ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Valorization of Agro-industrial Solid Waste by Two-stage Anaerobic Digestion for Biohythane Production

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Citation:
Tawantum, J., Muensit, N., Sarhapondecha, P., Mamimin, C. Valorization of agro-industrial solid waste by two-stage anaerobic digestion for biohythane production. *ASEAN J. Sci. Tech. Report.* **2024**, *27*(4), e253769. https://doi.org/10.55164/ajstr.v27i4.253769

Article history:
Received: April 21, 2024
Revised: May 20, 2024
Accepted: May 21, 2024
Available online: June 30, 2024

Abstract: This study investigated the valorization of agro-industrial solid waste for biohythane production through a two-stage anaerobic digestion process. Seventeen waste samples were characterized, revealing diverse physico-chemical properties suitable for anaerobic digestion. The highest biohythane yields were obtained from waste-activated sludge (WAS) from a frozen convenience food wastewater treatment plant (895.63 mL/g VS), WAS from a processed chicken wastewater treatment plant (835.73 mL/g VS), and WAS from a municipal wastewater treatment plant (830.79 mL/g VS). Kinetic analysis using the modified Gompertz model provided insights into the biohythane production potential, with predicted yields ranging from 0 to 111.85 mL/g VS and production rates from 0 to 21.37 mL/d. The comparative analysis highlighted the superior biohythane production potential of the studied waste materials compared to other substrates, such as food waste (180.5 mL/g VS) and sugarcane bagasse (165.2 mL/g VS). The highest hydrogen and methane contents in the produced biohythane were 26.57% and 67.85%, respectively. The techno-economic assessment of scaled-up biohythane production demonstrated the economic feasibility, with a payback period of 2.05 years for a plant capacity of 100 ton waste/day, a biohythane yield of 500 m^3/ton waste, and a biohythane production of 50,000 m^3/day. The capital cost was estimated at 15 million USD, with an operating cost of 0.2 USD/m^3 biohythane and a revenue of 0.6 USD/m^3 from biohythane sales. The results of this study demonstrate the high potential of agro-industrial solid waste valorization for biohythane production and its contribution to sustainable waste management and renewable energy production.

Keywords: Agro-industrial solid waste; Biohythane; Waste valorization; Sustainable energy; Circular economy

1. Introduction

The rapid growth of the global population, urbanization, and industrialization has led to an increasing demand for energy and the generation of vast amounts of waste. The world's energy consumption is expected to increase by nearly 50% between 2018 and 2050, with fossil fuels continuing to dominate the energy mix [1]. However, the reliance on fossil fuels has resulted in numerous environmental problems, such as greenhouse gas emissions, air pollution, and

climate change [2]. Moreover, the improper management of waste, particularly in developing countries, has caused severe environmental and public health issues, including water and soil contamination, air pollution, and the spread of diseases [3]. In response to these challenges, there has been a growing interest in the development of sustainable and renewable energy sources, as well as the implementation of effective waste management strategies. Anaerobic digestion (AD) has emerged as a promising technology for valorizing organic waste, as it can simultaneously address waste management and renewable energy production [4]. AD is a biological process in which microorganisms break down organic matter in the absence of oxygen, producing biogas, which primarily consists of methane (CH_4) and carbon dioxide (CO_2) [5]. Agro-industrial solid waste, generated from various agricultural and industrial activities, represents a significant portion of the global waste stream. The world is estimated to generate approximately 1.3 billion tons of agro-industrial solid waste annually, with a large portion disposed of in landfills or left to decay in the open environment [6]. This waste, however, contains a substantial amount of organic matter and nutrients, making it a suitable feedstock for AD [7].

Conventional AD processes produce methane-rich biogas, which can be used for heat and electricity generation. However, recent advancements in AD technology have led to the developing of two-stage AD systems, which produce biohythane, a mixture of hydrogen (H_2) and methane [8]. A two-stage AD system separates the process into two distinct stages: hydrogen and methane production. During the first stage, hydrogen-producing bacteria, such as Clostridium and Enterobacter, convert the organic matter into hydrogen and volatile fatty acids (VFAs). In the second stage, methanogens convert the VFAs and remaining organic matter into methane [9]. The production of biohythane through two-stage AD offers several advantages over conventional single-stage AD. First, biohythane has a higher energy content compared to methane-rich biogas, as hydrogen has a higher calorific value (122 kJ/g) than methane (50 kJ/g) [10]. Second, separating the hydrogen and methane production stages allows for optimizing process conditions, such as pH and hydraulic retention time (HRT), for each stage, improving overall process efficiency [11]. Third, hydrogen production in the first stage can enhance the biodegradability of the substrate, as it helps in the hydrolysis of complex organic compounds, making them more accessible for methanogens in the second stage [12]. Despite the promising potential of biohythane production from agro-industrial solid waste, challenges still need to be addressed. One of the main challenges is the variability in the composition and characteristics of the waste, which can affect the performance and stability of the AD process [4]. Another challenge is the optimization of process parameters, such as substrate concentration, inoculum ratio, and temperature, to maximize biohythane yield and quality [7]. Moreover, the scale-up and economic viability of biohythane production systems needs to be evaluated to ensure their successful implementation in real-world applications [8]. Several studies have investigated the potential of biohythane production from various agro-industrial solid waste streams. For example,[4] studied the co-digestion of pig manure and rice straw for biohythane production and achieved a maximum biohythane yield of 333.5 mL/g volatile solids (VS) with a hydrogen content of 38.6%. [7] investigated the use of palm oil mill effluent (POME) and oil palm empty fruit bunches (EFB) for biohythane production and obtained a maximum biohythane yield of 181.9 mL/g VS with a hydrogen content of 28.4%. [9] explored the potential of food waste for biohythane production and reported a maximum biohythane yield of 254.7 mL/g VS with a hydrogen content of 35.6%.

Therefore, this study aims to investigate the valorization of agro-industrial solid waste streams, including waste-activated sludge (WAS) from different wastewater treatment plants, expired seasoning powder, and plant residues, for biohythane production through a two-stage AD process. The specific objectives of this study are to characterize the physicochemical properties of the agro-industrial solid waste samples and evaluate their suitability for biohythane production, to investigate the biohythane production potential of the waste samples through a two-stage AD process, and determine the optimal process parameters for maximum biohythane yield and quality, to perform a kinetic analysis of the biohythane production.

2. Materials and Methods

2.1 Characterization of agro-industrial solid waste

Figures 1. Variety of agro-industrial solid waste materials

Solid waste materials were sourced from various agro-industrial sectors, including waste-activated sludge from sugar and starch products wastewater treatment plant, Purac (Thailand) Co., Ltd. (sample 1), waste-activated sludge from tapioca starch wastewater treatment plant, Ingredion (Thailand) Co., Ltd. (sample 2), waste activated sludge from frozen convenience food wastewater treatment plant, McKey Food Services (Thailand) Ltd. (sample 3), waste activated sludge from soft drink wastewater treatment plant, Suntory PepsiCo Beverage (Thailand) Co., Ltd. (sample 4), waste activated sludge from processed chicken wastewater treatment plant, GFPT Nichirei (Thailand) Co., Ltd. (sample 5), expired seasoning powder, Ajinomoto (Thailand) Co., Ltd. (sample 6), waste activated sludge from chemical product wastewater treatment plant, TPT Petrochemicals Public Co., Ltd. (sample 7), waste activated sludge, from municipal wastewater treatment plant, Pankornwattanakit Ltd., Part. (sample 8), waste-activated sludge from cleaning solution product wastewater treatment plant, Colgate Palmolive (Thailand) Ltd. (sample 9), waste-activated sludge from condiments processing wastewater treatment plant, Cofco Biochemical (Thailand) Co., Ltd. (sample 10), waste-activated sludge from the chemical product from the wastewater treatment plant, BBGI Biodiesel Co., Ltd. (sample 11), plans residue, McCormick (Thailand) Co., Ltd. (sample 12), waste activated sludge from sugar and starch products wastewater treatment plant, Purac (Thailand) Co., Ltd. (sample 13), waste activated sludge from feedstuff wastewater treatment plant, CPF (Thailand) Public Co., Ltd. (sample 14), waste activated sludge from processed chicken wastewater treatment plant, Sun Food International Co., Ltd. (sample 15), expired lime flavor seasoning powder, Ajinomoto (Thailand) Co., Ltd. (sample 16), waste activated sludge from sugar and starch products wastewater treatment plant, Purac (Thailand) Co., Ltd.

