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Comparative Study of Total Volume of Biohydrogen Produced and Yield across Reactors using Different Wood Materials

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Abstract

Research on the comparative investigation to determine the total volume of $BioH_2$ produced and $BioH_2$ yield across the reactors set-up was monitored by considering different species of wood sawdust as well as experimental design concepts. The investigation revealed that the biohydrogen gas production across the various biodigester set-up demonstrated the significance contribution of the substrate composition (word saw dust) and the available nutrient contained and the potential of the microorganism's capacity to degradation the for the production of the biohydrogen gas. The order of the magnitude of biohydrogen production ($BioH_2$) is UHP2 > USP2 > USHP2 > UHM2 > HSP1 > HMP3 > UMP3 > HSM1 > USM2 > HSHM1 > HSHP1 > USHM2 > HMP1 > HHM1 other products obtained alongside with biohydrogen gas are methane (CH_4), carbon dioxide (CO_2) and hydrogen sulphide (H_2S). The rate of products produced was dependent of the composition of the substrate formation as well as the function of microbial activity in the biodigester set-up. The critical comparative concepts were involved for the evaluation of the total volume of $BioH_2$ obtained in the bioreactors set-up and the unhydrolyzed hardwood (UHP2) of pure culture demonstrates pH value of 8.5 at particle size of 3.0 -mm, which revealed highest $BioH_2$ produced at 35, 711 ml. The research has well demonstrated the usefulness of words materials in the production of $bioH_2$, $bioCH_4$, $bioCO_2$, and bio H_2S and these products are of chemical important for industrial utilization and in some cases for energy application.

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INTRODUCTION

The greatest energy challenge of the 21st century is to develop sustainable energy resources which will meet future demands. In order to achieve energy sustainability, the approach should be geared towards decentralization of energy system. An energy production unit operation that is mobile, portable, easy to operate and reliance on local technology. Currently, fossil fuels are the primary energy sources used to satisfy the global energy demand. Following the oil crisis in 1973 and current energy crisis due to Russian-Ukrainian war, the need for developing alternative sustainable energy sources is more important now than ever to reduce the dependency on fossil fuels and assist with mitigating environmental problems due to the usage of fossil fuels. Changes in global

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environmental conditions due to greenhouse gases (GHG), especially increasing CO_2 and methane levels, can be linked to increasing use of fossil fuels [1–2]. In order to prevent increasing climatic issues, the need for researchers to develop economical and renewable fuels is evident. In response to these economic and environmental drivers, research on the use of hydrogen (H_2) as an energy carrier was initiated by the International Energy Agency [3].

Hydrogen is considered a strategically important energy carrier because of its high energy content (142 kJ g-1). Hydrogen is an alternative energy source because it can be produced from renewable feedstocks and H_2O the combustion byproduct of H_2 is a carbon neutral species [4]. A variety of feedstocks such as methane, natural gas, coal, water, alcohols, lignocellulosic biomass, glycerol, sugars, organic acids, wastewater and food waste can be used to produce H_2 using biological and non-biological methods [5–6]. Among these biological processes of H_2 production is preferred for using renewable sources such as organic waste or lignocellulosic biomass, which is more energy efficient in solving the growing energy needs [7]. Hydrogen is commercially produced using non-biological methods such as steam reforming, thermal cracking, gasification and pyrolysis of fossil fuels. Among the various non-biological methods, steam reforming of natural gas is the most widely used commercial method for producing H_2. According to the United States Department of Energy, the steam reforming method accounts for 90% of the H2 [8].

The United States Energy Information Administration has reported that approximately 10-11 million metric tonnes of H_2 are produced each year and can fuel 20-30 million cars [9]. However, the H_2 produced from non-biological sources is mainly used as a feedstock by chemical industries for the synthesis of ammonia, alcohol (methanol) and a variety of organic chemicals [10]. Steam reforming is an energy intensive process because of the high temperature (above 800 ºC) required for the reaction of steam with natural gas containing methane to produce H_2. Hydrogen gas production from water is considered a favourable renewable process, since H_2 is produced by the electrolysis of water. The disadvantage associated with electrolysis of the water is attributed to high capital cost and the operating cost which accounts for 80% of the production cost [11]. Other production methods which include thermal decomposition and photolysis are also energy intensive [11].

