens from the culture.

The pH of all the batch reactors was at pH 7.0 at the beginning of the experiment, which dropped to 4.5 when measured after 7 days (Table 1), due to the accumulation of VFA in this first phase of the reactors. The control reactor (0 g/L PSBC) had an average pH drop of 4.91 after 7 days, with a final pH of 6.91. Reactors with 3 g/L, 6 g/L and 9 g/L of PSBC additions had average pH drops to 5.03, 4.47 and 4.67, respectively, with final pH values of 6.95, 6.93 and 7.08. This shows that the buffering effect due to PSBC was greater in the second stage compared to the first stage.

Statistical analysis was conducted to see if the addition of PSBC significantly impacted hydrogen yield. One-way analysis of variance (ANOVA) followed by post hoc test analysis was done to check the significance between the results of different groups. A statistical significance level with a (p) value of 0.05 or less indicated that at least one of the hydrogen production mean values differed from the others, while (p) value greater than 0.05 indicated statistical non-significance of the comparison. The result of the statistical analysis is presented in Table 2, which includes the difference in mean values of the final hydrogen production of any two compared cultures, standard errors, and the associated p value.

Table 1. Average pH value at the end of the first and second stage tests.

Concentration of PSBC addition (g/L)	Average pH at the end of first stage	Average pH at the end of second stage
Control (0)	4.91	6.91
3	5.03	6.95
6	4.47	6.93
9	4.67	7.08

Table 2. One-way ANOVA and post hoc analysis on cumulative hydrogen production at varying concentrations of PSBC addition.

Concentration of PSBC addition (g/L)	Average cumulative hydrogen production (mL/g VS)	Standard deviation	p value
0 (control)	19.74	3.67	<0.05
3	37.84	7.66	<0.05
6	22.39	4.26	<0.05
9	26.00	4.87	<0.05

The fitted curves for cumulative hydrogen production are presented in Figure 3. The coefficient of determination (R^2) of all of the fittings ranged from 0.991 to 0.996, which indicates a strong alignment between experimental data and the model. The experimental data of hydrogen production in each reactor were fitted with a Modified Gompertz model to predict the lag phase (λ), maximum hydrogen potential (P_{max}), and maximum hydrogen production rate (R_{max}) over time. Their predicted values are presented in Table 3.

First of all, the lag phase (λ) decreased from 0.22 days for the control to the range of 0.05 to 0.15 days upon PSBC addition. Similarly, the R_{max} of batch reactors with 3 g/L and 6 g/L of PSBC addition (15.18 - 34.16 mL/g VS per day) are higher than that of the control (12.32 mL/g VS per day). Similarly, the potential (P) of all the batch reactors with different concentrations of PSBC addition is higher than control, i.e. without the addition of PSBC. Among all, the highest potential (P) value (36.44 mL/g VS) has been reported in the batch reactor with 3 g/L of PSBC addition. In general, the batch reactors supplemented with PSBC demonstrated a progressively higher hydrogen production potential than the control reactors (batch reactors without any biochar addition) starting from day 1. However, from day 3, hydrogen production displayed marginal differences between the reactors with and without PSBC addition. Notably, the pattern of hydrogen production for the batch reactor with 3 g/L PSBC addition is higher than that of other higher concentrations of PSBC addition ratios.

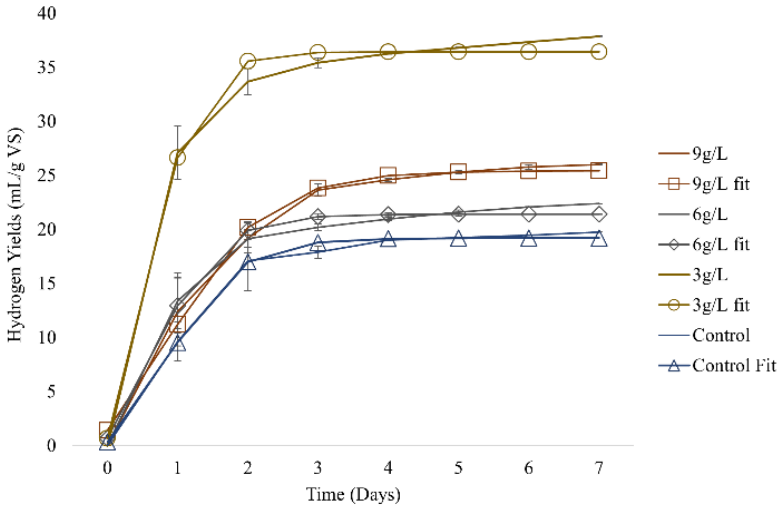

Fig. 3. Fitted curve for cumulative hydrogen production.

