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Enhanced Hydrogen Production via Co-Fermentation of Mixed Fruit Peels, Vegetable Waste, and Gelatinous Solid Waste

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Abstract

This study investigates the potential for enhanced hydrogen production (HP) from the co-fermentation of mixed fruit peels and vegetable waste (MFPVW) with gelatinous solid waste (GSW) as an alkaline supplement and nutrient source. Co-fermentation of mixed fruit peels and vegetable waste with gelatinous solid waste enhances hydrogen production.A series of batch experiments were conducted under thermophilic conditions, evaluating the addition of different GSW concentrations (0–10 g per 100 g of MFPVW) on hydrogen yield. The results revealed that adding 2 g of GSW significantly enhanced hydrogen production and achieved a yield of 94.67 mL H_2/g COD removed, which is a 1.96-fold increase over the control. The optimal GSW concentration not only accelerated microbial activity, drastically shortening the reaction time to 18 hours, but also improved substrate utilization, with 57% COD removal and 53% carbohydrate degradation. These findings suggest that GSW can effectively enhance biohydrogen production from organic wastes, providing a green and efficient solution for producing renewable energy.

Highlights

- Co-fermentation of mixed fruit peels and vegetable waste with gelatinous solid waste enhances hydrogen production.
- The optimal gelatinous solid waste concentration of 2 g/100 g of mixed fruit peels and vegetable waste led to a 1.96-fold increase in hydrogen yield.
- Mixed fruit peels and vegetable waste addition shortened the fermentation time, with a peak hydrogen production rate of 94.10 mL H_2/h.
- Improved substrate utilization was achieved, with 57% COD and 53% carbohydrate removal efficiencies.
- Gelatinous solid waste functions as an alkaline supplement, stabilizing microbial activity and enhancing fermentation efficiency

Keywords: Hydrogen production, Gelatinous solid waste (GSW), Mixed fruit peels,

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Carbohydrate-rich substrates, Microbial activity.

1 INTRODUCTION

In recent years, hydrogen has gained significant attention as a vital source of renewable energy due to its high energy density (122 kJ/g) and clean combustion, producing only water as a byproduct [1]. As a fuel and energy carrier, hydrogen is particularly efficient in fuel cells for electricity generation [2]. However, Conventional hydrogen production methods like electrolysis and hydrocarbon cracking are resource-heavy and expensive. For example, hydrogen production via water electrolysis requires significant electrical energy, and steam methane reforming (SMR), a chemical cracking process, also releases substantial carbon emissions. This has led to interest in biological methods such as dark fermentation and photo-fermentation, which offer a more sustainable, cost-effective pathway by utilizing organic waste and renewable energy sources [3]. A critical review by Martino et al., [4] discusses various hydrogen production methods, emphasizing the advantages of biological approaches, particularly in terms of sustainability and lower operational costs. Similarly, Agyekum et al., [5] highlight the potential of biological hydrogen production methods, such as those derived from biomass, as an eco-friendly and scalable alternative to traditional methods.

Among biological methods, anaerobic digestion (AD) of organic wastes is one of the most developed technologies for biohydrogen production, favored for its low energy consumption, minimal sludge production, and environmentally friendly nature [6]. Depending on the feedstock's total solids (TS) content, anaerobic digestion (AD) processes can be categorized into wet, semi-dry, or dry. Compared to wet AD, dry AD, which operates at a higher TS content (over 20%), has several advantages, such as requiring smaller reactor volumes and requiring less energy because it handles less water. Furthermore, dry AD systems are better suited for processing solid waste with less liquid content, which lowers the requirement for mixing and pumping a lot of water and increases energy efficiency [7].

However, Challenges in dark fermentation for hydrogen production, particularly due to the accumulation of hydrogen-consuming metabolites such as lactic and propionic acids, have been well documented. These metabolites often lead to reduced hydrogen yield, falling short of theoretical values. The interaction between hydrogen-producing bacteria (HPB) and lactic acid bacteria (LAB) is critical in such systems. Studies have shown that an imbalance, where LAB outcompete HPB, can divert fermentation pathways away from hydrogen production toward lactic acid or other metabolite formation, further reducing efficiency. This has been observed in both batch and continuous dark fermentation processes using various organic substrates [8], [9]. The accumulation of acetic and butyric acids has been associated with higher hydrogen yield, though excessive butyric acid may indicate instability [10]. Micronutrients such as Fe, Co, Ni, and Mo, as well as macronutrients like Na, K, and Ca, are critical for the metabolic activity of fermentative microorganisms [11].

Fruit and vegetable wastes (FVW) are highly degradable and rich in carbohydrates, making them an excellent feedstock for biohydrogen production. Studies have demonstrated that when managed effectively, these waste materials can be converted

into valuable energy resources through dark fermentation and other processes. The carbohydrate-rich nature of FVWs, along with their abundance, makes them a sustainable and cost-effective choice for hydrogen production, aligning with current goals for renewable energy development and reducing waste [12], [13]. Other studies have demonstrated promising hydrogen yield using mesophilic fermentation of mixed vegetable waste, with outputs reaching up to 89 mL H_2/gCOD [14].