(sample 17). The visualization of solid waste is shown in Figure 1. All samples were collected from each source using sterile containers and stored at 4°C until further analysis. The chemical and physical composition of the waste materials was determined using standard methods. Total solids, volatile solids, moisture, and ash content were determined according to [13]. Carbohydrate content, protein, total sugar, oil, and grease were analyzed using standard methods for examining water and wastewater [13]**.** The cellulose, hemicellulose, and lignin were analyzed following the protocol described by Van Soest et al. [14]. Volatile fatty acids (VFAs) were analyzed using a gas chromatograph (GC).

2.2 Preparation of inoculum for hydrogen and methane production

Seed sludge for hydrogen and methane production was collected from a local anaerobic digester and pretreated by the load-shock method to remove unwanted materials and enhance microbial activity [15]. Briefly, the sludge was mixed with a nutrient solution containing glucose (10 g/L), yeast extract (2 g/L), and peptone (5 g/L) and incubated at 40°C for 24 hours to activate the hydrogen-producing bacteria. For methane production, methane-producing microorganisms were obtained from the biogas production system and mixed with POME as a substrate at a ratio of 4:1 (v/v). The mixture was adjusted to a pH range of 7-8 using 1M NaOH and 1M HCl. The alkalinity was maintained at 5 g/kg of $CaCO_3$. The inoculum was incubated at 40°C for two weeks in a 1 L anaerobic reactor. Biogas production was monitored daily using a gas counter. Biogas composition was analyzed weekly using gas chromatography with a thermal conductivity detector (GC-TCD) (Gas Chromatography, CG-8A, Shimadzu). The inoculum was considered ready for use when it achieved a stable biogas production rate and a microbial sludge concentration of at least 50 g/L [7]. The microbial communities of inoculum, sampling each inoculum, were extracted for DNA using PowerSoil DNA kit (MO BIO, Carlsbad, CA, USA) and were analyzed with 16s rRNA sequencing using MiSeq technology in the V3–V4 regions of the bacterial and archaeal 16S rRNA gene fragments as described by [16].

2.3 Evaluation of hydrogen and methane production potential

The potential of various solid waste materials to produce hydrogen and methane was evaluated using a two-step fermentation process. Each solid waste material was mixed with the hydrogen-producing inoculum at a 4:1 VS basis ratio with a working volume of 200 mL in a 500 mL fermentation bottle. The bottles were purged with N_2:CO_2 (80:20) to maintain the anaerobic condition and sealed with silicone and aluminum caps using a hand crimper. The bottles were incubated at 40°C for 7 days, and the volume of biogas produced was measured daily using the water displacement method [15]. After the hydrogen fermentation step, the effluent from each bottle was mixed with the methane-producing inoculum at a 2:1 VS basis ratio. The bottles were purged with N_2:CO_2 (80:20), sealed, and incubated at 40°C for 45 days. Biogas production was monitored daily, and biogas composition was analyzed weekly using GC-TCD. All experiments were conducted in triplicate, and the results were expressed as mean± standard deviation.

2.4 Analytical methods

The contents of cellulose, hemicellulose, and lignin in wastes were determined following the protocol described by [14]. Alkalinity, chemical oxygen demand (COD), pH, total solids (TS), and volatile solids (VS) were determined following the standard methods described by [13]. The volume of biogas produced in the headspace of the fermentation bottles was measured daily using the water displacement method. The volume of gas produced was equivalent to the amount of water displaced in the cylinder. Continuous biogas volume was measured via a digester headspace connection to the gas counter using the water displacement method [17]. Biogas composition was analyzed by gas chromatography (GC-8A Shimadzu) equipped with Thermal Conductivity Detectors (GC-TCD) and fitted with a 2.0 m packed column (Shin-Carbon ST 100/120 Restek). Argon was used as a carrier gas at a flow rate of 15 mL min^{-1}. The injection port, oven, and detector temperatures were set at 120 ºC, 50 ºC, and 100 ºC, respectively [18]. Gas samples of 0.5 mL were injected in duplicate. The temperature of the injection port was 230 ºC. The chromatography program was as follows: 70 ºC for 1 min, ramping from 70 to 180 ºC at a rate of 20 ºC $min^{-1,}$ and holding at 180 ºC for 6 min. The detector temperature was 250 ºC. Gas measurements were reported

under STP conditions (standard temperature and pressure, 273 K, 1.01325 Pa). Volatile fatty acids (VFAs) were analyzed using a Gas Chromatograph (GC) equipped with a Flame Ionization Detector (FID). The GC system was an Agilent 7890A (Agilent Technologies, USA) fitted with a DB-FFAP capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness; J&W Scientific, USA).

2.5 Kinetic model and determination of hydrolysis constant

The kinetics of biogas production were described using a first-order kinetic model, as reported in a previous study. The first-order kinetic model is given by Equation 1

$$ln\frac{B_{\infty}-B}{B_{\infty}} = -K_h t \quad (1)$$

Where K_h is the constant biogas rate (d^{-1}), B_∞ is the final methane production value, B is the methane produced at a given time, and t is the production time. To determine the hydrolysis constant (K_h), the experimental data of cumulative methane production (B) at different time points (t) were fitted to Equation 1 using linear regression. The slope of the linear regression line represents the hydrolysis constant (K_h). The lag phase before the start of methane production was determined using the modified Gompertz equation, as described in a previous study. The modified Gompertz equation is given by Equation 2:

$$M = P \cdot exp\{-exp\left[\frac{R_m \cdot e}{P}(\lambda - t) + 1\right]\} \quad (2)$$

Where M is the cumulative methane production, P is the methane production potential, Rmax is the maximum methane production rate, λ is the lag phase, t is the time, and e is the mathematical constant (exp (1) = 2.7183). The experimental data of cumulative methane production (M) at different time points (t) were fitted to Equation 2 using non-linear regression analysis in SigmaPlot ® 11.0 software [19]. The model parameters (P, R_{max}, and λ) were estimated by minimizing the sum of squared errors between the experimental data and the model predictions. The goodness of fit of the first-order kinetic model and the modified Gompertz model was evaluated using the coefficient of determination (R^2) and the root mean square error (RMSE). A p-value less than 0.05 was considered statistically significant. All statistical analyses were done using SigmaPlot ® 11.0 software [19].

3. Results and Discussion

3.1 Composition and characteristics of agro-industrial solid waste

The composition and characteristics of the agro-industrial and agricultural solid waste samples varied significantly, as shown in Table 1. The total solids (TS) content ranged from 10.04% to 95.81%, with samples 6, 12, and 15 having the highest TS values of 95.81%, 93.23%, and 95.78%, respectively. The volatile solids (VS) content also varied widely, from 7.94% to 83.97%, with samples 6, 12, and 15 exhibiting the highest VS values of 58.15%, 76.65%, and 83.97%, respectively. The high TS and VS contents in these samples indicate a higher organic matter content, which is advantageous for biogas production through anaerobic digestion [20]. The moisture content of the samples ranged from 4.19% to 89.96%, with most samples having moisture content above 70%. High moisture content is typical for agro-industrial and agricultural waste materials, and it can affect the performance of anaerobic digestion processes [21]. The ash content varied from 1.05% to 37.66%, with sample 6 having the highest ash content of 37.66%. High ash content can lead to operational problems in anaerobic digesters, such as reduced practical volume and clogging [22]. The carbohydrate content of the samples ranged from 0.05 g/L to 700 g/L, with samples 12 and 16 having the highest values of 645 g/L and 700 g/L, respectively. Carbohydrates are essential for biogas production, as they are readily biodegradable and can be converted into volatile fatty acids (VFAs) by acidogenic bacteria [5]. The protein content also varied significantly, from 50.25 g/L to 398.69 g/L, with samples 6 and 12 having the highest protein content of 330.69 g/L and 398.69 g/L, respectively. Proteins can contribute to biogas production, but their degradation can also lead to the formation of ammonia, which can inhibit the anaerobic