3.3 Effect of PSBC Addition on Methane Production in the Second Stage

Figure 4 shows the cumulative methane production in the second phase batch reactors of 2S-AD with and without PSBC addition over the sixty-five days of operation. Adding PSBC to the second phase batch reactors increased cumulative methane production compared to the batch reactors without PSBC addition. Methane production in all the second-phase batch reactors started from Day 8. However, the daily methane production rates exhibit fluctuation from Day 10 till Day 30, attaining the peak of 44.86 mL/g VS shown by a batch reactor with a PSBC concentration of 6 g/L (Figure 5). Yet, in batch reactors with PSBC concentrations of 6 g/L and 9 g/L, methane production started to decline on Day 10, contrasting with the increasing methane production observed in batch reactors with PSBC concentrations of 3 g/L and the control group (batch reactors without PBSC supplement).

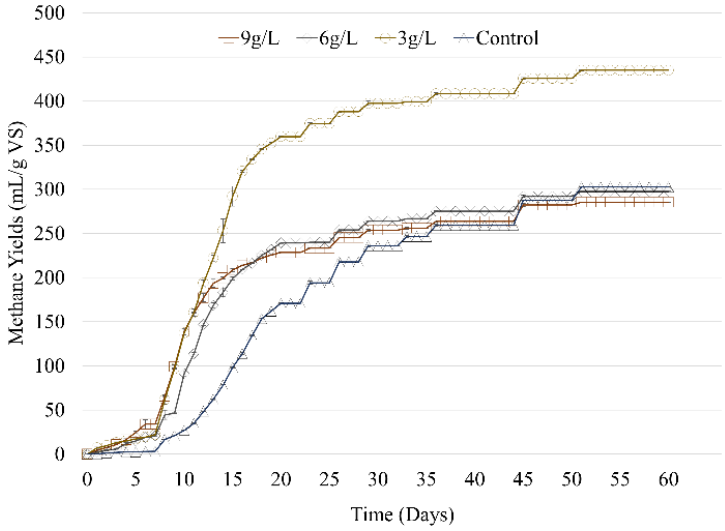

Fig. 4. Cumulative production of methane during the batch tests at concentrations of PSBC 3 g/L, 6 g/L and 9 g/L, and control, respectively.

Simultaneously, the production rate for a batch reactor with a PSBC concentration of 3 g/L attains a daily production rate in the range of 28.26 mL/g VS to 40.51 mL/g VS from Day 8 to Day 16. After sixty-five days, the highest cumulative methane production was achieved from the batch reactors with 3 g/L PSBC addition, while the control groups exhibited the lowest cumulative methane production. The ANOVA analysis of the cumulative methane production data shows a significant difference in methane production among the various treatments, as shown in Table 3. The ANOVA post hoc test suggested that the cumulative methane yields of batch reactors with 6 g/L and 9 g/L PSBC addition were not significantly different between each other and with control, but they significantly differ with the 3 g/L PSBC added test.

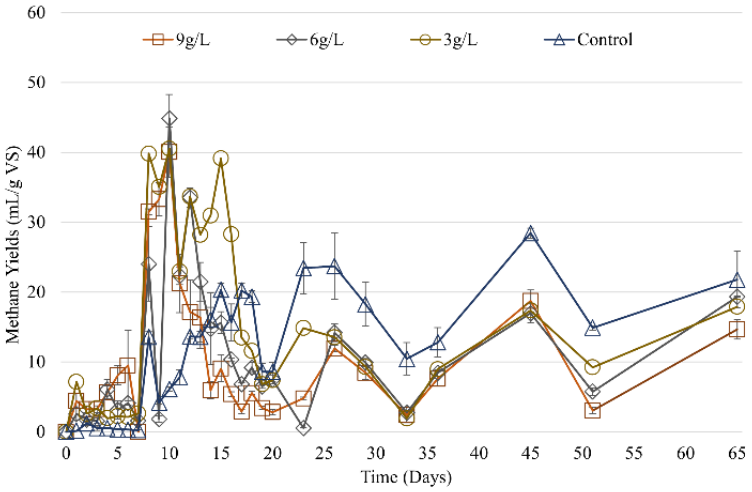

Fig. 5. Daily methane production rates during the batch tests at concentrations of PSBC 3 g/L, 6 g/L and 9 g/L, and control, respectively.

Table 3. Values of all the parameters for first phase batch reactors with varying concentrations of PSBC addition.

Concentration of PSBC addition (g/L)	λ (day)	R_{max} (mL/g VS per day)	P (mL/g VS)	R^2
Control (0)	0.22	12.32	19.21	0.99
3	0.15	34.16	36.44	0.99
6	0.12	15.18	21.41	0.99
9	0.05	11.85	25.44	0.99

The cumulative methane production experimental data were fitted in the Modified Gompertz model, as shown in Figure 6. The R^2 of all of the fit ranged from 0.97 to 0.99, suggesting a good fitting between experimental data and the model. Thus, the predicted values of lag phase (λ), maximum methane potential (P) and Maximum methane production rate (R_{max}), calculated using the Modified Gompertz model, are presented in Table 4

Table 4. One-way ANOVA and post hoc analysis on cumulative methane production varying concentrations of PSBC addition.