Maintaining a pH between 5.0 and 6.0 is critical for enhancing hydrogenase enzyme activity during biohydrogen production. Proper control of pH has been shown to improve hydrogen yield in fermentation processes. A study by Ginkel and Sung (2001) discusses how pH and substrate concentration impact biohydrogen production, emphasizing the importance of optimizing fermentation conditions for better yield [15]. Gelatinous solid waste (GSW), a byproduct of the food and pharmaceutical industries, has potential in co-fermentation as an inorganic nutrient and buffering agent. Its ability to stabilize pH while improving microbial growth has been linked to more efficient thermophilic fermentation processes, which enhance hydrogen production. Research by Elbeshbishy et al. [16] explores the mitigation of inhibitors in dark fermentation and provides insights into the importance of stable fermentation conditions, which could be relevant when using GSW. Thermophilic fermentation, carried out at elevated temperatures, accelerates substrate degradation and supports the growth of thermophilic bacteria, which typically produce higher hydrogen yield than mesophilic organisms. This process has been widely discussed in the literature for its efficiency in biohydrogen production. Liu et al. [17] provide an overview of recent advances in fermentative biohydrogen production, focusing on the role of temperature and other parameters in optimizing hydrogen yield.

Several research papers emphasize the importance of biohydrogen production as a waste management and energy recovery solution in reducing greenhouse gas emissions and supporting a circular economy. Biohydrogen production through the valorization of organic waste, such as food and agricultural residues, has been shown to not only provide renewable energy but also minimize environmental impacts associated with waste disposal, especially through the reduction of landfill emissions. This process is crucial for moving toward a low-carbon bioeconomy, contributing to sustainable energy systems, and enhancing waste-to-energy strategies. Studies highlight how integrating biohydrogen production into waste management can significantly mitigate carbon footprints while contributing to the broader transition to a circular economy [18]. This study aims to explore the potential of co-fermenting mixed fruit peels and vegetable waste (MFPVW), and GSW to enhance hydrogen production. By optimizing fermentation conditions and incorporating GSW as a nutrient and alkaline supplement, this research seeks to advance biohydrogen production processes and contribute to the development of sustainable energy solutions.

2 MATERIALS AND METHODS

2.1 Characteristics of Substrate and Inoculum Sludge

In this study, two types of substrates were used: (1) MFPVW, and (2) GSW.

- **MFPVW:** This substrate comprised equal weights of orange peels, banana peels, tomato peels, peas, and spinach. The MFPVW was collected from Faragalla Industrial Company in New Borg El Arab, Egypt. The collected MFPVW was crushed using an electrical grinder to minimize particle size and prevent dilution. The crushed material

was preserved at 4°C to prevent biodegradation. Afterward, it was sieved using a stainless-steel sieve with 2.0 mm gaps, and the filtrate was used for the batch experiments.

- **GSW:** GSW was collected from a gelatin manufacturing company in Alexandria, Egypt. The GSW was dried at 70°C for 4 hours, disaggregated, and sieved through a 200-mesh screen before being used in the fermentation experiments to enhance hydrogen production from MFPVW. GSW primarily consists of C_2H_4, CaO, and $CaCO_3$ with small amounts of copper, iron, magnesium, and potassium, alongside trace elements. The chemical characteristics of MFPVW and GSW are summarized in Table 1.

The inoculum sludge used in this study was collected from the thickener tank of a wastewater treatment plant in Alexandria, Egypt. The sludge was further concentrated by incubating it under anaerobic conditions for two months, followed by filtration through a sieve No 10 to remove coarse particles. The seed sludge was pre-heated at 70°C for 30 minutes which is widely used in biohydrogen production studies to suppress methanogenic activity, which would otherwise convert hydrogen into methane, and to enrich spore-forming anaerobes [19]. This process is critical in enhancing hydrogen yield during dark fermentation. Pre-heating selectively targets methanogens, allowing for more efficient biohydrogen production by favoring hydrogen-producing bacteria [20], [21]. Studies highlight this method's effectiveness in maintaining anaerobic environments conducive to hydrogen production from organic wastes like food residues or agricultural biomass [21]. The mixed liquor volatile suspended solids (MLVSS) concentration and pH of the adapted sludge were 25.34 g/L, 21.49 ± 1.83 g/L, and 6.77, respectively.

Table 1. Characteristics of MFPVW and GSW.