In order to avoid an undesirable scenario of using depleting resources to generate fuel with the energy intensive and GHG-contributing methods, H_2 production from renewable feedstocks has emerged as an alternative energy source. Bio- H_2 may prove to be a sustainable fuel as H_2 can be produced from organic waste. In comparison to the potential negative environmental impacts associated with the use of fossil fuels as well as the increase in fuel prices, the commercial production of H_2 from biomass has become very important [12]. Although pyrolysis, gasification and steam gasification techniques are available for converting biomass to a useful form of energy, increasing concern about global warming has increased, research interest in the development of environmentally friendly biological methods for H_2 production from biomass [13]. The contribution to global warming potential (GWP) by Pyrolysis, gasification and steam gasification process was reported to be on average of 1.8E+02 kg CO_2-equivalent. In each case the GWP contribution was majorly from the energy required at start up [14].

Biological H_2 production can be achieved by photolysis, photo-fermentation and dark- fermentation using green algae, Cyanobacteria, photosynthetic bacteria and anaerobic fermentative bacteria, respectively. Photo fermentation from different waste materials requires a light source and low photochemical conversion efficiency results in low H_2 Production Rates (HPR), while bio- photolysis from water produces O_2 in addition to H_2. A major disadvantage of the bio-photolysis process is the inhibitory effect of O_2 on hydrogenase leads to reduced HPRs. Thus, among different methods of hydrogen production, dark fermentation is a more sustainable and environmentally viable.

Dark fermentation is an anaerobic biodegradation of organic matter to produce hydrogen gas, organic acids and other bye products. Dark- fermentative H_2 production using a variety of carbon sources offers high HPR and produces high-value liquid metabolites (acetic, butyric and lactic acid) and lower alcohols

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(ethanol and butanol), which have some commercial value [15–17]. A major issue for commercial implementation of dark fermentation is the cost of feedstock that is required for H2 production [18]. Avoiding high cost in the production process can be accomplished using low value feedstocks or organic wastes. Dark fermentation can utilize a wide variety of renewable biomass sources which could be used in larger scale systems. Biomass sources include agricultural waste, woody and non-woody biomass [19–21], municipal solid waste [22] and food waste [23].

MATERIALS AND METHODS

Materials and Reagents

Different materials and reagents will be used for this research work as shows in Table 1.

Methods

This chapter details the experimental approach employed to investigate biohydrogen production from different wood sources using mixed microbial cultures in an anaerobic bioreactor. The selection criteria were based on their structural differences and availability of the wood material. Despite significant progress in fermentative H_2 production research and development, still technical and economic issues remain to be resolved before the technology can be adopted for producing H_2 from lignocellulosic biomass in large scale. In general, existing concerns for fermentative H_2 production are related to optimizing H_2 production by manipulating various factors affecting the performance of mixed anaerobic cultures. In order to achieve energy sustainability through hydrogen economy, the approach should be geared towards decentralization of hydrogen production system. An energy production system that is mobile, portable, easy to operate and reliance on local technology.

Also, there is another major issue affecting fermentative H_2 production using mixed anaerobic cultures which is the syntrophic association between H_2-producers and H_2-consumers (homoacetogens and hydrogenotrophic methanogens) as well as non-H_2 producers (such as acetoclastic methanogens, Ethanoligens and acidogens) which are linked to undesirable byproducts. In order to achieve optimal H_2 yields, pretreatment techniques are employed to enrich H_2-producers. A pretreatment technique that could be feasible and applied on a large scale to combat the H_2 consumers without affecting H_2 producers is required. Physical treatment such as heat and chemical inhibitors such as acetylene, 2-bromoethanesulfonate (BES) and long chain fatty acids (LCFAs) to suppress the activity of H_2 consumers is widely used, an innovative heat shock will be explored in this research. A system where *Clostridium* sp. was isolated from a medium and the medium was exposed to 150^{o}C to deactivate H_2-consumers in the medium for a period of one hour, and the H_2-producers will be reintroduced into the medium again after cooling. The use of heat shock is economically less feasible on large scale, while synthetic inhibitors such as BES could cause environmental effects if effluents from bioreactors are discharged into receiving water bodies.