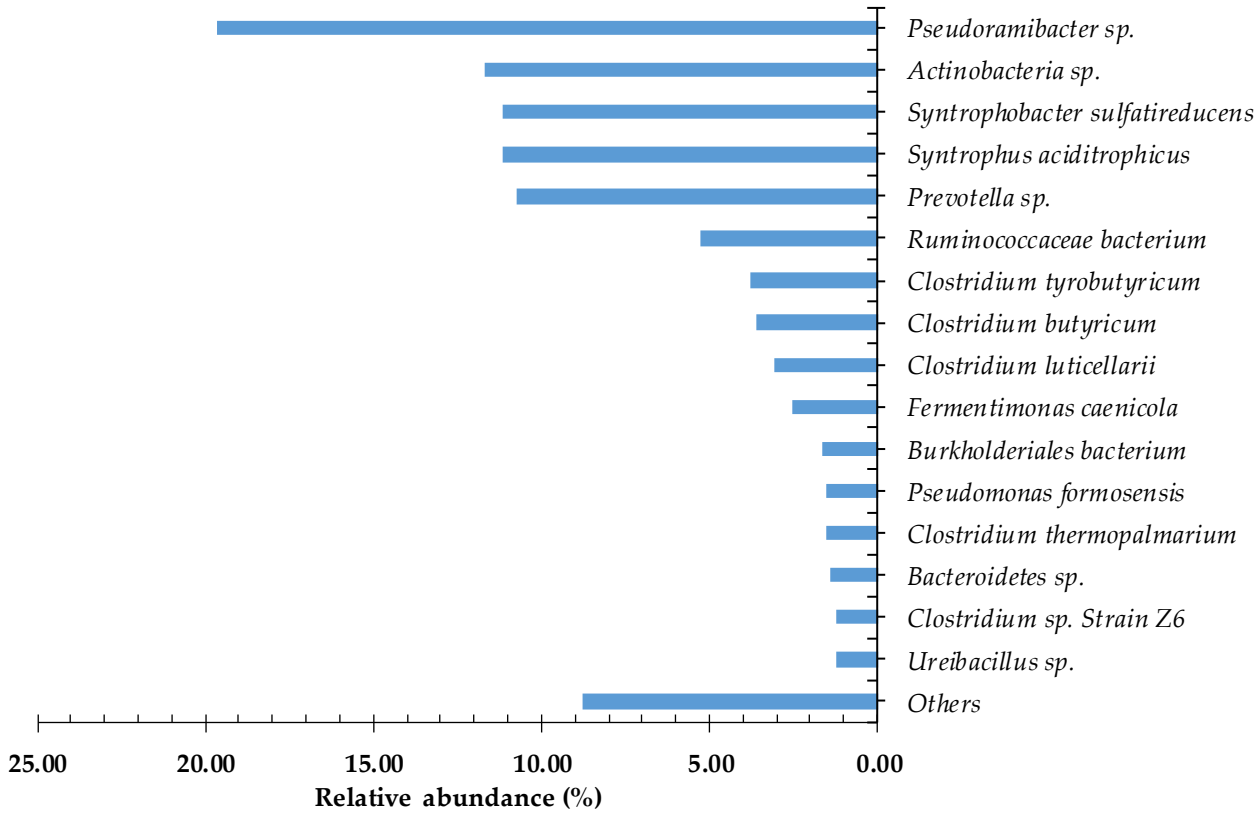
digestion process at high concentrations [23]. The VFA content of the samples ranged from 0.10 g/L to 18.70 g/L, with sample 15 having the highest VFA content of 18.70 g/L. VFAs are intermediate products in the anaerobic digestion process and are essential for the stability and efficiency of the process [24]. The total sugar content varied from 0.36 g/L to 481.73 g/L, with sample 16 having the highest total sugar content of 481.73 g/L. Sugars are readily biodegradable and can contribute significantly to biogas production [25]. The oil and grease content of the samples ranged from 1.77% to 63.73%, with samples 5 and 8 having the highest values of 56.84% and 63.73%, respectively. High oil and grease content can inhibit the anaerobic digestion process by limiting the mass transfer of substrates and metabolites and causing flotation of biomass [26]. The composition of the agricultural solid waste samples varied significantly, with some samples having high organic matter content (e.g., samples 6, 12, and 15), while others had high moisture content (e.g., samples 4, 14, and 17).

3.2 Microbial community of inoculum

The microbial community analysis of the hydrogen production stage inoculum for biohydrogen production in a two-stage anaerobic digestion system revealed a diverse consortium of bacteria (Figure 2). The most abundant microorganisms were *Pseudoramibacter* sp. (19.64%), *Actinobacteria* sp. (11.70%), *Syntrophobacter sulfatireducens* (11.14%), *Syntrophus aciditrophicus* (11.14%), and *Prevotella* sp. (10.72%). Other notable members of the community included *Ruminococcaceae* bacterium (5.29%), *Clostridium tyrobutyricum* (3.76%), *Clostridium butyricum* (3.62%), and *Clostridium luticellarii* (3.06%). The remaining microorganisms had relative abundances ranging from 0.42% to 2.51%. The results of the microbial community analysis provide valuable insights into the complex interplay of bacteria involved in the dark fermentation stage of a two-stage anaerobic digestion system for biohydrogen production. The presence of a diverse range of bacteria highlights their crucial roles in the efficient production of hydrogen. *Pseudoramibacter* sp., the most abundant microorganism in the community, is known to be involved in the fermentation of carbohydrates and the production of hydrogen [28]. Its high abundance suggests it plays a key role in dark fermentation. *Actinobacteria* sp., the second most abundant microorganism, is also involved in the fermentation of various organic compounds and hydrogen production [29]. *Syntrophobacter sulfatireducens* and *Syntrophus aciditrophicus*, both equally abundant in the community (11.14%), are known to engage in syntrophic relationships with hydrogen-utilizing microorganisms [30,31]. These bacteria are capable of degrading complex organic compounds into simpler substrates, such as acetate and hydrogen, which can be utilized by other microorganisms in the community. The high abundance of these syntrophic bacteria highlights their crucial role in the efficient functioning of the dark fermentation stage. *Prevotella* sp., the fifth most abundant microorganism (10.72%), is known to be involved in the fermentation of carbohydrates and the production of hydrogen [32]. Its presence suggests that it contributes significantly to the overall hydrogen production in the dark fermentation stage. The presence of various *Clostridium* sp., such as *C. tyrobutyricum* (3.76%), *C. butyricum* (3.62%), and *C. luticellarii* (3.06%), is not surprising, as these bacteria are well-known hydrogen producers [33] [34]. Their presence further supports the importance of hydrogen production in the dark fermentation stage. Other bacteria, such as *Fermentimonas caenicola* (2.51%), *Ruminococcaceae bacterium* (5.29%), and *Bacteroidales bacterium* 6E (0.84%), are likely involved in the fermentation of complex organic matter into simpler compounds that the hydrogen-producing bacteria can utilize [35,36,37].