Concentration of PSBC addition (g/l)	Average cumulative methane production (ml/g VS)	Standard deviation	P value
0 (control)	324.46	0.52	<0.05
3	452.79	2.25	<0.05
6	317.17	1.05	<0.05
9	300.07	1.19	<0.05

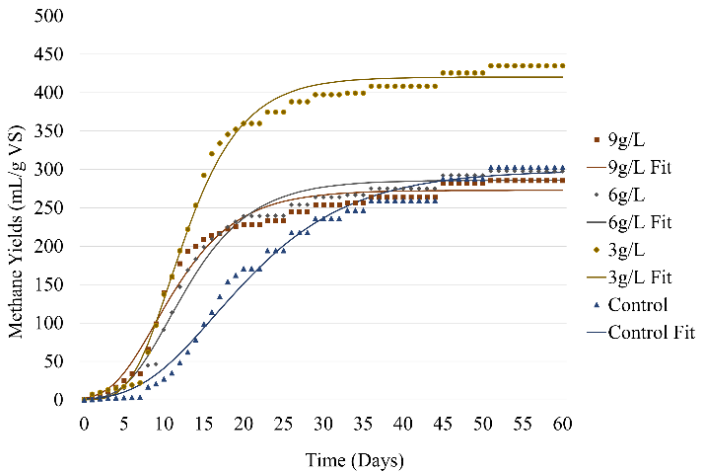

Fig. 6. Fitted curve for methane.

4. DISCUSSION

The outcome of this study establishes that the efficiency and effectiveness of a 2S-AD system, to produce both hydrogen and methane can be enhanced after the supplementation of PSBC. Biochar is well known for its capacity to enhance hydrolysis in anaerobic digester by facilitating the breakdown of complex organic matter and enabling the in-situ removal of CO_2 from biogas, thereby increasing methane concentration [30], [31], [32]. The addition of biochar also shortens the lag phase of biogas production, resulting in faster start-up of the digestion process. While biochar can promote VFA production, it also helps in accelerating their degradation, which is crucial for maintaining process stability [30], [33]. Furthermore, biochar can help buffer the pH of the AD process, which is significantly affected by VFA concentration [30], [34], [35]. Due to its large surface area and porous structure, biochar facilitates microbial colonization, supporting the growth and immobilization of bacterial cells [30], [35], [36], [37]. Additionally, being a conductive material, biochar can promote Direct Interspecies Electron Transfer (DIET) between microorganisms, such as acetogens and methanogens, which increases the microbial accumulation of genes related to certain methanogens that facilitate methane formation [35], [38]. Besides various benefits of biochar in anaerobic digestion, its properties-such as pyrolysis temperature, size, and dosage require optimization to achieve maximum performance [35], [39], [40].

The hydrogen and methane yields obtained from this study are comparable to the data reported in the existing literature, which typically utilized FW, glucose, OFMSW, and farm feedstocks as feedstocks [34], [37], [41], [42], [43], supporting our establishment. Table 5 compares the results of this study with 2S-AD studies done previously. The result of this study seems comparable to previous studies done using FW. However, the difference in H_2 and CH_4 yields suggests the difference in FW composition, *i.e.* content of carbohydrates, soluble sugars, protein, and lipid content, which may have impacted the production of H_2 and CH_4. For instance, a study by [44] using batch mesophilic conditions reported a higher H_2 yield but a lower CH_4 yield compared to our study. Conversely, another study [45] using continuous mesophilic conditions with FW and brown water achieved significantly higher yields of both H_2 and CH_4. This underscores the impact of feedstock composition and process conditions on biogas production. In addition, the variations in process types, such as batch versus continuous and mesophilic versus thermophilic conditions, also contribute to the differences in yields. For example, the recirculation DF with thermophilic DF and mesophilic AD reported by [46] yielded 135 mL H_2/g VS and 510 mL CH_4/g VS, highlighting the potential benefits of thermophilic conditions for hydrogen production. Overall, the comparative analysis in Table 5 emphasizes the importance of optimizing both feedstock composition and process parameters to enhance biogas yields in 2S-AD systems.

Table 5. Comparison of biogas yield by DF+AD of FW.