Table 1. Materials and reagents.

S.N.	Materials and reagents	Quantity
1	Gas sampling bag (Tedlar^{R} bag)	14
2	Gas chromatography (GC: LGC-B18-Labtron)	1
3	High Powered liquid chromatograph	1
4	Laboratory scale bioreactors	14
5	Pilot scale bioreactor	1
6	Biomass material (Mahogany, Agba and Ako wood)	90% of reactor volume
7	Microbial cultures and single strain *Clostridium* sp.	Varied from low to high
8	Pretreatment (sulphuric acid, 2.5M)	0.5%v/v
9	Gas delivery tubes	As required by the design
10	Laboratory Glass wares	As required by the design
11	pH meter (PH/ION S220)	1
12	Bio-nutrient (Urine)	As required by the design
13	Sampling bottles (For VFA analysis)	14 X 3

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In comparison, LCFAs are biodegradable and preferred for selective enrichment of H_2-producers and suppression of methanogens in full-scale bioreactors, for this research palmitic acid was used for that purpose.

Selective large-scale enrichment involves a combination of several operational parameters as well as culture pretreatment. Furthermore, commercialization of dark- fermentation techniques for bio-H_2 production requires the achievement of high HPR in order for the technology to be economically feasible. Factors affecting HPRs are mainly the organic loading rate (OLR) and the hydraulic retention time (HRT) for a continuous bioreactor. Although several studies have examined the optimization of operational parameters and culture enrichment for maximal H_2 production, conflicting experimental outcomes have been reported. Linking the impact of multiple factors on the performance of reactors and diversity of microbial populations in full-scale reactors using low value feed stocks is lacking in the literature.

A detailed characterization of the process (i.e., the culture and byproducts formed under different operating conditions) will assist in optimizing H_2 production and developing an economically feasible full-scale process. Future research challenges to develop economically feasible bio-H_2 production methods will include effective conversion of lignocellulosics to consumable sugars during biomass pretreatment and increased substrate conversion efficiency in terms of H_2 production to overcome the inhibition caused by toxic substance (furfural, 5-hydroxymethylfurfural (5-HMF) and phenols. The performance of microbial communities is affected by environmental factors (pH, temperature), operational factors (OLR, HRT), substrate feed (type and concentration) and inoculum treatment. Assessing the impact of these factors on the performance of mixed microbial communities using genomic tools is important in optimizing H_2 production.

Apart from the use of hydrogen gas as fuel cells, there are other industrial processes that relies on hydrogen as a key component such as the syntheses of ammonia and the manufacture of nitrogenous fertilizers, methanol production, hydrochloric acid, Steel making, and hydrogenation of unsaturated vegetable oils among other applications, the use of green hydrogen as an alternate starting material for those processes was encouraged through the result of this research.The beauty of this research work lies in the fact that the variables selected for manipulation of the bioreactor will lead to different valuable products such as composite gas which is a mixture of methane gas and hydrogen gas. Composite gas can be used for cooking, also it can be separated into its constituent gas through the process called Pressure Swing Adsorption.

RESULTS AND DISCUSSION

Comparative Total Volume of BioH_2 Produced and BioH_2 Yield Across Reactors

The biohydrogen gas yield across the different biodigester configurations shows clear performance variations that reflect the influence of substrate composition, nutrient supplementation, and microbial activity within each unit. Among the digesters without urine bio-nutrient supplementation, yields generally ranged from low to moderately high. For instance, HHP1 and HHM1 produced some of the lowest hydrogen yields (33.19 ml of H_2/g of biomass and 30.24 ml of H_2/g of biomass), indicating suboptimal fermentation conditions, likely due to nutrient limitations, slow substrate hydrolysis, and inhibitory structural characteristics of the Mahogany wood material. Slightly better performance was observed in HSHP1 (68.24 ml of H_2/g of biomass), HSHM1 (71.51 ml of H_2/g of biomass), and HSM1 (85.36 ml of H_2/g of biomass), showing that those biodigester combinations were able to support moderate microbial activity even without additional nutrients. The highest yields within the non-supplemented category appeared in HSP1 (117.40 ml of H_2/g of biomass) and HMP3 (110.54 ml of H_2/g of biomass), suggesting that these digesters had more favourable configurations, possibly due to wood type, inoculum strength, and improved fermentability of the substrate.