Table 1. Physico-chemical characteristics of agro-industrial solid waste samples

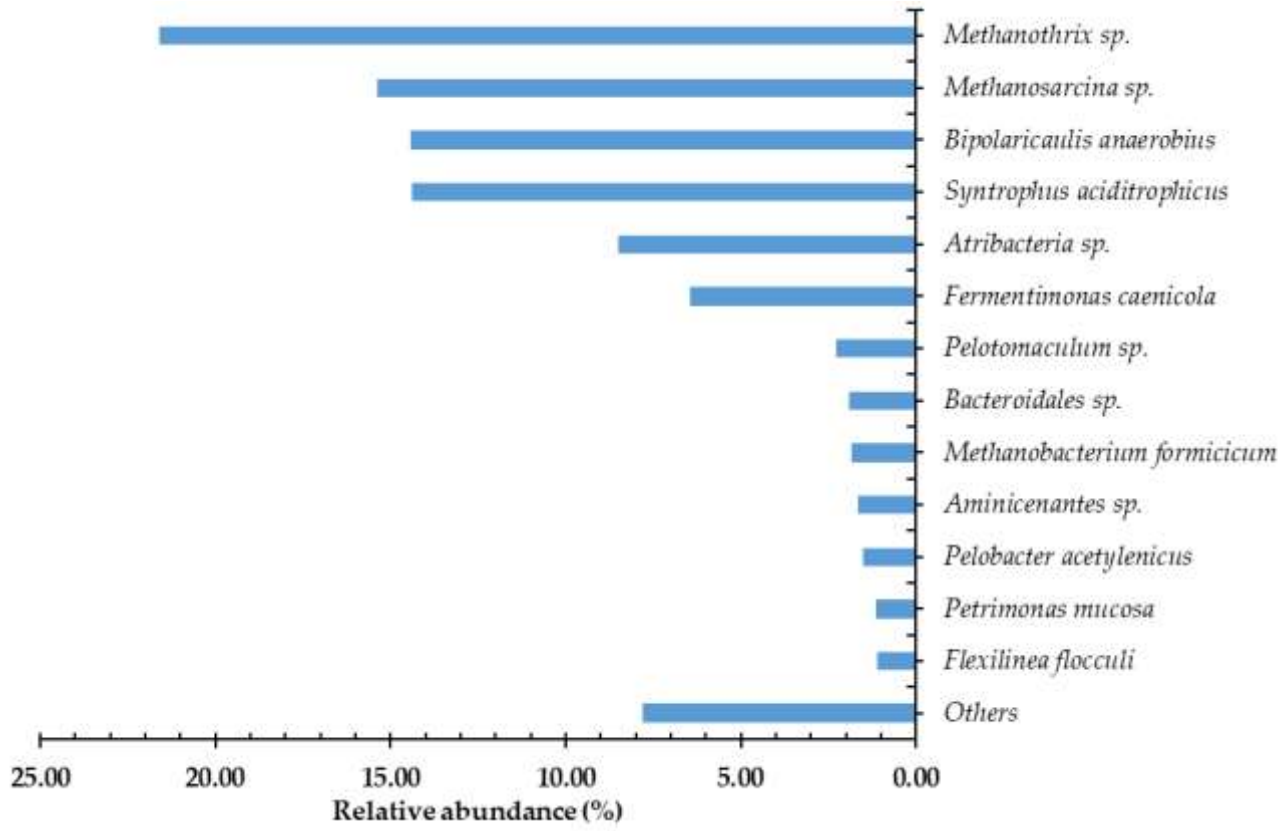
Sample	Total Solids (%)	Volatile Solids (%)	Moister (%)	Ash (%)	Carbo hydrate (g/L)	Protein (g/L)	Volatile fatty acids (g/L)	Total Sugar (g/L)	Oil and Grease (%)
1	33.46 ± 0.23	9.85 ± 0.03	66.54 ± 0.20	23.61 ± 0.23	0.64 ± 0.02	71.94 ± 0.22	2.20 ± 0.07	0.70 ± 0.00	7.70 ± 1.55
2	15.65 ± 0.94	15.65 ± 0.94	84.35 ± 0.13	13.46 ± 0.36	2.85 ± 0.09	50.25 ± 0.15	2.10 ± 0.06	1.93 ± 0.09	7.84 ± 1.22
3	17.63 ± 1.50	15.33 ± 0.04	82.37 ± 0.33	2.31 ± 1.50	0.05 ± 0.06	139.63 ± 0.42	2.54 ± 0.08	0.36 ± 0.00	48.66 ± 0.34
4	10.76 ± 1.08	7.94 ± 0.80	89.24 ± 0.27	2.82 ± 1.08	0.14 ± 0.01	150.25 ± 0.45	0.46 ± 0.01	0.58 ± 0.03	6.51 ± 0.24
5	15.37 ± 1.21	14.31 ± 1.14	84.63 ± 0.07	1.05 ± 1.21	0.06 ± 0.00	142.63 ± 0.43	0.39 ± 0.01	3.47 ± 0.06	56.84 ± 0.41
6	95.81 ± 0.14	58.15 ± 0.77	4.19 ± 0.78	37.66 ± 0.14	220.00 ± 0.00	330.69 ± 0.99	11.52 ± 0.35	290.42 ± 0.03	1.77 ± 0.15
7	16.66 ± 0.91	10.83 ± 0.75	83.34 ± 0.70	5.83 ± 0.91	0.25 ± 0.02	191.38 ± 0.57	0.11 ± 0.00	0.46 ± 0.03	3.74 ± 0.22
8	27.02 ± 0.40	25.59 ± 0.47	72.98 ± 0.09	1.43 ± 0.40	0.42 ± 0.03	179.63 ± 0.54	0.75 ± 0.02	2.31 ± 0.25	63.73 ±0.64
9	21.45 ± 0.80	13.27 ± 0.43	78.55 ± 0.37	8.18 ± 0.80	1.57 ± 0.02	82.88 ± 0.25	0.30 ± 0.01	1.85 ± 0.00	18.86 ± 0.48
10	17.59 ± 1.55	10.69 ± 0.71	82.41 ± 0.84	6.90 ± 1.55	5.60 ± 0.28	367.50 ± 1.10	0.18 ± 0.01	1.24 ± 0.21	7.10 ± 0.57
11	16.10 ± 2.08	14.20 ± 1.71	83.90 ± 0.26	1.90 ± 2.08	0.74 ± 0.04	184.38 ± 0.55	0.10 ± 0.00	1.08 ± 0.13	4.93 ± 1.16
12	93.23 ± 0.33	76.65 ± 0.12	6.77 ± 0.16	16.58 ± 0.33	645.00 ± 1.41	398.69 ± 1.20	0.60 ± 0.02	18.55 ± 0.07	2.27 ± 2.09
13	13.38 ± 0.38	9.92 ± .12	86.62 ± 0.26	3.45 ± 0.38	0.29 ± 0.03	210.50 ± 0.63	0.17 ± 0.01	0.47 ± 0.01	3.07 ± 2.29
14	10.41 ± 0.71	9.24 ± 0.60	89.59 ± 0.12	1.18 ± 0.71	193.50 ± 2.12	185.63 ± 0.56	0.11 ± 0.00	1.47 ± 0.01	3.41 ± 1.50
15	95.78 ± 0.02	83.97 ± 0.51	4.22 ± 0.49	11.81 ± 0.02	168.00 ± 1.41	303.50 ± 0.91	18.70 ± 0.56	1.54 ± 0.00	9.96 ± 0.37
16	12.74 ± 0.33	8.87 ± 0.20	87.26 ± 0.13	3.88 ± 0.33	700.00 ± 0.00	62.44 ± 0.19	17.68 ± 0.53	481.73 ± 0.00	8.31 ± 0.38
17	10.04 ± 0.20	8.94 ± 0.14	89.9 6± 0.06	1.10 ± 0.20	168.00 ± 1.41	111.00 ± 0.33	0.37 ± 0.01	1.21 ± 0.000	14.83 ± 2.33



Figures 2. Taxa relative abundance in the species of the hydrogen-producing inoculum

The microbial community analysis of inoculum for the methane production stage in a two-stage anaerobic digestion system revealed a diverse consortium of archaea and bacteria (Figure 3). The most abundant microorganisms were *Methanothrix* sp. (21.60%), *Methanosarcina* sp. (15.39%), *Bipolaricaulis anaerobius* (14.41%), and *Syntrophus aciditrophicus* (14.37%). Other notable members of the community included *Atribacteria* sp. (8.51%), *Fermentimonas caenicola* (6.47%), and *Pelotomaculum* sp. (2.27%). The remaining microorganisms had relative abundances ranging from 0.08% to 1.93%. The results of the microbial community analysis provide valuable insights into the complex interplay of microorganisms involved in the methane production stage of a two-stage anaerobic digestion system. The presence of both archaea and bacteria highlights the importance of their synergistic interactions in the efficient production of methane. *Methanothrix* sp. and *Methanosarcina* sp., the most abundant microorganisms in the community, are methanogenic archaea known for utilizing acetate as a substrate for methane production [38] [39]. Their high abundance suggests that acetate is a key intermediate in methane production. The presence of *Methanobacterium formicicum* (1.85%) and *Methanosaeta harundinacea* (0.49%), also known as methanogens, further supports the importance of methanogenesis in this stage of anaerobic digestion [40]. *Bipolaricaulis anaerobius* and *Syntrophus aciditrophicus*, the third and fourth most abundant microorganisms, are known to engage in syntrophic relationships with methanogens [30] [31]. These bacteria can degrade complex organic compounds into simpler substrates, such as acetate and hydrogen, which the methanogens can utilize for methane production. The abundance of these syntrophic bacteria highlights their crucial role in the efficient functioning of the methane production stage. The presence of *Atribacteria* sp. (8.51%), *Fermentimonas caenicola* (6.47%), and *Pelotomaculum* sp. (2.27%) suggests that fermentative bacteria also play a significant role in the methane production process. These bacteria are likely involved in the fermentation of complex organic

matter into simpler compounds that syntrophic bacteria and methanogens can utilize [41,42]. Other bacteria, such as *Aminicenantes* sp. (1.66%), *Pelobacter acetylenicus* (1.51%), and *Petrimonas mucosa* (1.13%), are known to be involved in the degradation of various organic compounds, further contributing to the overall efficiency of the methane production process [43,44].

