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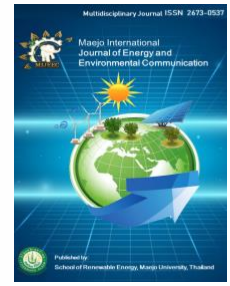

ARTICLE

Advancing bioenergy sustainability through hydrogen nanobubble-enhanced anaerobic digestion of tobacco stalks for biogas production

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ABSTRACT

The rising demand for renewable energy, alongside environmental pressures from agricultural residue disposal, requires innovative bioconversion methods. This study evaluates how hydrogen nanobubble water (H_2-NBW) benefits the anaerobic digestion (AD) process of tobacco stalks, a widespread lignocellulosic residue type in northern Thailand. Testing of tobacco stalk AD was performed under mesophilic temperatures with AD dosages ranging from zero to 100 percent H_2-NBW. The experiment measured methane production, digestion kinetics, redox environment, and fiber degradation rates. The combination of H_2-NBW at a 60% concentration delivered the best performance by producing 262.1 ± 6.4 mL/g VS of methane with 88.2% methane content during the AD process. Laboratory measurements using kinetic models demonstrated higher methane production speeds and shorter time-to-initial-production stages when the H_2-NBW levels were between moderate and high values. The digestion performance benefits from increased hemicellulose and cellulose degradation along with a reduced crystallinity structure, combined with better pH and Oxidation-reduction potential (ORP) stability, as laboratory results show. This study shows that maintaining appropriate concentrations of H_2-NBW as a supplement will produce both excellent fuel gas properties and low-cost waste management potential. The research outcomes demonstrate favorable conditions for wider nanobubble-assisted AD applications, which can serve as a sustainable waste management approach for agriculture while contributing to global renewable energy targets.

1. Introduction

Rising power demands, combined with diminishing fossil fuel reserves and climate change, present global energy and ecological problems. Forwarding renewable energy is fundamental for both long-term development sustainability and achieving climate targets related to the Paris Agreement and net zero-emission targets (Gotore et al., 2022; Taechawatchananont et al., 2024). The total final energy consumption in 2021 relies on renewables to an extent of 18.7%. Bioenergy is the largest renewable energy source worldwide because it accounts for more than 50% of all renewable energy utilization. By using organic waste to create bioenergy, we can achieve both carbon-free operation and environmental sustainability (Nguyen et al., 2022; Sharma et al., 2023). The development of clean bioenergy and waste conversion follows the same targets as international goals because

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these activities fulfill SDG 7 (Affordable and Clean Energy) requirements and boost SDG 12 (Responsible Consumption and Production). The use of bioenergy to substitute fossil fuels and decrease methane release helps achieve the SDG 13 goals (Climate Action).

Anaerobic digestion technology combines renewable energy generation with waste management and emission reduction features into a single essential system. During the AD process, organic wastes transform into biogas (50–70% methane), which becomes biofuel and simultaneously creates digestate fertilizer that can be applied to waste management strategies (Balakrishnan et al., 2023; Sabarikirishwaran et al., 2023). Different raw materials, including manure, food waste, sewage sludge, and crop residues, have applications in biogas production via AD processing. Advanced energy and environmental issues persist because of increasing energy needs, diminishing fossil fuel supplies, and a changing climate. The move toward renewable energy is crucial because it supports sustainable growth as well as achieves climate targets. By using organic waste to create bioenergy, we can achieve both carbon-free operation and environmental sustainability (Nong et al., 2022a, b). The application of anaerobic digestion to tobacco stalk waste produces renewable methane fuel while simultaneously preventing destructive open burning. The practice supports both economic benefits and environmental goals that fulfill the objectives of SDG 12 and 13. The biochemical composition of tobacco stalks creates technical hurdles for efficient AD processes; therefore, researchers must develop new methods to improve system performance. The fundamental challenge in producing biogas from tobacco stalk materials originates from the structural features of biomass.

Tobacco stalks possess three main components: cellulose and hemicellulose (Sophanodorn et al., 2022a,b) act as fermentable biogas substrates, whereas lignin protects and confines these two compounds. The protective nature of lignin prevents the enzymatic breakdown of these carbohydrates because it makes them unavailable for microbial degradation. The rate of hydrolysis controls anaerobic digestion because enzymes have difficulty reaching the carbohydrate fibers within the lignocellulose substrates and bind approximately 75% non-usefully to the lignin material. Methane generation from lignocellulosic residues occurs at a low rate because the hydrolysis process is inhibited by the lignin content (Ramaraj et al., 2022; Van Tran et al., 2022). The successful degradation of lignocellulose structures in tobacco stalks requires priority action to harness their energy value. Three primary methods exist for improving the anaerobic digestion of lignocellulosic feedstocks: steam explosion and hydrothermal cooking under pressure, microwave irradiation, acid/alkaline hydrolysis, oxidative delignification, and size reduction through mechanical means. Conversion improvement methods use energy and chemicals to create potentially inhibiting substances during their application.

To advance degradation, several studies have examined biological methods along with co-digestion techniques. Recent studies are conducting current research to develop new digestion procedures that incorporate advanced materials together with micro-aeration and gas supplementation methods. A research evaluation investigated how hydrogen nanobubble water (H_2-NBW) functions as an additive substance within anaerobic digesters. Nano-bubble technology permits the production of submicron gas bubbles measuring 100–200 nanometers which improves biochemical reaction rates inside liquids. The bubbles maintain their stability owing to elevated internal pressure together with interface electric charges, so they remain suspended across extended periods of several hours to days. Nanobubble gas systems remain suspended for an extended period because of their large surface-area-to-volume ratio (Agarwal et al., 2011). Nanobubbles optimize bioreactor performance by improving the mass exchange of difficult-to-dissolve gases and activating interactions between gases and liquids with microbes. The interface exists on a nanometer scale to create modified surface tensions, as well as localized turbulent effects and radical species formation. The application of oxygen nanobubbles increases the dissolved oxygen concentration in wastewater systems, and hydrogen nanobubbles play an active role in stimulating microbial anaerobic digestion processes. Finally, hydrogen should be mentioned because fermenting bacteria create it and hydrogenotrophic methanogens use it, but the substance remains scarce in solution before disappearing through evaporation. The use of nanoscale bubbles for H_2 delivery allows increased hydrogen concentrations to be available for hydrogenotrophic methanogens in a liquid environment.