In contrast, biodigesters (Figure 1), supplemented with urine-based bio-nutrient, consistently

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produced higher hydrogen yields, sharply distinguishing them from the non-supplemented systems. For example, UHM2 (119.06 ml of H_2/g of biomass), USHM2 (72.73 ml of H_2/g of biomass), USM2 (84.57 ml of H_2/g of biomass), and UMP3 (87.20 ml of H_2/g of biomass) represent stable and improved yields across their configurations, indicating the positive impact of nutrient-rich urine on microbial performance. More notably, three biodigesters, USP2 (142.45 ml of H_2/g of biomass), USHP2 (134.41 ml of H_2/g of biomass), and UHP2 (148.39 ml of H_2/g of biomass), achieved the highest yields in the entire experiment as shown in Figure 2.

These units benefited greatly from the balanced nitrogen, urea, phosphate, and trace minerals present in the bio-nutrient, which enhanced microbial proliferation, enzyme activity, and substrate conversion efficiency. In these digesters, the synergy between nutrient supplementation and substrate degradability resulted in markedly superior hydrogen production. A clear trend emerges when comparing the two groups: biodigesters without bio-nutrients show wider variability and generally lower yields, while those supplemented with urine produce consistently higher and more stable hydrogen volumes.

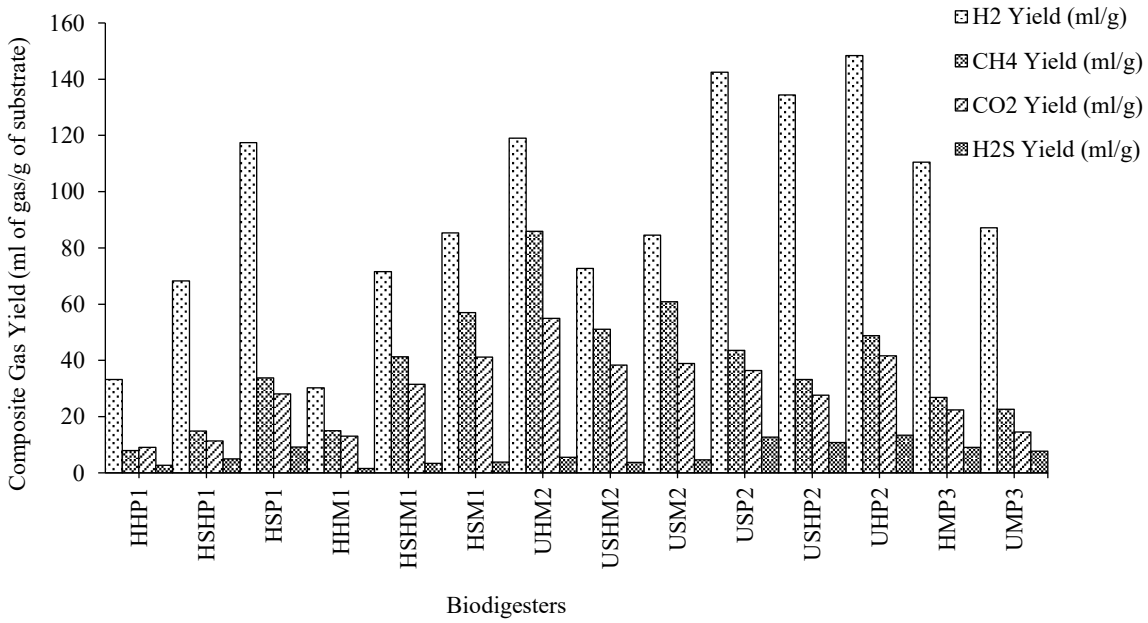


Figure 1. Gas yields from different wood materials.