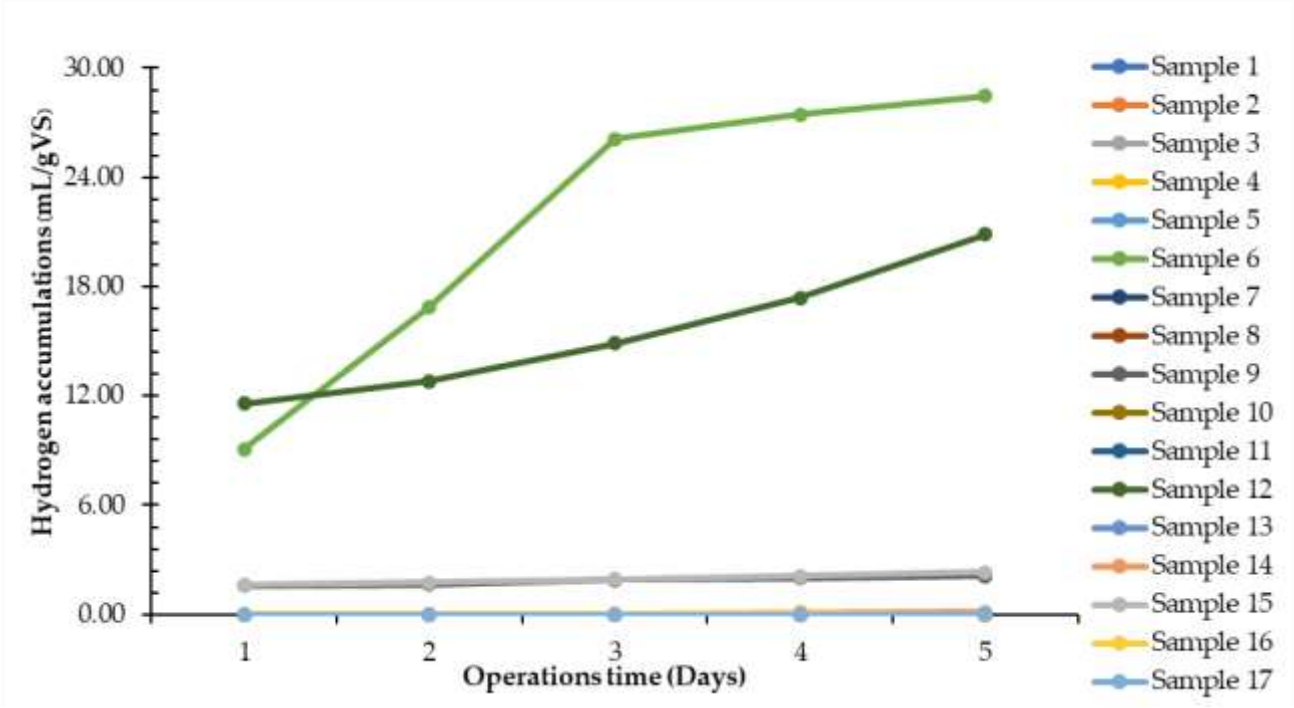


Figures 3. Taxa relative abundance in species in methane-producing inoculum

3.3 Performance of hydrogen production stage

Table 2 summarizes the hydrogen production performance from various agro-industrial solid waste samples. The hydrogen yield varied significantly among the samples, ranging from 0 to 28.46 mL H_2/g VS. Sample 6 exhibited the highest hydrogen yield of 28.46 mL H_2/g VS, followed by Sample 12 with 20.86 mL H_2/g VS and Sample 15 with 2.34 mL H_2/g VS. The remaining samples showed negligible or no hydrogen production, with yields below 2.10 mL H_2/g VS. The hydrogen concentration in the produced biogas also varied considerably, ranging from 0% to 15.27%. Sample 6 had the highest hydrogen concentration of 15.27%, followed by Sample 12 with 11.06% and Sample 9 with 2.33%. The low hydrogen concentrations in most samples indicate the presence of other gases, such as carbon dioxide, which can be attributed to the activity of non-hydrogen-producing microorganisms [45]. The hydrogen production per ton of waste ranged from 0 to 16.55 m^3/ton, with Sample 6 showing the highest production of 16.55 m^3/ton, followed by Sample 12 with 15.99 m^3/ton and Sample 15 with 1.96 m^3/ton. The low hydrogen production in most samples can be attributed to the low hydrogen yields and non-biodegradable components, such as lignin and cellulose, limiting fermentable substrates' availability [46]. Figure 4 presents the time course of hydrogen yield from the various agro-industrial solid waste samples. Samples 6 and 12 exhibited rapid hydrogen production, reaching their maximum yields within 1 and 4 days, respectively. This rapid hydrogen production can be attributed to readily fermentable substrates, such as sugars and carbohydrates, quickly consumed by hydrogen-producing bacteria [12]. In contrast, Sample 15 showed a slower hydrogen production rate, possibly due to more complex substrates requiring longer hydrolysis times [47]. The decomposition time for

hydrogen production varied from 1 to 4 days, with most samples achieving complete decomposition within 2 days. The short decomposition times indicate the rapid activity of hydrogen-producing bacteria, such as *Clostridium* sp. and *Enterobacter* sp., which are known to convert fermentable substrates into hydrogen [48] efficiently. The hydrogen production performance varied significantly among the agro-industrial solid waste samples, with Sample 6 and Sample 12 exhibiting the highest hydrogen yields, concentrations, and production rates. The low hydrogen production in most samples can be attributed to the presence of non-biodegradable components and the activity of non-hydrogen-producing microorganisms. The rapid decomposition times in most samples highlight the potential for fast hydrogen production from readily fermentable substrates. However, pretreatment methods may be necessary to improve the biodegradability of complex substrates and enhance hydrogen production from these waste materials [49].



Figures 4. Time crouse for yield hydrogen from various agro-industrial solid waste.

Table 3 presents the kinetic parameters for hydrogen production from various agro-industrial solid waste samples using the modified Gompertz model. The model coefficients, including the hydrogen production potential (Kh), predicted hydrogen yield, hydrogen production rate, and lag time, were determined to evaluate the hydrogen production kinetics of each sample. The hydrogen production potential (Kh) varied significantly among the samples, ranging from 0 to 0.6754 days. Sample 6 exhibited the highest Kh value of 0.6754 days, followed by Sample 7 with 0.5274 days and Sample 4 with 0.5267 days. The high Kh values in these samples indicate their potential for extended hydrogen production periods, which can be attributed to the presence of slowly biodegradable substrates or the efficient activity of hydrogen-producing bacteria [45]. The predicted hydrogen yield ranged from 0 to 326.9316 mL H_2/g-VS, with Sample 6 showing the highest expected yield of 326.9316 mL H_2/g VS, followed by Sample 12 with 229.0055 mL H_2/g-VS and Sample 15 with 45.5818 mL H_2/g-VS. These predicted hydrogen yields are consistent with the experimental results presented in Table 2, confirming the accuracy of the modified Gompertz model in describing the hydrogen production kinetics [50]. The hydrogen production rate varied from 0 to 9.7385 mL H_2/d, with Sample 12 exhibiting the highest production rate of 9.7385 mL H_2/d, followed by Sample 6 with 9.2789mL H_2/d and Sample 15 with 2.8996 mL H_2/d.

The high hydrogen production rates in these samples can be attributed to readily fermentable substrates, such as sugars and carbohydrates, which are rapidly consumed by hydrogen-producing bacteria [12]. The lag time represents the initial adaptation period of the hydrogen-producing bacteria to the substrate and environmental conditions. Most samples exhibited no lag time, indicating the rapid initiation of hydrogen production. However, Samples 3, 5, and 1 showed lag times of 19.7097, 13.9578, and 8.5884 days, respectively, suggesting the presence of more complex substrates or the need for a more extended adaptation period for the hydrogen-producing bacteria [51]. The coefficient of determination (R^2) values ranged from 0.000 to 0.9998, indicating a moderate to good fit of the modified Gompertz model to the experimental data. The relatively low R^2 values in some samples may be attributed to the complexity of the substrate composition and the presence of non-biodegradable components, which can affect the hydrogen production kinetics [47]. The modified Gompertz model's kinetic analysis provided valuable insights into the agro-industrial solid waste samples' hydrogen production potential, predicted yield, production rate, and lag time. Samples 6, 12, and 15 exhibited the highest hydrogen production potential and predicted yields, consistent with their experimental performance. The high hydrogen production rates in Samples 6, 12, and 15 highlight their potential for rapid hydrogen production from readily fermentable substrates. The kinetic parameters obtained from this analysis can be used to optimize the design and operation of hydrogen production systems using these waste materials.