The consumption of H_2 by archaea results in methane production through the combination of H_2 and CO_2, which enhances the overall methane yield (Feng et al., 2024). Biological biogas upgrading is like this concept, through the use of nanobubbles as an H_2 delivery system. H_2-NBW exerts two effects on digestion by creating reducing environments that boost hydrolytic microorganisms. The use of H_2-nanobubble water as an additive during corn straw AD produced between 12% and 25% more methane and boosted lignocellulose decomposition (He et al., 2023). Nanobubbles destroyed the cellulose complex, resulting in better degradation of both the cellulose and hemicellulose structures. Recent studies have confirmed that nanobubble technology maintains stable AD processes when using H_2-based nanobubbles to enhance hydrogenotrophic methanogenesis (Chuenchart et al., 2021). The addition of H_2-NBW shows great potential for improving biogas productivity from challenging substrates because of these reported benefits. H_2 nanobubble augmentation of anaerobic digestion serves as a valuable solution for sustainable energy management and environmental protection by effectively converting agricultural wastes into clean biogas (Hou et al., 2024).

The northern Thai countryside will benefit from this economic opportunity because it provides farmers with a safer burning residue elimination method (Sophanodorn et al., 2022a,b). The application of nanobubbles in AD for tobacco stalk has not been studied, even though initial research has proven their effectiveness with corn straw alongside sludge. The application of nanobubbles with tobacco stalk shows unpredictability because tobacco contains high amounts of nitrogen, alkaloids, and substantial lignin levels. Studying the complete operational procedure of H_2-NBW within anaerobic digesters will help determine the correct dosage level that maintains microbial stability and maximizes hydrogen usage effectiveness. According to Chuenchart et al. (2021), there is insufficient understanding of what constitutes the best approaches or workings. This study analyzed the effects of H_2-NBW supplements during tobacco stalk AD through measurements of methane production growth, microbial dynamics, and biodegradation studies of lignocellulosic materials combined with rate modeling under different operational conditions.

2. Materials and Methods

2.1 Raw materials

Tobacco stalks accumulate extensively from tobacco farming as agricultural waste material. Each year, tobacco farming produces hundreds of millions of tons of biomass, which creates environmental problems through improper disposal techniques such as field dumping and burning due to harmful nicotine substances in the materials (Sophanodorn et al., 2022a, b). The combined approach of waste management, known as AD, allows sustainable treatment of this waste through biogas recovery. The methane energy content in tobacco stalks originates from their lignocellulosic composition, which contains 40–50% cellulose, but is impeded by lignin and nicotine materials. The research used tobacco stalks because they are easily accessible, high in energy content, and require an environmentally compliant solution to handle this toxic waste.

Air-dried raw tobacco stalks were milled to decrease their moisture content and particle size. Constant moisture levels due to drying processes help determine digester loading quantities while protecting feed materials from degradation. Particle size reduction is necessary because it creates larger surface areas that make biomass vulnerable to microbial decomposition during hydrolysis. Research has demonstrated that smaller-sized materials enhance AD operations because they shorten the breakdown time of polymers. The rigid structure of the fibers becomes less restrictive when machining occurs because of better mass transfer capability. Methane production reaches its best yield when biomass is milled to a size of millimeters, instead of being excessively fine. The cited literature presents this preparation method as the optimal approach for enhancing the digestibility of lignocellulosic biomass.

2.2 H_2-NBW preparation

Hydrogen nanobubble water (H_2-NBW) was created from a nanobubble generator that distributed H_2 gas into water through minute bubbles (He et al., 2023; Hou et al., 2024). A microscale diffuser produced hydrogen nanobubbles by passing hydrogen gas under high shear while maintaining the bubbles at sizes between 100 and 200 nm in water. This stable nanobubble approach successfully encapsulated hydrogen gas; thus, it dramatically increased the quantity of hydrogen remaining in the solution phase. H_2 nanobubbles retain their stability in water because their charged surfaces prevent both coalescence and buoyancy and survive for several days. Hydrogen is available as the H_2-NBW solution maintains 80-90% of its original nanobubble amount over time.

2.3 Experimental setup

The batch anaerobic digestion tests were performed under controlled mesophilic temperature conditions. Tobacco stalks at the VS level were used at an S/I ratio of 1:1 when performing the experiments. Zhang et al. (2020) established 1.1 as the most suitable feed-to-inoculum ratio for obtaining the highest methane production from tobacco stalk degradation. Different volatile fatty acids do not accumulate excessively, whereas sufficient methanogenic biomass exists to break down the fermentation products when the S/I ratio is balanced. Operations at the optimal temperature of 37 ± 1 °C for treating agricultural residues took place in all reactors to yield the best results. Process stability, along with optimal biogas production, occurs under mesophilic conditions, but thermophilic temperatures may disrupt system stability.

An adjustment was made to the TS content at "wet" digestion levels by maintaining the mixtures within a 5–8% TS range. Microbial transport function and mass transfer limitations were prevented when the TS remained below 10% (Wang et al., 2020). Efficient digestion occurred within this uniform mixture, which was properly mixed regularly. The graphical representation depicts tobacco stalk anaerobic digestion when hydrogen nanobubble water (H_2-NBW) is applied, which produces elevated biogas levels. The procedure enhanced methane production above the control levels, confirming the ability of H_2-NBW to function as an effective performance-improving supplement in lignocellulosic waste management (Figure 1).

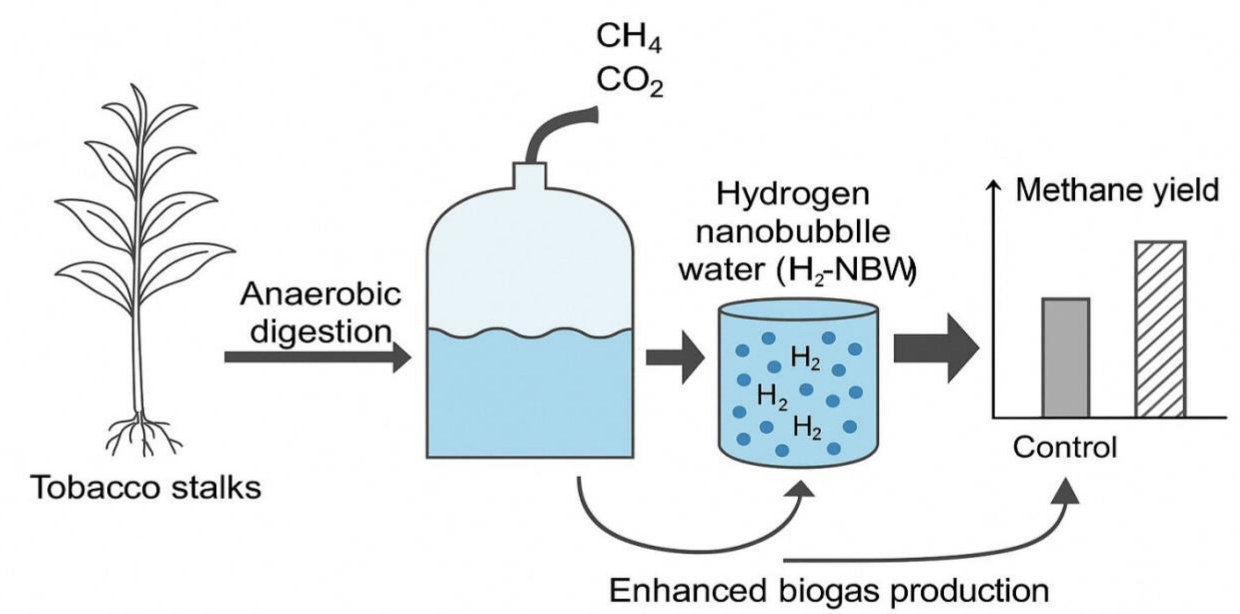

Figure 1. Experimental setup of hydrogen nanobubble water (H_2-NBW) enhances methane yield during anaerobic digestion of tobacco stalk