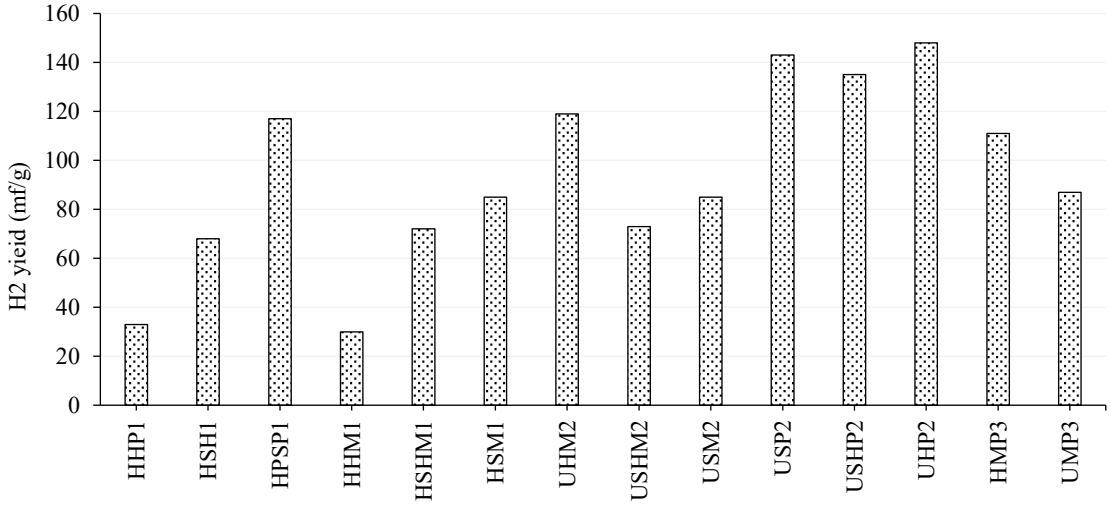


Figure 2: Biohydrogen gas yield (ml of H_2/g of biomass).

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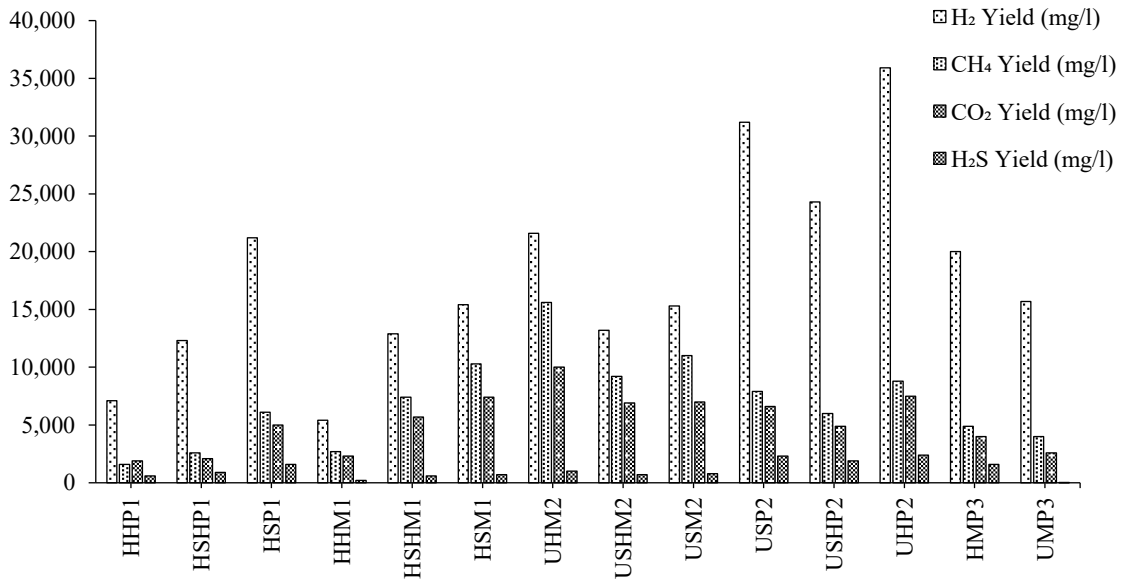