Table 2. A summary of the study on hydrogen gas production from agro-industrial solid waste

Samples of agro-industrial solid waste	Hydrogen yield (mL-H_2/g-VS)	Hydrogen concentration (%)	Hydrogen production (m^3/ton of waste)	Decomposition time (Days)
Sample 1	0.00	0.21	0.00	1
Sample 2	0.15	0.89	0.02	2
Sample 3	0.00	0.05	0.00	1
Sample 4	0.00	0.20	0.00	1
Sample 5	0.00	0.05	0.00	1
Sample 6	28.46	15.27	16.55	1
Sample 7	0.00	0.15	0.00	3
Sample 8	0.00	0.27	0.00	3
Sample 9	2.10	2.33	0.28	1
Sample 10	0.04	0.48	0.00	1
Sample 11	0.00	0.27	0.00	1
Sample 12	20.86	11.06	15.99	4
Sample 13	0.04	0.44	0.00	1
Sample 14	0.14	0.42	0.01	1
Sample 15	2.34	2.29	1.96	1
Sample 16	0.00	0.00	0.00	-
Sample 17	0.02	0.40	0.00	3

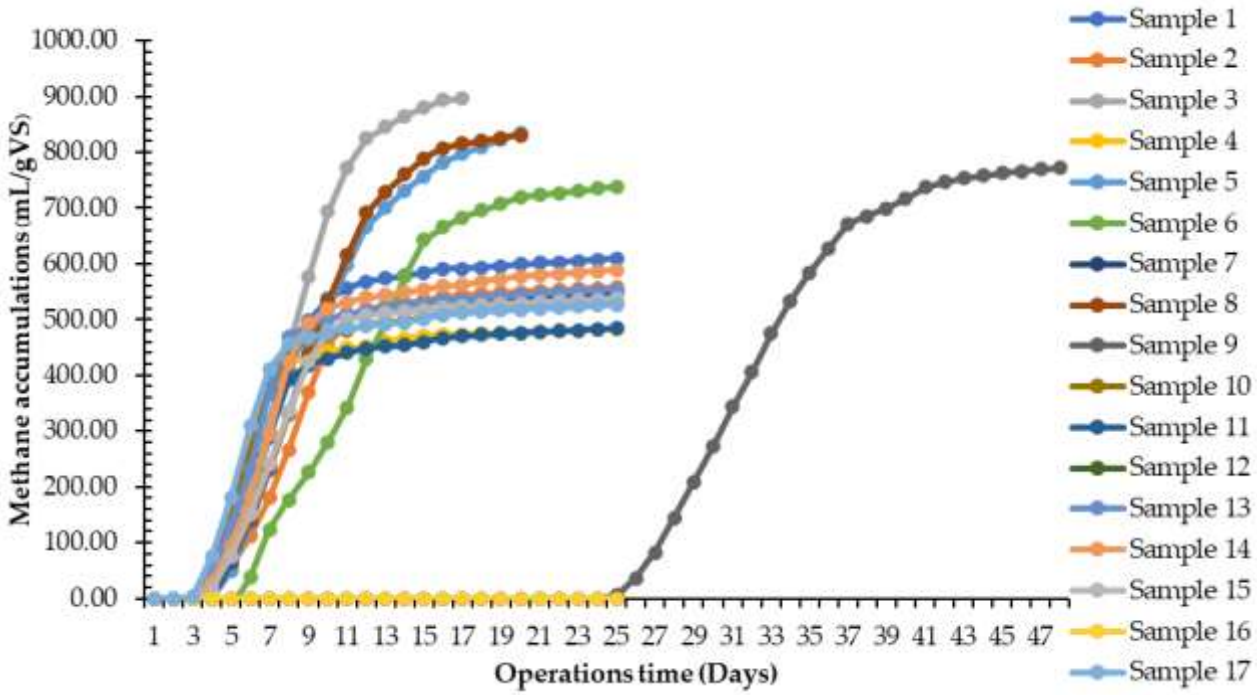
Table 3. Kinetic parameters for hydrogen production from agro-industrial solid waste using the modified Gompertz model

Agro-industrial solid waste	Gompertz coefficients of Hydrogen stage				K_h (days)
	Predicted hydrogen yield (ml-H_2/g VS)	Hydrogen production rate (ml-H_2/d)	Lag time (days)	Rsqr (R^2)	
Sample 1	0.2378	0.0000	8.5884	0.0000	0.4322
Sample 2	6.2869	1.3003	0.3493	0.9884	0.4925
Sample 3	0.1954	0.0000	19.7097	0.0000	0.4683
Sample 4	2.1446	0.329	0.0000	0.9966	0.5267
Sample 5	0.2683	0.0000	13.9578	0.0000	0.4978
Sample 6	326.9316	9.2789	0.0000	0.4357	0.6754
Sample 7	1.8781	0.2457	0.0000	0.9959	0.5274
Sample 8	2.3332	0.3179	0.2456	0.9997	0.4592
Sample 9	34.6487	2.9713	0.0000	0.9995	0.4728
Sample 10	5.6475	0.8613	0.0000	0.997	0.5222
Sample 11	4.8602	0.443	0.0000	0.999	0.4928
Sample 12	229.0055	9.7385	0.0000	0.7649	0.3838
Sample 13	5.6019	0.7602	0.0000	0.9974	0.5148
Sample 14	6.3411	0.7087	0.0000	0.9971	0.551
Sample 15	45.5818	2.8996	0.0000	0.9998	0.4334
Sample 16	0.0000	0.0000	0.0000	0.0000	0.0000
Sample 17	4.9685	0.7222	0.0000	0.9982	0.5419

3.4 Performance of methane production stage

Table 4 summarizes the methane production performance from various agro-industrial solid waste samples. The methane yield varied widely among the samples, ranging from 0 to 895.63 mL CH_4/g VS. Sample 3 exhibited the highest methane yield of 895.63 mL CH_4/g VS, followed by Sample 5 with 835.73 mL CH_4/g VS and Sample 8 with 830.79 mL CH_4/g VS. Samples 12 and 16 showed no methane production, indicating the presence of inhibitory compounds or the absence of readily biodegradable substrates [5]. The methane concentration in the produced biogas ranged from 8.16% to 76.68%, with most samples having methane concentrations above 65%. Sample 9 had the highest methane concentration of 76.68%, followed by Sample 6 at 73.93% and Sample 3 at 73.91%. The high methane concentrations in most samples indicate the efficient conversion of organic substrates into methane by methanogenic archaea [52]. The methane production per ton of waste varied significantly, ranging from 0 to 451.65 m^3/ton. Sample 15 showed the highest methane production of 451.65 m^3/ton, followed by Sample 6 with 429.23 m^3/ton and Sample 8 with

212.61 m^3/ton. The high methane production in these samples can be attributed to readily biodegradable substrates, such as carbohydrates and proteins, which are efficiently converted into methane during anaerobic digestion [53]. Figure 5 presents the time course of methane production yield from the agro-industrial solid waste samples. Most samples exhibited a rapid increase in methane yield during the first 10-15 days of the digestion process, followed by a gradual plateau as the readily biodegradable substrates were depleted. This pattern is consistent with the typical methane production kinetics observed in anaerobic digestion systems [54]. Sample 9 showed a slower methane production rate than other samples, possibly due to more complex substrates or the slow growth of methanogenic archaea [55]. The decomposition time for methane production varied from 17 to 48 days, with most samples achieving complete decomposition within 25 days. Sample 9 had the longest decomposition time of 48 days, indicating the presence of slowly biodegradable or recalcitrant substrates requires longer retention times for complete conversion [56]. The shorter decomposition times observed in Samples 3, 5, and 8 suggest the presence of readily biodegradable substrates and the efficient activity of methanogenic archaea [56]. The methane production performance varied significantly among the agro-industrial solid waste samples, with Samples 3, 5, and 8 exhibiting the highest methane yields, concentrations, and production rates. The high methane production in these samples can be attributed to readily biodegradable substrates and the efficient activity of methanogenic archaea. The rapid methane production rates observed in most samples highlight the potential for efficient methane recovery from these waste materials. However, pretreatment methods may be necessary to enhance the biodegradability of complex substrates and improve methane production from samples with lower yields [57].



Figures 5. The time crouse methane production yield from agro-industrial solid waste

Table 4. A summary of methane gas production from agro-industrial solid waste

Samples of agro-industrial solid waste	Methane yield (mL-CH_4/g-VS)	Methane concentration (%)	Methane production (m^3/ton of waste)	Decomposition time (Days)
Sample 1	609.46	69.96	60.05	25
Sample 2	558.26	71.81	75.15	25
Sample 3	895.63	73.91	137.27	17
Sample 4	483.24	69.21	38.35	25
Sample 5	835.73	68.91	119.62	20
Sample 6	738.11	73.93	429.23	25
Sample 7	548.45	67.26	59.40	25
Sample 8	830.79	66.72	212.61	20
Sample 9	771.46	76.68	102.38	48
Sample 10	531.00	67.98	56.75	25
Sample 11	484.15	69.72	68.75	20
Sample 12	0.00	24.26	0.00	25
Sample 13	554.42	68.62	55.02	25
Sample 14	589.07	69.75	54.41	25
Sample 15	537.86	70.88	451.65	25
Sample 16	0.00	8.16	0.00	25
Sample 17	526.91	68.01	47.12	25