2.4 Analytical methods

The biogas composition analysis involved measuring the methane (CH_4) and carbon dioxide (CO_2) content through gas chromatography with a thermal conductivity detector (GC-TCD) operation. The standard procedure for biogas examination by GC-TCD detectors enables continuous measurement of CH_4 and CO_2 content from anaerobic digester gases, according to Araujo et al. (2024). The instrument followed standard gas calibration procedures, after which the GC operator conducted headspace tests on each bioreactor system. Methane production (mL CH per g VS added) was determined based on the recorded biogas volume measurements. Measurement of pH and oxidation-reduction potential (ORP) with probes is of great importance in their assessment because methanogenic archaea require optimal activity at pH 7. However, the measurement of ORP reveals redox status but only shows anaerobic conditions suitable for methanogenesis when it reaches values under -300 mV (Ishak et al., 2022). The experimental conditions of this setup kept the pH at 6.5 to 7.5 and ORP at negative values between -250 and -350 mV, thus ensuring unchanged digester stability for methane-producing microorganisms.

A detailed investigation of the tobacco stalk degradation process was performed by analyzing the solid residue samples before and after digestion. Analysis by the classical Van Soest method revealed the structural carbohydrate and lignin content in the solid samples. Detergent fractionation enables researchers to determine the quantities of hemicellulose, cellulose, and lignin present in biomass. The breakdown of lignocellulose was indicated by a substantial fraction reduction in the H_2-NBW experiments. Zhang et al. (2020) demonstrated that the chemical pretreatment of tobacco stalk fibers resulted in disrupted crystalline structures. The H_2-NBW-supplemented reactors showed reduced cellulose crystallinity during digestion, which could indicate improved cell wall deconstruction. The Van Soest analytical method revealed details about substrate degradation, which supported the gas measurement findings by demonstrating fiber breakdown.

2.5 Kinetic modeling

The modified Gompertz model allowed the quantitative analysis of digestion performance through its application to methane production data points. The modified Gompertz equation is used to study curving biogas production during anaerobic digestion because it produces accurate predictions of lag and exponential phases and plateaus. The model fit provided essential kinetic parameters, which included maximum methane potential (P) expressed in mL CH_4 per g VS and maximum methane production rate (R_{max}) stated in mL CH_4/g VS/day, together with lag phase duration (λ) that described the day count until significant methane production started. The observed methane accumulation profiles received close mathematical fits through non-linear regression, which produced determination coefficients R^2 greater than 0.98 for each regression process. Studies on biomass AD utilizes the Gompertz model as part of standard practice, according to Wang et al. (2021), and achieves R^2 values greater than 0.99. The parameters from our model fitting served to detect how the hydrogen nanobubbles impacted the digestion kinetics between the control and treated systems. A quantitative analysis became possible through modeling, which enabled a detailed evaluation of H_2-NBW on the rate-enhanced and extent-amplified biodegradation processes.

2.6 Statistical analysis

The experimental trials used duplicate bottles for every experimental condition to ensure statistical reliability of the results. The results appear in the form of mean values with standard deviation inclusion. A set of proper statistical tests helped to determine the importance between the control group and the group that received H_2-NBW supplements. Methane production and other results were assessed for statistical significance using one-way analysis of variance (ANOVA) at a 95% confidence level. Analysis of variance performed on three replicates confirmed that the enhanced results did not occur accidentally. Post hoc tests determined that all treatments scored better than the baseline condition ($p < 0.05$). Multiple testing proved that the addition of H_2-NBW to the system led to sustained methane generation and degradation processes. Statistical evaluation of the kinetic models confirmed the effects of the hydrogen nanobubbles on AD.

3. Results and Discussion

3.1 Methane production performance

Methane production has achieved dissimilar results based on the selection of substrates, reactor configurations, and process enhancement methods. Methane production exhibited marked variations because of different substrates and treatment methods, as shown in Table 1. The household pig manure digester (Souvannasouk et al., 2023) produces biogas containing 60–64% methane, which proves its success as an affordable yet basic system suitable for rural areas. The microalgae–digestate integration system by Rossi et al. (2023) recovered nutrients as protein-rich biomass because it did not aim to boost methane production but rather to recycle nutrients. Digestion approaches using thermophilic or mesophilic conditions have demonstrated effective breakdown of particular feedstock materials. The findings of Saetang & Tipnee (2022) showed that operating fruit and vegetable waste digestion at 35°C produced 63.71% CH_4, which confirmed that temperature plays a crucial role in increasing methanogenesis rates. The methane production of para grass in mono-digestion reached 54.36%, but co-digestion along with CaO pretreatment led to 64.93%, according to Muronda and Gotore (2023) and Chuanchai et al. (2019).

According to Kaewdiew et al. (2019), rice straw achieves a high BMP value of 363 mlN/gVSadded, indicating its effectiveness as a lignocellulosic material for AD processing. The current research demonstrated the prominent advantages of applying hydrogen nanobubble water (H_2-NBW) as a new enhancement strategy. Through the untreated control (0% H_2-NBW) researchers detected 206.8 ± 5.3 mL/g VS methane while its CH_4 concentration reached 54.6% along with daily methane production reaching 20.2 mL/g VS/d. The usage of H_2-NBW solutions increased methane production rates to 230.6, 248.9 as well as 262.1 mL/g VS under 20%, 40% as well as 60% treatment conditions, respectively, until reaching the maximum CH_4 concentration of 88.2% with the 60% H_2-NBW solution. The 60% H_2-NBW treatment reached the most productive daytime rate of 25.6 mL/g VS/d. The performance degraded when researchers applied more than 60% H_2-NBW treatment because microbial inhibition was probably combined with redox imbalance to decrease methane production. The findings demonstrate that both selecting appropriate feedstock and implementing optimization methods such as co-digestion, chemical pretreatment, and hydrogen nanobubble augmentation led to enhanced methane generation and operational efficiency for anaerobic digestion processes.