Figure 3: Total volume of gas produced (ml)

This demonstrates that nutrient availability is a major limiting factor in lignocellulosic hydrogen fermentation. The supplemented digesters provided microorganisms with the metabolic requirements needed to efficiently break down the wood-based substrates and generate higher hydrogen outputs. Overall, the biodigester performance patterns strongly highlight the crucial role of nutrient enhancement in maximizing hydrogen yield, with the supplemented systems outperforming the non-supplemented ones across virtually all configurations.

A critical comparative analysis total volume of $BioH_2$ produced across the biodigester configurations revealed substantial variability depending on process parameters, most notably the hydrolysis condition, microbial strain, reactor pH, and biomass particle size. Figure 3 shows the experimental gas yields and total volume produced, while this section focuses on dissecting the factors contributing to the highest and lowest H_2 outputs, with a specific spotlight on UHP2, the best-performing configuration in terms of hydrogen production. Among all the tested configurations, UHP2 (Unhydrolyzed Hardwood with Pure Culture at pH 8.5 and 3.0-mm particle size) exhibited the highest H_2 produced at 35,711 ml.

This was significantly higher than other setups, including both hydrolyzed and unhydrolyzed systems, indicating that certain unhydrolyzed substrates can outperform hydrolyzed ones under optimal conditions. The second-best performer, USP2, also an unhydrolyzed pure-culture system, produced total volume of 31,041 ml of H_2. These findings highlight the potential of raw biomass in biohydrogen processes when matched with the right microbial strains and environmental conditions. This outcome contrasts with conventional assumptions that hydrolysis, as a pretreatment step, universally enhances fermentability and H_2 yields.

While hydrolysis generally improves solubilization of complex polysaccharides into fermentable sugars [24], the results here demonstrate that under specific reactor conditions particularly alkaline pH and the use of tailored pure microbial cultures raw biomass can be equally or even more effective. This challenges the established paradigm and supports findings by Kapdan & Kargi [25], who noted that overprocessing biomass might release inhibitory compounds or alter microbial pathways unfavourably. The role of pH was particularly prominent.

All top-performing configurations maintained an initial pH of 8.5, suggesting that alkaline conditions favour fermentative hydrogen production, consistent with reports by Antonopoulou *et al*. (2020) [26] and O-Thong *et al*. (2019) [27].

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Alkaline pH values have been associated with enhanced microbial enzymatic activity, reduced methanogenesis, and improved suppression of hydrogen-consuming pathways [28]. The notable contrast in total volume of hydrogen gas produced between HHP1 (7035.5 ml at pH 6.5) and UHP2 (35,711 ml at pH 8.5) reinforces the criticality of pH control in biohydrogen systems. The type of microbial inoculum also proved influential. Pure cultures consistently outperformed mixed consortia in most unhydolyzed setups. UHP2, USP2, and USHP2 (all pure culture systems) recorded significantly higher H_2 yields compared to their mixed-strain counterparts such as USHM2 and UHM2. This supports assertions by Das and Veziroglu [2] and Levin *et al* [3] that pure cultures though less resilient to environmental fluctuations can offer superior specificity and metabolic efficiency under controlled conditions. The selectivity of pure strains ensures that metabolic flux is directed toward H_2 generation rather than competing pathways such as methanogenesis or lactic acid fermentation [20]. Interestingly, hydrolyzed systems like HHP1, HSHP1, and HSHM1 produced relatively lower H_2 despite using the same microbial strains and similar particle sizes as their unhydrolyzed counterparts. One plausible explanation, as discussed by Antonopoulou *et al*. [26], is that hydrolyzed substrates can accumulate fermentation inhibitors such as furfurals, phenolics, or volatile fatty acids at levels that suppress microbial hydrogenase activity. Moreover, Varanasi, J.L. et al. [29] note that complex syntrophic interactions in mixed communities often redirect electron flow toward methane production, which is evident from the higher CH_4 yields in hydrolyzed setups using mixed strains. The particle size of biomass also appeared to influence the outcomes. Configurations with larger particles (3.0 mm), particularly under alkaline pH, had higher hydrogen yields than those using smaller particles. For instance, UHP2 (3.0 mm) outperformed USHP2 (2.0 mm) and USP2 (1.0 mm). Larger particles may offer slower hydrolysis rates but prolonged substrate availability, thereby aligning with the kinetics of hydrogen-producing pathways. However, this relationship is likely non-linear and highly dependent on substrate structure and microbial affinity.