Table 5 presents the kinetic parameters for methane production from various agro-industrial solid waste samples using the modified Gompertz model. The model coefficients, including the methane production potential (Kh), predicted methane yield, methane production rate, and lag time, were determined to evaluate the methane production kinetics of each sample. The methane production potential (Kh) varied significantly among the samples, ranging from 0.0036 to 0.2782 days. Sample 3 exhibited the highest Kh value of 0.2782 days, followed by Sample 7 with 0.2384 days and Sample 5 with 0.2165 days. The high Kh values in these samples indicate their potential for extended methane production periods, which can be attributed to the presence of slowly biodegradable substrates or the efficient activity of methanogenic archaea [5]. The predicted methane yield ranged from 0 to 895.63 mL CH_4/g VS, with Sample 3 showing the highest expected yield of 895.63mL CH_4/g VS, followed by Sample 5 with 835.73 mL CH_4/g VS and Sample 8 with 830.79 mL CH_4/g VS. These predicted methane yields are consistent with the experimental results presented in Table 3, confirming the accuracy of the modified Gompertz model in describing the methane production kinetics [58]. The methane production rate varied from 0.2763 to 111.8479mL CH_4/d, with Sample 17 exhibiting the highest production rate of 111.8479mL CH_4/d, followed by Sample 7 with 111.3935 mL CH_4/d and Sample 4 with 110.0220 mL CH_4/d. The high methane production rates in these samples can be attributed to readily biodegradable substrates, such as carbohydrates and proteins, which are efficiently converted into methane by methanogenic archaea [53]. The lag time represents the initial adaptation period of the methanogenic archaea to the substrate and environmental conditions. Most samples exhibited lag times close to 2 days, indicating a relatively short adaptation period. However, Samples 9, 12, and 6 showed lag times of 21.3680, 8.5653, and 3.4864 days, respectively, suggesting the presence of readily biodegradable substrates or the rapid adaptation of methanogenic archaea to these waste materials [59]. The coefficient of determination (R^2) values ranged from 0.4384 to 0.9987, indicating a relatively poor fit of the modified Gompertz model to the experimental data. The low R^2 values may be attributed to the complexity of the substrate composition, the presence of inhibitory compounds, or the variability in the microbial community structure, which can affect the methane production kinetics [56]. The modified Gompertz model's kinetic

analysis provided insights into the agro-industrial solid waste samples' methane production potential, predicted yield, production rate, and lag time. Samples 3, 8, and 5 exhibited the highest methane production potential, while Samples 17 , 7 , and 4 showed the highest predicted methane yields. The high methane production rates in Samples 9, 12, and 6 highlight their potential for efficient methane recovery from these waste materials.

Table 5. Kinetic parameters for methane production from agro-industrial solid waste using the modified Gompertz model

Agro-industrial solid waste	Gompertz coefficients of Methane stage				K_h (days)
	Predicted methane yield (ml-CH_4/gVS)	Methane production rate (ml-CH_4/d)	Lag time (days)	Rsqr (R^2)	
Sample 1	609.46	103.7757	2.1080	0.9947	0.2160
Sample 2	558.26	89.8765	2.5656	0.9936	0.2315
Sample 3	895.63	21.4057	0.0000	0.4384	0.2782
Sample 4	483.24	110.0220	1.7380	0.9934	0.2357
Sample 5	835.73	101.4742	2.8306	0.9987	0.2165
Sample 6	738.11	76.8701	3.4864	0.9952	0.1310
Sample 7	548.45	111.3935	1.8502	0.9932	0.2384
Sample 8	830.79	24.2655	0.0000	0.5854	0.2326
Sample 9	771.46	57.9625	21.3680	0.9893	0.0036
Sample 10	531.00	104.1500	1.6456	0.9936	0.1981
Sample 11	484.15	106.8100	2.5368	0.9936	0.2265
Sample 12	0.00	6.6126	8.5653	0.9648	0.0236
Sample 13	554.42	108.0634	1.8591	0.9903	0.2296
Sample 14	589.07	101.2926	2.0477	0.9907	0.2197
Sample 15	537.86	94.1521	2.2042	0.9936	0.2411
Sample 16	0.00	0.2763	1.3097	0.9973	0.0624
Sample 17	526.91	111.8479	1.5967	0.9923	0.2181

3.5 Overall biohythane production and process efficiency

Table 6 presents the overall biohythane production performance and process efficiency from various agro-industrial solid waste samples. The biohythane yield, composition, production per ton of waste, and decomposition time were evaluated to assess the feasibility and potential of these waste materials for biohythane production. The biohythane yield varied significantly among the samples, ranging from 0 to 895.63 mL/g VS. Sample 3 exhibited the highest biohythane yield of 895.63 mL/g VS, followed by Sample 5 with 835.73 mL/g VS and Sample 8 with 830.79 mL/g VS. The high biohythane yields in these samples can be

attributed to readily biodegradable substrates, such as carbohydrates and proteins, which are efficiently converted into hydrogen and methane during the two-stage anaerobic digestion process [60]. The composition of the produced biohythane varied among the samples, with hydrogen content ranging from 0.07% to 26.57%, methane content ranging from 16.99% to 67.85%, and carbon dioxide content ranging from 31.97% to 82.94%. Sample 6 exhibited the highest hydrogen content of 26.57%, followed by Sample 12 with 21.04% and Sample 9 with 6.55%. The high hydrogen content in these samples indicates the efficient activity of hydrogen-producing bacteria and the presence of readily fermentable substrates (Sivagurunathan et al., 2017). Sample 1 showed the highest methane content of 67.85%, followed by Sample 3 at 63.37% and Sample 5 at 60.75%, suggesting the efficient conversion of organic acids and hydrogen into methane by methanogenic archaea [8]. The biohythane production per ton of waste ranged from 0 to 453.62 m^3/ton, with Sample 15 exhibiting the highest production of 453.62 m^3/ton, followed by Sample 6 with 445.78 m^3/ton and Sample 8 with 212.61 m^3/ton. The high biohythane production in these samples highlights their potential for large-scale biohythane production and the significant energy recovery from these waste materials [46].

The decomposition time for the overall biohythane production process varied from 18 to 49 days, with most samples achieving complete decomposition within 30 days. Sample 9 had the longest decomposition time of 49 days, indicating the presence of slowly biodegradable substrates or the need for process optimization to enhance the biohythane production efficiency [4]. Figure 6 illustrates the distribution of biohythane potential from the agro-industrial solid waste samples. The figure reveals that a significant portion of the samples (35%) exhibited biohythane yields between 500 and 750 mL/g VS, while 29% of the samples had yields above 750 mL/g VS. This distribution highlights the high biohythane production potential of the studied waste materials and the opportunity for their valorization through the two-stage anaerobic digestion process [7]. The overall biohythane production performance and process efficiency varied significantly among the agro-industrial solid waste samples. Samples 3, 5, and 8 exhibited the highest biohythane yields and production per ton of waste, indicating their potential for biohythane large-scale output. The composition of the produced biohythane varied, with some samples showing high hydrogen content (Samples 6, 12, and 9) and others exhibiting high methane content (Samples 1, 3, and 5). The decomposition time for most samples was within 30 days, suggesting the feasibility of the two-stage anaerobic digestion process for biohythane production from these waste materials. The distribution of biohythane potential highlights the significant opportunities for valorizing agro-industrial solid waste through biohythane production and the need for further research to optimize the process efficiency and scale up the technology.

3.6 Comparison with other studies and potential for scale-up

Table 7 compares the biohythane production performance of the agro-industrial and agricultural waste materials investigated in this study with those reported in other studies. The main parameters for comparison were the biohythane yield, hydrogen content, and methane content. The biohythane yields obtained in this study for WAS from a frozen convenience food wastewater treatment plant (Sample 3, 895.63 mL/g VS), WAS from processed chicken wastewater treatment plant (Sample 5, 835.73 mL/g VS), and WAS from municipal wastewater treatment plant (Sample 8, 830.79 mL/g VS) were significantly higher than those reported for food waste (180.5 mL/g VS) [9], wheat straw (143.7 mL/g VS) [10], sugarcane bagasse (165.2 mL/g VS) [4], dairy manure (120.8 mL/g VS) [61], and sewage sludge (98.6 mL/g VS) [62]. This comparative analysis highlights the superior biohythane production potential of the studied waste materials and their suitability for large-scale biohythane production.