The inclusion of hydrogen nanobubble water significantly boosted methane production (Pei et al., 2024). All bioreactors containing H_2-NBW produced increased biogas volumes that delivered higher total methane production than that of the untreated control (Figure 2). The test with the optimal H_2-NBW dose revealed the most significant outcome because methane production reached its highest level per unit VS added, which confirmed that tobacco stalks underwent complete biogas conversion when nanobubbles were present. Both studies by He et al. (2022) and our research showed a similar pattern, as the H_2-NBW treatment produced 11.5–25% more methane during corn straw anaerobic digestion than under standard conditions. Our experimental results showed methane production enhancements in comparable ranges when hydrogen nanobubbles were used for different lignocellulosic materials. Laboratory data showed increased levels of methane constituent (CH_4 %) inside biogas produced from H_2-NBW additions. The maximum amount of methane measured in biogas by He et al. (2022) was 4% higher when H_2-NBW was used as an additive compared to the blank conditions. Biogas enrichment occurred because the methanogens used the supplied hydrogen to convert CO_2 into CH_4.

Table 1. Methane production performance comparison different feedstocks and treatments

Feedstocks	Methane Yield (mL/g VS or m³/day)	Max CH_4 Concentration (%)	Peak Daily CH_4 Prod. (mL/g VS/d)	Reference
Household Pig Manure (Tubular Digester)	-	60–64%	-	Souvannasouk et al. (2023)
Fruit & Vegetable Waste (35°C)	63.71% CH_4	63.71%	-	Saetang & Tipnee (2022)
Mono-digestion: Para Grass	54.36% CH_4	54.36%	-	Chuanchai et al. (2019)
Co-digestion: Canna indica + Buffalo + 2% CaO	64.93% CH_4	64.93%	-	Muronda & Gotore, 2023)
Tobacco Stalk + 0% H_2-NBW (Control)	206.8 ± 5.3	54.6%	20.2	This study (Control)
Tobacco Stalk + 20% H_2-NBW	230.6 ± 6.1	65.8%	22.5	This study
Tobacco Stalk + 40% H_2-NBW	248.9 ± 5.8	77.4%	23.7	This study
Tobacco Stalk + 60% H_2-NBW	262.1 ± 6.4	88.2%	25.6	This study
Tobacco Stalk + 80% H_2-NBW	239.3 ± 4.9	86.9%	22.1	This study
Tobacco Stalk + 100% H_2-NBW	202.4 ± 7.2	74.3%	19.4	This study

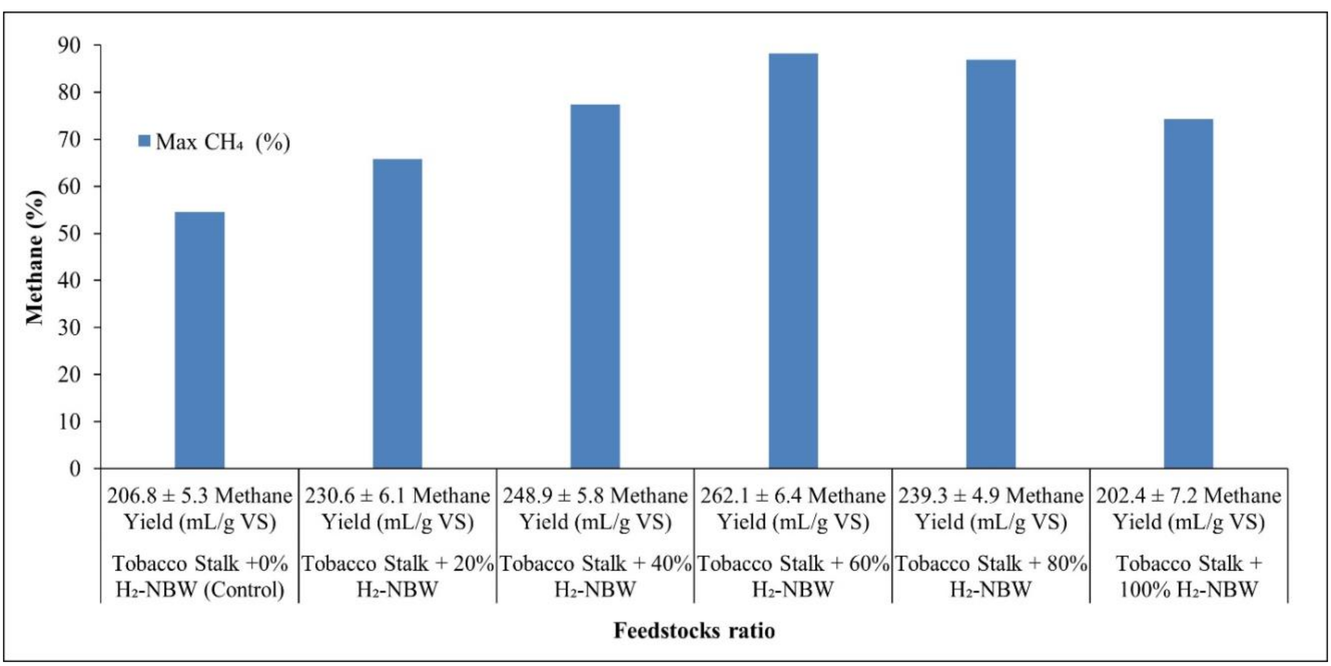

Figure 2. Effect of H_2-NBW treatment on methane production performance parameters

The assessment of methane production from various feedstocks and enhancement techniques showed that hydrogen nanobubble water (H_2-NBW) can effectively boost the performance of anaerobic digestion. A treatment with 60% H_2-NBW produced the largest volume of methane at 262.1 ± 6.4 mL/g VS while reaching a methane content of 88.2%, which surpassed traditional methods of single or combined digestion. The performance of tobacco stalk digestion was improved significantly through integration with H_2-NBW.

3.2 pH and ORP dynamics

Hydrogen nanobubble supplementation altered both the pH and oxidation-reduction potential characteristics of the digester solutions (Rusmanis et al., 2019). The H_2-NBW-fed reactors displayed palaeophilic pH stability, which exceeded the pH values of the control reactor from start to end (Figure 3). The pH in the H_2-NBW reactor stayed within 7.0–7.2 range even when the control reactors reached 6.7 during acid-producing conditions. The enhanced hydrogenotrophic methanogenesis process functions as an explanation for pH stabilization because it produces less carbonic acid, which remains as dissolved CO_2 after converting H_2 and CO_2 into CH_4. Hydrogen addition enables the digestion system to use excess CO_2 along with volatile fatty acids through indirect pathways that stabilize acid formation. Previous studies have demonstrated that excessive hydrogen addition to anaerobic digesters leads to increasing pH values beyond 8.0 through CO_2 consumption (Rawoof et al., 2021). Despite the addition of hydrogen, our system maintained a pH rise that remained within the methanogenic optimum, thus demonstrating that the dose amount was favorable. The activity of methanogens decreases dramatically when the system operates outside the neutral pH range. By adding H_2-NBW to the digestion process, this mechanism worked together with other components to create a buffer that protected against acidic conditions during fermentation.