Feedstock	Process/type	H_2 yield from first phase (mL H_2/g VS)	CH_4 yield from AD (mL CH_4/g VS)	Reference
FW	DF+AD (batch, mesophilic)	53.5	276.5	[44]
FW	Recirculation DF (thermophilic)+AD (mesophilic)	135	510	[46]
FW+WAS	DF+AD (Batch, mesophilic)	42.0	329.8	[47]
Artificial FW	DF+AD (Batch,	30.3	190.3	[48]
FW and brown water	Continuous DF+AD (mesophilic)	99.8	728	[45]
FW	DF+AD (Batch, mesophilic)	37.84	452.79	This study

WAS-Waste activated Sludge

In the first phase of 2S-AD, a short lag phase of 0.05 days and 0.12 days was observed for the batch reactor with 9 g/L and 6 g/L, respectively. This short lag phase indicates a rapid production and accumulation of VFA in this batch reactor, meaning the reactor attained a very high rate of acidogenesis. The higher rate of acidogenesis leads to higher nicotinamide adenine dinucleotide ($NADH/NAD^+$), and to maintain a balanced $NADH/NAD^+$ ratio, this reactor activates propionic acid fermentation spontaneously, [49], resulting in following the propionic pathway [50]. A study done by [51] reported the hydrogen production from the batch reactors using PSBC pyrolyzed at 700°C following propionate and valerate type fermentation. These fermentation processes are H_2 consuming, which explains the minimal cumulative hydrogen production for this batch reactor (first phase batch reactor with 9g/l and 6 g/l PSBC addition), leaving us ambiguous about the role of PSBC addition.

In the second stage of 2S-AD, the maximum methane production rate (R_{max}) was attained by the batch reactor with 3 g/L PSBC addition, which is greater than the others PSBC supplemented reactors. The production rate for methane increased drastically, as shown by the first peak from day 8 to day 11 in Figure 2, which could be due to the rapid breakdown of VFA by methanogens, while the second peak of the batch reactor with 3g/l was due to methane generation from the remaining substrate in the reactor. The control groups showed a slight rise in

production rates from day 7 to day 9, which justifies the statement above.

The supplementation of PSBC to the two-stage anaerobic digestion process utilizing FW has demonstrated significant enhancement in both hydrogen and methane production. This can be attributed to several factors elucidated in the literature. The pyrolysis of PSBC was done at a higher temperature (700 ℃);, therefore, it may have resulted in increased surface area with nano-pores [52]. Such a porous structure of biochar promotes the growth of biofilms, creating a more favorable environment for microbial activity [53]. This enhanced microbial environment is critical for efficient anaerobic digestion. However, this also leads the PSBC to adsorb methane emissions, as evident in a study by [54], thereby decreasing the methane emission in the batch reactors with PSBC concentrations of 6 g/L and 9 g/L.

Secondly, the porous biochar with abundant functional groups facilitates direct electron or hydrogen transfer between syntrophic bacteria and methanogens, thereby improving the efficiency of the anaerobic digestion process [53], [55]. However, due to the pyrolysis of PSBC at higher temperatures, some neutral or basic aromatic group formation occurs [49], [56] that lowers the cation exchange capacity of biochar, negatively impacting the exchange of species with a positive charge [57]. This may have lowered the yield of methane in batch reactors with 6g/l and 9g/l PSBC. Additionally, the presence of trace metals in biochar, such as Mn, Co, Ni, Zn, and Fe, plays a crucial role in stimulating methanogenic microorganisms by catalyzing key metabolic reactions [58]. These essential elements support enzyme activity and microbial growth, preventing digester failure and ensuring a steady production of biogas. This aligns with the observed enhancement in methane production in reactors with PSBC addition, corroborating findings that trace metals are critical for the methanogenesis process. However, it is important to note that this study is limited in characterizing all the trace elements present in PSBC. Therefore, a detailed chemical characterization and economic analysis of PSBC in future research is recommended to fully understand its chemical and economic viability on the anaerobic digestion process.

5. CONCLUSION

This study aimed to investigate the effect of peanut shell biochar supplementation on H_2 and CH_4 production in a batch two-stage mesophilic anaerobic digestion system using FW. The findings revealed that peanut shell biochar supplementation could enhance H_2 and CH_4 yields by improving gas production rate and reducing the lag phase. During the first phase, the lag phase was reduced by 31.5-77.1%, H_2 production rate was enhanced by 32.4-177.3% and production potential increased by 30.18-47.28%. In the second phase, peanut shell biochar supplementation shortened the lag phase by 17.6-51.3%, boosted the production rate by 41.8-61.1%, and raised CH_4 production potential by 40.6%. This improvement suggests that peanut shell biochar acts as a microbial facilitator and buffering agent, creating favorable conditions for hydrogen and methane production. However, the study was limited to specific conditions and did not explore variations in feedstock composition or other operational parameters. Future research should prioritize continuous reactor systems, thermophilic conditions, and diverse organic waste streams to validate the scalability and robustness of biochar assisted anaerobic digestion.

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