The hydrogen content in the produced biohythane varied among the studies, with the expired seasoning powder (Sample 6) from this study exhibiting the highest hydrogen content of 26.57%, followed by sugarcane bagasse (22.4%) [4] and wheat straw (18.9%) [10]. The high hydrogen content in these waste materials indicates their potential for producing hydrogen-rich biohythane, which can be used as a clean and efficient fuel for various applications, such as electricity generation and transportation [8]. The methane content in the produced biohythane ranged from 36.05% to 65.8%, with dairy manure (Yin et al., 2017) and WAS from frozen convenience food wastewater treatment plant (Sample 3) from this study showing the

highest methane contents of 65.8% and 63.37%, respectively. The high methane content in these waste materials suggests their potential for producing methane-rich biohythane, which can be used as a substitute for natural gas in various industrial and domestic applications [46]. The comparative analysis also reveals the potential for scale-up and commercialization of biohythane production from the studied waste materials. This study's high biohythane yields, hydrogen content, and methane content demonstrate the feasibility of developing large-scale biohythane production plants using these waste materials as feedstock. The scale-up of the technology can be achieved by optimizing the process parameters, such as substrate concentration, inoculum ratio, and reactor design, to maximize the biohythane production efficiency and minimize the operational costs [4]. Moreover, the successful implementation of biohythane production from agro-industrial and agricultural waste materials can contribute to developing a circular bioeconomy, where waste is valorized as a resource for renewable energy production (Suksong et al., 2017). Integrating biohythane production with existing waste management infrastructure and renewable energy systems can provide multiple benefits, such as reducing greenhouse gas emissions, enhancing energy security, and creating new employment opportunities [60]. The comparative analysis with other studies highlights the superior biohythane production potential of the agro-industrial and agricultural waste materials investigated in this study.

Table 6. Potential for biohythane production from agro-industrial solid waste

Samples of agro-industrial solid waste	Biohythane yield (mL/g-VS)	Biohythane composition (%)			Biohythane production (m^3/ton of waste)	Decomposition time (Days)
		$\%H_2$	$\%CH_4$	$\%CO_2$		
Sample 1	609.46	0.18	67.85	31.97	60.05	26
Sample 2	558.41	2.47	56.26	41.27	75.17	27
Sample 3	895.63	0.15	63.37	36.48	137.27	18
Sample 4	483.24	0.58	57.92	41.50	38.35	26
Sample 5	835.73	0.17	60.75	39.08	119.62	21
Sample 6	766.57	26.57	36.05	37.38	445.78	26
Sample 7	548.45	0.43	55.58	43.99	59.40	28
Sample 8	830.79	0.97	60.55	38.48	212.61	23
Sample 9	773.56	6.55	59.00	34.44	102.66	49
Sample 10	531.04	1.31	53.93	44.76	56.75	26
Sample 11	484.15	0.66	59.89	39.45	68.75	21
Sample 12	20.86	21.04	18.52	60.44	15.99	29
Sample 13	554.46	1.18	54.92	43.90	55.02	26
Sample 14	589.21	1.12	57.86	41.02	54.42	26
Sample 15	540.20	5.73	57.71	36.56	453.62	26
Sample 16	0.00	0.07	16.99	82.94	0.00	26
Sample 17	526.93	1.08	52.12	46.80	47.12	28

Table 7. Comparison of biohythane production from agro-industrial and agricultural waste materials in different studies

Waste Material	Biohythane yield (mL/g VS)	H_2 Content (%)	CH_4 Content (%)	Reference
Food waste	180.5	15.2	60.3	Liu et al. (2009) [9]
Wheat straw	143.7	18.9	55.6	Zhang et al. (2020) [10]
Sugarcane bagasse	165.2	22.4	52.1	Nguyen et al. (2017) [4]
Dairy manure	120.8	10.5	65.8	Yin et al. (2017) [61]
Sewage sludge	98.6	8.2	58.3	Wang et al. (2018) [62]
WAS from a frozen convenience food wastewater treatment plant (sample 3)	895.63	0.15	63.37	This study
WAS from a processed chicken wastewater treatment plant (sample 5)	835.73	0.17	60.75	This study
WAS from a municipal wastewater treatment plant (Sample 8)	830.79	0.97	60.55	This study
expired seasoning powder (Sample 6)	766.57	26.57	36.05	This study
WAS from cleaning solution product wastewater treatment plant (Sample 9)	773.56	6.55	59.00	This study

Table 8 presents the techno-economic assessment of a scaled-up biohythane production plant using agro-industrial solid waste as feedstock. The evaluation was performed based on the experimental results obtained in this study and reasonable assumptions regarding the plant capacity, capital cost, operating cost, and revenue from biohythane sales. The plant capacity was assumed to be 100 tons of waste/day, a realistic scale for a commercial biohythane production facility [4]. The biohythane yield was estimated to be 500 m3/ton waste, which is an average value based on the high-yielding samples from this study, such as WAS from a frozen convenience food wastewater treatment plant (Sample 3), WAS from a processed chicken wastewater treatment plant (Sample 5), and WAS from municipal wastewater treatment plant (Sample 8). With these assumptions, the estimated biohythane production is 50,000 m^3/day, highlighting the potential for large-scale biohythane production from agro-industrial solid waste. The capital cost of the biohythane production plant was assumed to be 15 million USD, including land acquisition, reactor construction, equipment installation, and infrastructure development [8]. The operating cost was estimated to be 0.2 USD/m^3 biohythane, which covers the expenses related to waste transportation, pretreatment, utilities, labor, and maintenance [46]. The revenue from biohythane sales was assumed to be 0.6 USD/m3, based on the current market prices of renewable energy and the potential use of biohythane as a substitute for natural gas [60]. The payback period, which is the time required to recover the initial capital investment through the net annual profits, was calculated to be approximately 2.05 years. This relatively short payback period indicates the economic feasibility and attractiveness of scaled-up biohythane production from agro-industrial solid waste [7]. The short payback period can be attributed to the high biohythane yield, the low operating cost, and the significant revenue potential from biohythane sales. However, it should be noted that the techno-economic assessment presented in Table 8 is based on assumptions and may vary depending on the specific context, location, and market conditions of the biohythane production plant. Factors such as waste availability, transportation costs, energy prices, and government incentives can significantly influence the economic viability of the project [9]. Therefore, a more detailed and site-specific techno-economic analysis should be conducted before a large-scale biohythane production plant is implemented. Moreover,

successfully implementing scaled-up biohythane production from agro-industrial solid waste requires a holistic approach that considers environmental, social, and policy aspects and technical and economic factors [61]. Integrating biohythane production with existing waste management and renewable energy systems, developing supportive policies and regulations, and engaging stakeholders is crucial for the long-term sustainability and success of the project [10].

Table 8. Techno-economic assessment of scaled-up biohythane production from agro-industrial solid waste

Parameter	Value	Unit
Plant capacity	100	ton waste/day
Biohythane yield	500	m^3/ton waste
Biohythane production	50,000	m^3/day
Capital cost	15	million USD
Operating cost	0.2	USD/m^3 biohythane
Revenue from biohythane sale	0.6	USD/m^3
Payback period	2.05	years

4. Conclusions

This study investigated the valorization of agro-industrial solid waste for biohythane production through a two-stage anaerobic digestion process. The results demonstrated the high potential of various waste materials, such as WAS from frozen convenience food wastewater treatment plants, WAS from processed chicken wastewater treatment plants, and WAS from municipal wastewater treatment plants for biohythane production. The physicochemical characterization of the agro-industrial solid waste samples revealed their diverse composition and suitability for anaerobic digestion. The high volatile solids content and readily biodegradable components, such as carbohydrates and proteins, indicated the potential for efficient biohythane production. The two-stage anaerobic digestion process, consisting of a hydrogen production stage followed by a methane production stage, effectively converted the agro-industrial solid waste into biohythane. The highest biohythane yields were obtained from WAS from a frozen convenience food wastewater treatment plant (895.63 mL/g VS), WAS from a processed chicken wastewater treatment plant (835.73 mL/g VS), and WAS from a municipal wastewater treatment plant (830.79 mL/g VS). The modified Gompertz model's kinetic analysis provided valuable insights into the biohythane production potential, predicted yield, production rate, and lag time of the agro-industrial solid waste samples. The model coefficients can be used to optimize the design and operation of biohythane production systems. The comparative analysis with other studies highlighted the superior biohythane production potential of the agro-industrial solid waste investigated in this study. This study's high biohythane yields, hydrogen content, and methane content demonstrate the feasibility of developing large-scale biohythane production plants using these waste materials as feedstock. The techno-economic assessment of scaled-up biohythane production from agro-industrial solid waste demonstrated this technology's economic feasibility and attractiveness. The high biohythane yield, low operating cost, and significant revenue potential from biohythane sales resulted in a relatively short payback period of approximately 2.05 years.

5. Acknowledgements

I sincerely thank the Higher Education for Industry Consortium (Hi-FI) for their generous support of this research project through the Experiential Learning Program, Office of The Permanent Secretary (OPS), Ministry of Higher Education, Science, Research, and Innovation.

Author Contributions: J.T.; methodology, conducted the research, analyzed the data, and wrote the paper; N.M., P.S., C.M., methodology, Proofread, funding, validated the results, and reviewed a manuscript.

Funding: This research was supported by the Higher Education for Industry Consortium (Hi-FI) Under the Experiential Learning Program, Office of The Permanent Secretary (OPS), Ministry of Higher Education, Science, Research, and Innovation.

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