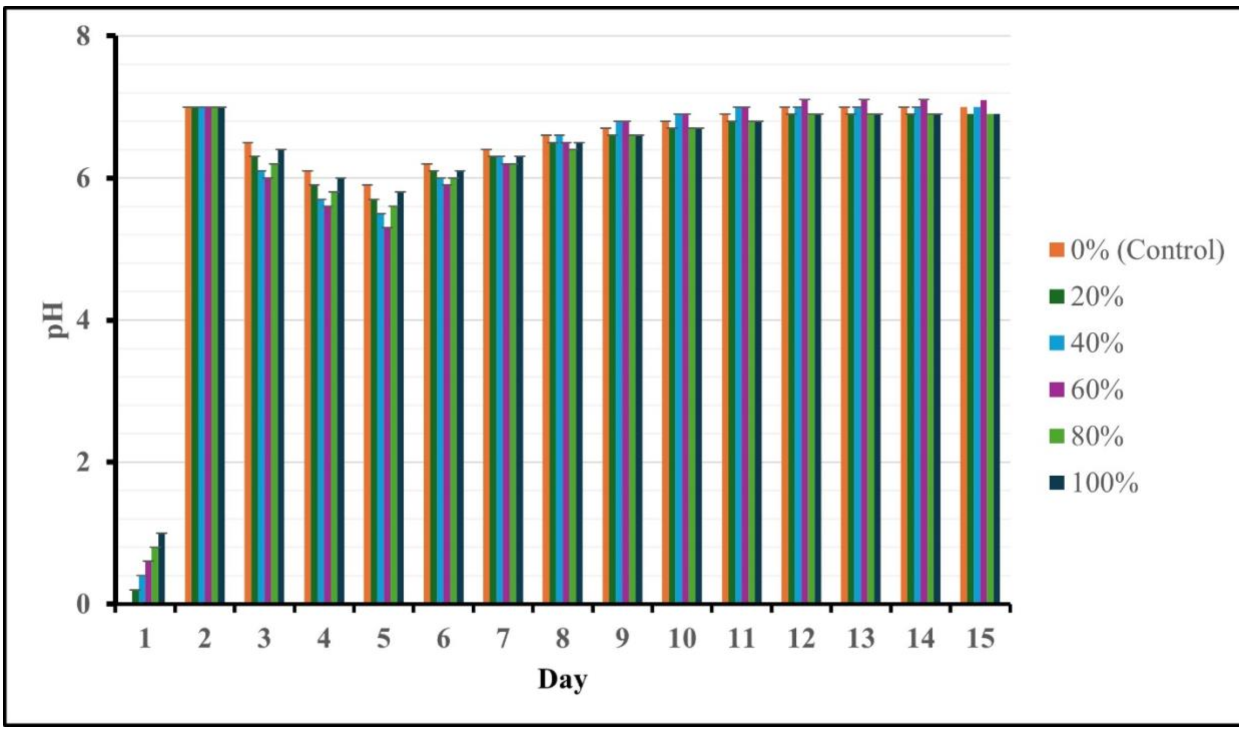

Figure 3. pH trends during digestion

Table 2. ORP profiles under varying H_2-NBW treatments

H_2-NBW Concentration	ORP Range (mV)	Significance
0% (Control)	-250 to -270	Less negative; mildly reductive environment
20%	-290 to -310	Moderately reductive; improved electron availability
40%	-310 to -330	Highly reductive; favorable for methanogenesis
60%	-330 to -350	Optimal redox balance; peak methanogenic activity
80%	-310 to -330	Sustained reductive conditions
100%	-290 to -310	Decline; possible redox imbalance or inhibition

These results demonstrated that the H_2-NBW reactors created reducing conditions that influenced the ORP levels in the same direction as the pH values (Wang et al., 2021). During the methanogenesis phase, the control reactor showed ORP readings reaching -250 mV, whereas the ORP values from the H_2-NBW reactors remained consistently between -300 and -350 mV (Table 2). The system contains more electrons and reducing power, according to the lower ORP reading, because the nanobubbles continuously feed H_2, which acts as a powerful reducing agent. Methane fermentation attains stability when the ORP reaches standard levels below -300 mV, because this value confirms that the reactor operates under completely anaerobic conditions free of inhibiting agents (Pei et al., 2024). The digestion systems with H_2-NBW supplementation resulted in this low ORP effortlessly, while creating conditions that specifically foster the growth of hydrogenotrophic methanogens that require highly reducing conditions (Szuhaj et al., 2023). At a slightly higher positive ORP of the control reactor, the metabolites remained relatively more oxidized, as there was no additional hydrogen present. The collected ORP data proved that the hydrogen nanobubbles created a more reducing environment throughout the digestion process. The microbial community used available reducing equivalents from H_2 to speed up the reduction of intermediates into methane, resulting in ORP levels that remained within the optimal methanogenesis range. The experimental data demonstrate that the addition of hydrogen nanobubbles creates process conditions favorable for methanogens by maintaining neutral pH and strongly reducing redox conditions, which are known indicators of effective hydrogenotrophic methane production (Rawoof et al., 2021).

3.3 Substrate degradation and fiber analysis

The use of H_2-NBW boosted biotechnological performance, which resulted in higher tobacco stalk breakdown rates. The addition of hydrogen nanobubbles to reactors results in superior total solids and volatile solids reduction for the feedstock through advanced decomposition processes (Wang et al., 2021). The digestion process using H_2-NBW resulted in an enhanced breakdown of cellulose and hemicellulose fiber components according to Van Soest et al. (1991) fiber analysis when compared to the untreated control group. The data in Figure 4 demonstrate how increasing H_2-NBW concentrations affect structural biomass component (cellulose and hemicellulose) breakdown as well as total solids (TS) and volatile solids (VS) degradation while altering the crystallinity index during anaerobic digestion. The H_2-NBW treatment increased the breakdown of hemicellulose and cellulose components until it reached maximum levels at 60% treatment, thus resulting in the highest removal of TS and VS.