Claassen *et al*. [7] and Saifuddin, N. *et al*. [17] emphasized that substrate surface area affects not just degradation rates but also microbial attachment and reactor mixing efficiency. Another dimension is the composite gas yields, which serve as a proxy for overall gas productivity. While UHP2 had the highest hydrogen yield, it also exhibited elevated levels of CH_4 (8786 ml) and CO_2 (7498 ml), suggesting that the system supported multiple gas-forming metabolic routes. This underlines the need for process integration strategies to selectively enhance hydrogen production while suppressing methanogenesis, perhaps through pH shock, selective inhibitors, or staged fermentation [30–31]. Comparing the performance of UMP3 (Unhydrolyzed Mixed Strain at pH 8.5) with HMP3 (Hydrolyzed Mixed Strain at pH 6.5), further reinforces these observations. Despite sharing similar biomass and strain characteristics, UMP3 yielded 15,696 ml H_2, nearly double that of HMP3 (19898 L). The poorer performance of HMP3 could be due to the combination of hydrolysis induced inhibitors and sub-optimal pH for hydrogenase activity [32]. From an application perspective, the dominance of unhydrolyzed-pure systems is promising in terms of reducing pretreatment costs and processing time. This aligns with the economic and environmental assessments by Khoo [14] and Momirlan & Veziroglu [4], who argued for leaner, more direct conversion routes in waste-to-hydrogen frameworks. Additionally, the minimal need for extensive hydrolysis steps in configurations like UHP2 could support decentralized bioreactor designs in rural or industrial settings, reducing infrastructure burden [33–35].

CONCLUSION

The following conclusions can be achieved from the research as shown below

- BioH_2 can be produced using wood material under the process of biodigester application
- The microorganisms capable of carrying out these functions are identified as anaerobic type of organisms.
- The pH of the system and particle size of the wood saw dust influenced the rate of production and quantity of yield to be achieved at the end of the process.
- The types of microorganisms built up in the biodigester can be influenced by the concentration of the substrate.

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LIST OF ABBREVIATIONS

Abbreviation Full Meaning

HHP1 Hydrolyzed Mahogany wood Pure Clostridium sp.
HSHP1 Hydrolyzed Abga wood Pure Clostridium sp.
HSP1 Hydrolyzed Ako wood Pure Clostridium sp.
HHM1 Hydrolyzed Mahogany wood Mixed Clostridium sp.
HSHM1 Hydrolyzed Abga wood Mixed Clostridium sp.
HSM1 Hydrolyzed Ako wood Mixed Clostridium sp.
UHM2 Unhydrolyzed Mahogany wood Mixed Clostridium sp.
USHM2 Unhydrolyzed Abga wood Mixed Clostridium sp.
USM2 Unhydrolyzed Ako wood Mixed Clostridium sp.
USP2 Unhydrolyzed Ako wood Pure Clostridium sp.
USHP2 Unhydrolyzed Abga wood Pure Clostridium sp.
UHP2 Unhydrolyzed Mahogany wood Pure Clostridium sp.
HMP3 Hydrolyzed Mixture of the three woods Pure Clostridium sp.
UMP3 Unhydrolyzed Mixture of the three woods Pure Clostridium sp.
$BioH_2$ Bio-hydrogen gas
OLR Organic Loading Rate
HRT Hydraulic Retention Time

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