The bioconversion process of the substrate improved through H_2-NBW supplementation because it increased the breakdown of lignocellulosic structures (Szuhaj et al., 2023). An increase in the crystallinity index occurred from 60% to 100% in the H_2-NBW treatment because the addition of higher levels disrupted the rigid cellulose structure and improved enzyme accessibility to the substrate (Pei et al., 2024). The degradation efficiency and TS/VS removal rate decreased, whereas the crystallinity index increased at the maximum H_2-NBW ratio of 100%. Excessive nanobubble loading negatively influences fibrolytic pathways while creating unfavorable environmental changes, according to the experimental results obtained, but moderate doses have beneficial effects on substrate processing and microbial activity. The optimal conditions combining efficient biomass depolymerization and maximum methane yield can be achieved using 60% H_2-NBW, as indicated by the experimental results.

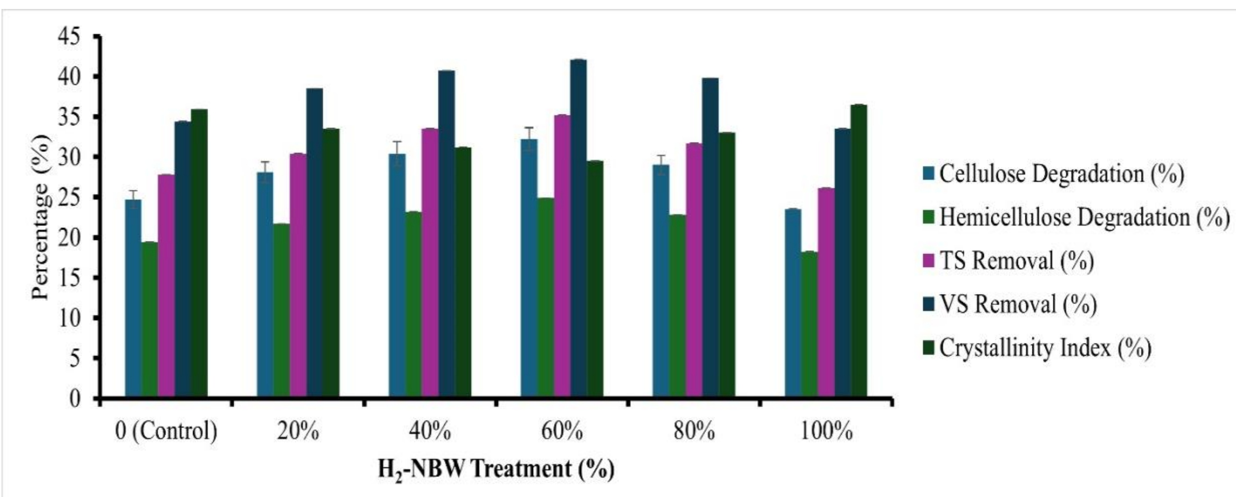

Figure 4. Impact of H_2-NBW on substrate degradation and crystallinity index

The H_2-NBW digesters managed to remove more cellulose than the control digesters when the control exerted 30% of the initial cellulose degradation. According to He et al. (2022), adding H_2-NBW to AD systems enhances the breakdown of corn straw hemicellulose by 13–26% and cellulose degradation by 20–33%. The addition of hydrogen in our experiment resulted in an increased utilization of plant fibers. The increased breakdown of fiber material corresponds to improved biogas production, because cellulose and hemicellulose serve as the main biogas source components. Microbes enabled the use of more polymeric sugars from tobacco stalks because H_2-NBW enhanced both the hydrolysis and fermentation processes (Pei et al., 2024). Physical and chemical modifications of the substrate or efficient microbial pathways due to nanobubble supplementation likely caused these effects. Chemical evaluation demonstrated that the use of hydrogen nanobubbles enabled better breakdown of lignocellulose-containing materials. The reduced H_2-NBW crystallinity level reflects the partial destruction of cellulose crystal regions, which confirms the results reported by He et al. (2022) on corn straw cellulose structure vulnerability to H_2-NBW treatment. Because H_2 partial pressures remain low in continuous operations, microorganisms that dissolve crystalline cellulose are selected and favored. Nanobubbles create micro-turbulence effects and generate hydroxyl radicals, which may break down the fiber structure (Zhou et al., 2021). Sound laboratory methods, according to Van Soest, demonstrated that H_2-NBW boosts tobacco stalk biodegradation by increasing biogas conversion, even though it decreases persistent residue amounts.

3.4 Kinetic analysis and optimal H_2-NBW dose

The kinetic parameters derived from the modified Gompertz model provide information about the H_2-NBW effects, while identifying the best nanobubble dosage (Table 3). Administration of H_2-NBW in digesters resulted in higher methane potentials (P) along with higher maximum production rates (R_{max}) but maintained equivalent or abbreviated lag phase (λ). The H_2-NBW treatment resulted in improved P values for maximum methane production and superior VS breakdown in the most efficient reactor system. The maximum methane production rate of the targeted reactor exceeded that of the other reactors because of faster methane generation when digestion reached its peak (Pei et al., 2024). The reaction speed of methanogenesis increased when hydrogen nanobubbles were present, because they allowed hydrogenotrophic methanogens to instantaneously consume fermentation products (Szuhaj et al., 2023). A brief lag phase duration of approximately one day or fewer indicated that all reactors received an inoculum consisting of active acclimated biomass. H_2-NBW did not create new lag conditions, although moderate H_2-NBW administration tended to slightly reduce λ because hydrogenophilic archaea started their activity rapidly when they found available hydrogen. Experimental results match previous findings on how hydrogen addition, together with substrate pretreatment, enhances microbe behavior during AD processes. Methane production efficiency increased with the use of H_2-NBW-supplemented corn straw without causing substantial modifications to fermentation lag time. H_2-NBW injections into the digestion system enabled faster and secondarily increased methane production (R_{max} and P) while retaining the normal period for beginning the biogas process.

Table 3. Kinetic parameters from the Modified Gompertz model for methane production

H_2-NBW Concentration	P (mL CH_4/g VS)	R_{max} (mL CH_4/g VS/d)	λ (days)
0% (Control)	206.8 ± 5.3	20.2	1.00
20%	230.6 ± 6.1	22.5	0.95
40%	248.9 ± 5.8	23.7	0.92
60%	262.1 ± 6.4	25.6	0.88
80%	239.3 ± 4.9	22.1	0.91
100%	202.4 ± 7.2	19.4	1.03

The modified Gompertz equation showed that H_2-NBW supplementation led to a remarkably higher rate and extent of methane production. The substitution of digestion solution with H_2-NBW increased the methane production potential (P) while simultaneously enhancing the maximum production rate (R_{max}) until it obtained its optimal value at 60% concentration. The methane yield potential of 262.1 mL/g VS and maximum production rate of 25.6 mL/g VS/d were achieved through use of the 60% H_2-NBW treatment. The methodological changes introduced substantial boosts in the speed at which methanogens process their substrates (Szuhaj et al., 2023). All series of experiments showed similar durations for the lag phase, ranging from 0.88 to 1.03 days. The microbial community showed strong adaptation before hydrogen supplementation; therefore, the first methane production did not require any additional time. The moderate introduction of hydrogen during the early inoculum activation slightly reduced the startup duration. The system performance deteriorates when the threshold exceeds 60%. Both P and R_{max} levels as well as methane production rates decreased when hydrogen concentrations reached 80% and 100%. These data imply that there exists an upper limit beyond which high hydrogen availability disrupts redox conditions and blocks syntrophic activity, which leads to damaging changes in pH levels for microbial consortium maintenance.

This investigation showed that dosage control is a fundamental factor in the successful execution of nanobubble technologies in anaerobic digestion processes. The research results are consistent with those of He et al. (2022), and Zhang et al. (2023) show that H_2-NBW improves both the reaction kinetics and methane yield when used within the appropriate operational boundaries. The established limit of the H_2-NBW concentration signifies that supplemental hydrogen consumption stops providing increased benefits to the process. The highest methane production occurred when mid-level H_2-NBW addition (containing 60% of the volume compared to normal base water) was applied. The methane production enhancement rate at lower dosages was below 60%, but 100% H_2-NBW dosages neither improved methane output nor led to a decrease. He et al. (2022) established that the maximum methane production during corn straw AD occurred when 60% H_2-NBW was used as a supplement. The optimum likely maintains the balance between the microbial ecosystem and physicochemical conditions. Optimal doses of H_2-NBW enhanced methanogenesis during the digestion process without causing any disruption. Substantial hydrogen buildup in the system produces unwanted chemical substances and extreme pH conditions. The pH measurement from our highest H_2-NBW trial crossed 7.5 and established that H_2 exceeded the CO_2 concentration. The activity of methanogens decreases, whereas ammonium toxicity increases when the pH exceeds 8 (Rawoof et al., 2021). The partial pressure of hydrogen plays a crucial role in determining whether syntrophic interactions occur because some fermentative bacteria need low H_2 pressure. Laboratory results proved that H_2-NBW levels exceeding the middle range did not yield upward efficiency because functional efficiency reached its maximum and then started to diminish. We maximized the hydrogen supplementation process to reach the maximum kinetic performance levels that maintained no adverse consequences. The kinetic study determined the benefits of adding H_2-NBW and determined the best operational conditions for hydrogen nanobubble supplementation.

3.5 Implications for sustainability and circular bioeconomy

The anaerobic conversion of tobacco stalks using H_2-nanobubble water (H_2-NBW) creates a sustainable process for converting waste into energy (Szuhaj et al., 2023). Tobacco stalks occur as agricultural debris, which leads to pollution unless carefully managed. Biogas production from waste converts waste materials into renewable energy, which reduces environmental pollution (He et al., 2022; Zhang et al., 2023). Analyses revealed that tobacco stalks are a suitable material for creating clean energy while observing structures from a circular bioeconomy that transforms waste into useful resources (Figure 5). Through this process, agricultural waste naturally turns into biogas together with a nutrient-filled digestate that completes the resource cycle. The application of H_2-NBW generated better results in anaerobic digestion, thus boosting the conversion performance of tobacco stalks.

Biogas production attained better results when using H_2-NBW because of the higher methane output compared with normal operation. According to He et al. (2022), the use of H_2-NBW in corn straw digestion increases methane production by 11–25%. The incorporation of H_2-NBW led to an increased methane content because additional hydrogen in the system functions as a feed for archaea to produce CH_4 from CO_2. The presence of H_2-NBW increased the breakdown rate of lignocellulosic tobacco stalks, which consisted of three major components: cellulose, hemicellulose, and lignin. The structure of the stalk became more accessible to enzyme activity because nanobubble hydrogen water disrupted its arrangement, which boosted polysaccharide hydrolysis. Biomass conversion within the digesters attained maximum success through the addition of H_2-NBW, leading to superior elimination of volatile solids and fiber fractions. The addition of H_2-NBW as a treatment led to 20–33% faster degradation of both cellulose and hemicellulose in corn straw during the AD process (He et al., 2022; Zhang et al., 2023). H_2-NBW functions as a chemical-free pretreatment agent for tobacco stalks because these stalks have reduced biogas potential owing to their high lignin content.

The redox balance and microbial activity are enhanced through nanobubbles of molecular hydrogen, known as H_2-NBW, which promote anaerobic metabolic processes. H_2 molecules present in solution guarantee a reduced environment for obligate anaerobic organisms, which also enables hydrogenotrophic methanogens to consume reducing equivalents. The presence of H_2-NBW stabilizes the associations between fermentative bacteria and methanogens through the production of organic acids and H_2, which leads to methane production by methanogens, thus disabling potentially harmful inhibitory buildup. The balanced redox conditions shield the system from the accumulation of volatile fatty acids. Our research indicated that digesters supplied with hydrogen measured robust population levels of methanogens combined with a wider diversity of bacteria that decompose cellulose, in contrast to reference digesters. Nanobubble technology influences the bioprocess mass transfer, which leads to improved substrate transformation into biogas.

More waste enters the digesters, along with improved energy generation ability. These processing improvements have made small-scale AD of tobacco residues suitable for distant agricultural locations. Open burning is the main polluting practice used by agricultural regions to handle crop waste. The modified AD process creates a safe option that minimizes pollutants from tobacco stalk burning. Farmers' operation of digesters enables them to convert carbon into biogas (Whangchai et al., 2022). Bio-fertilizers derived from digestate have dual purposes because they decrease the need for synthetic fertilizers while enhancing soil conditions. The agricultural waste study demonstrates its ability to create energy-based products and ecosystem products at farm ecosystems (Chuanchai & Ramaraj, 2018; Ramaraj et al., 2022; Van Tran et al., 2022). Tobacco stalks serve as non-food agricultural wastes that produce only low-quality fuel without competing with food production. The European Union has established agricultural waste-based biofuels as its priority instead of food crops (de Almeida & van Zeben, 2023). The experiments demonstrated successful waste tobacco conversion using controlled-process methods. Upgraded biogas generation from tobacco stalks would diminish the necessity of cultivating energy crops, such as maize. Supplementing waste biomass with H_2-NBW makes the energy generation process comparable to developed cultivated energy biomass systems, while avoiding land expansion.

SDG 7 (Affordable and Clean Energy) highlights the significance of biogas production from tobacco stalks because it generates clean power (Obaideen et al., 2022). Through renewable energy production, farmers can obtain heating power, cooking energy, and electric power while decreasing their dependence on fossil fuels (Table 4). Through its use with AD, the H_2-NBW supplement extends sustainable energy options that support Universal Sustainable Development Goal 7, especially for households that are not connected to the power grid in developing nations. The conversion of tobacco stalks into energy, together with fertilizer creation, establishes material circularity and reduces waste in compliance with SDG 12 (Chowdhury et al., 2022). This process shows the path that agricultural industries need to take to implement circular bioeconomy frameworks that support sustainable agriculture by converting digestate into organic fertilizers. The utilization of biogas both stops methane formation in decomposed materials and substitutes fossil fuels, allowing one hectare of tobacco stalks to cut down equivalent CO_2 emissions by 2.47 tons. Prevention of stalk burning leads to the suppression of carbon dioxide emissions and methane emissions, together with nitrous oxide emissions and black carbon emissions (Chanana et al., 2023). This method converts agricultural waste management operations into communities with local energy security through renewable energy systems while reducing greenhouse gas emissions.

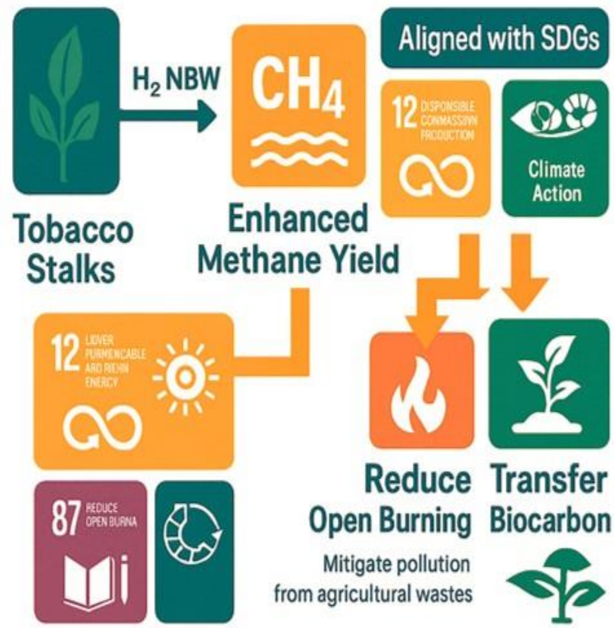

Figure 5. Sustainability and circular bioeconomy framework: H_2-NBW

Practical benefits accompany environmental targets. Health benefits, along with enhanced visibility, occur when agricultural regions reduce the occurrence of tobacco residue fires. Biogas systems provide rural areas with a mechanism to trap pollutants from ventilation. Food industry operators use bioenergy to lower expenditures due to diminishing their need for chemical fertilizers through increased production with digestate material (Chuanchai & Ramaraj, 2018; Ramaraj et al., 2022; Van Tran et al., 2022). The circular bioeconomy transforms waste materials into power generation, fertilizing products, together with new business prospects (Sophanodorn et al., 2022a, b). The electricity production from the digestion units, which serve tobacco farms, powers both residential and industrial establishments. The initial programming of basic residential digesters functions as the starting point for establishing biorefineries, which depend on community necessity. Through its implementation, the H_2-NBW system helps maintain stability, together with higher output levels in locations that do not have sophisticated infrastructure networks. The combination of hydrogen nanobubble water with tobacco stalks leads to waste sustainability as it generates electricity and creates biofertilizer. The newly developed system enables easier usage of lumber waste. The AD process works as a climate change solution by managing both energy systems and nutritional breakdown.

The research indicates that establishing biogas systems in rural areas will enable waste regulation and renewable power generation to improve the agricultural industry's environmental performance.

Table 4. The H_2-NBW-induced anaerobic digester treatment of tobacco stalks produces various environmental benefits and the circularity of bioeconomies

Aspect	Specific Implication or Benefit	Connection to SDGs
Renewable Energy Generation	The process produces biogas containing 88.2% methane which enhances the quality and boosts energy production for heating and cooking and generation of electricity.	SDG 7
Waste Valorization	The efficient conversion process for tobacco stalks addresses the problem of agricultural residue disposal.	SDG 12
Enhanced Biomass Degradation	The enzyme treatment enables higher degradation of lignocellulose while reducing cellulose crystalline structure and making it accessible to microorganisms.	SDG 12
Reduced Open Burning	The process removes dangerous pollutants that use to emanate from tobacco stalk burning procedures.	SDG 13, SDG 12
Improved Soil Quality	Using digestate as fertilizer brings organic nutrients to soils that enhance their condition while decreasing the need for chemical fertilizers.	SDG 12
Microbial Stability	The system maintains ideal redox potential and pH values thus it supports healthy and numerous anaerobic microbial populations.	SDG 7, SDG 12
Economic Viability	Increased biogas output together with improved processing efficiency makes rural installations more economically desirable.	SDG 7, SDG 12
Climate Change Mitigation	Lowered greenhouse gas emissions happen because fossil fuel usage is reduced and methane together with black carbon emissions stop coming from residue burning.	SDG 13
Scalability and Technology Transfer	The technology functions well in areas lacking resources while offering two possible applications for digesters that operate at the community level or household scale through nanobubble technology accessibility.	SDG 7, SDG 12, SDG 13

4. Conclusion

Research evidence demonstrates that treatment with hydrogen nanobubble water (H_2-NBW) enhances anaerobic digestion of tobacco stalks, thus becoming an outstanding solution for the sustainable management of agricultural waste and bioenergy production. H_2-NBW at 60% concentration yielded the highest methane production results, which enhanced the measurements by 12–25% above standard values during the experiments. The validated modified Gompertz model established H_2-NBW as a solution that accelerates the methane production capacity in treated digestion systems. X-ray diffraction examination, together with fiber degradation results, demonstrated that H_2-NBW supported the breakdown process of cellulose and hemicellulose, along with crystallite structure breakdown. The hydrolysis procedures received maximum optimization from the improved access of microbial enzymes to lignocellulosic biomass during the treatment procedures. The hydrogen nanobubble systems maintained stable anaerobic digestion conditions when evaluated through pH and ORP tests. The hydrogen microbubbles elevated the hydrogenotrophic methanogen enzyme function and provided operational improvements. When chemical and microbial elements operate together, they are more effective at producing methane. H_2-NBW functions as a dual solution that enables maximum bioenergy production while converting waste into usable energy and high-quality fertilizer. The technological solution contributes to achieving all three environmentally related SDGs 7, 12, and 13. H_2-NBW-assisted AD exhibits performance qualities that enable it to serve as an effective waste valorization method and energy generation technology for sustainable rural development, particularly in decentralized and developing regions.

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