Parameter	Unit	MFPVW	GSW
pH	-	4.96 ± 0.19	12.76 ± 0.19
Total solids (TS)	%, (w/w)	15.4 ± 1.6	74.5 ± 1.6
Volatile solids (VS)	%, (w/w)	12.74 ± 1.3	31.4 ± 2.6
Chemical oxygen demand (COD)	g/L	166.38 ± 10.4	357.17 ± 6.7
Carbohydrate	g/L	90.93 ± 6.3	13.75 ± 6.3
Total Kjeldahl nitrogen (TKN)	g/L	2.47 ± 0.11	35.52 ± 1.3
C/N ratio	-	67.2 ± 2.9	12.01 ± 0.90
Protein	g/L	13.78 ± 1.3	281.25 ± 1.3
Ammonia (NH_4-N)	mg/L	265 ± 15.2	686 ± 24.6
Calcium (Ca^{2+})	g/kg	0.690 ± 0.078	270.75 ± 8.9
Potassium (K^+)	mg/kg	3218 ± 98	33.2 ± 2.37

Copper (Cu)	mg/kg	1.175 ± 0.3	4.85 ± 0.27
Iron (Fe^{2+})	mg/kg	11.08 ± 1.6	40 ± 0.09
Magnesium (Mg^{2+})	mg/kg	344 ± 76	1278 ± 3.9
Manganese (Mn^{2+})	mg/kg	4.3175 ± 0.28	Not detected
Zinc (Zn^{2+})	mg/kg	4.68 ± 0.31	Not detected

2.2. Experimental Setup

Batch fermentation experiments were conducted in 250 mL serum bottles, each with a working volume of 150 mL and 100 mL of headspace to minimize the adverse effects of hydrogen partial pressure. The batch reactors were initially filled with 50 mL of inoculum (enriched sludge). Various ratios of MFPVW to GSW were tested in the following ratios: 100g:0g, 100g:1g, 100g:2g, 100g:4g, 100g:6g, 100g:8g, and 100g:10g (v/v). To create an anaerobic environment, the bottles were purged with pure nitrogen gas for 5 minutes before being sealed with rubber stoppers and aluminium caps. The batches were incubated at thermophilic conditions (55 ± 2 °C). All experiments were conducted in triplicate to ensure reliability.

2.3. Analytical Methods

In this study, several analytical methods were employed to assess various parameters. Chemical Oxygen Demand (COD), Total Solids (TS), Volatile Solids (VS), Total Kjeldahl Nitrogen (TKN), and calcium ions were quantified following standard methods [22]. Protein content was determined using the formula: Protein = 6.25 × (TKN - Ammonia Nitrogen) [23]. Carbohydrates were measured using the phenol-sulfuric acid method [24]. Volatile fatty acids (VFAs) were analysed through high-performance liquid chromatography (HPLC) with a column oven temperature of 40°C, utilizing a 4 mM H_2SO_4 solution as the mobile phase at a flow rate of 0.5 mL/min for 22 minutes, followed by 0.4 mL/min for 8 minutes. Hydrogen gas content in the produced biogas was measured using a gas chromatograph (GC-2014, Shimadzu, Japan), equipped with a thermal conductivity detector and a Shin Carbon column. The operational temperatures of the injection port, column oven, and detector were set at 100°C, 120°C, and 150°C, respectively, with helium as the carrier gas at a flow rate of 25 mL/min. Additionally, particle size distribution was determined using laser diffraction spectroscopy (Beckman Coulter LS230).

3 Results And Discussion

3.1 Cumulative Hydrogen Production (CHP) from the Co-Fermentation of MFPVW with GSW

Fig 1. investigates the CHP from co-fermentation of MFPVW with varying concentrations of GSW. The experiment evaluated the hydrogen production over time with different amounts of GSW (0 g, 1 g, 2 g, 4 g, 6 g, 8 g, and 10 g) added to the MFPVW substrate. In the mono-fermentation process, the CHP was limited to 454.0 mL, with a hydrogen yield (HY) of 74.55 mL H_2/g COD removed after 47 hours. The modified Gompertz equation was used to model the results, yielding a strong correlation (R^2 = 0.990) and a maximum hydrogen production rate (R_m) of 27.70 mL/h. Notably, MFPVW has a high C/N

ratio (67.2), which is less favorable for hydrogen production, as research has shown that the optimal C/N ratio for maximum hydrogen yield is between 26 and 31 [25] Additionally, the low initial pH of MFPVW (4.96) hampers hydrogen production, with optimal pH ranges falling between 5.5 and 7.5 [14]

Conversely, co-fermentation of MFPVW with GSW led to a notable improvement in hydrogen production. The highest CHP of 886 mL, representing a 75% increase over mono-fermentation, was achieved with the addition of 2 g of GSW (0.157 g GSW/g VS MFPVW). This also shortened the fermentation time from 28 hours (in mono-fermentation) to 18 hours. The modified Gompertz equation was applied to model these results, yielding high correlation coefficients (R^2 = 0.990–0.997), indicating a strong fit between the experimental and modelled data [26]. The highest hydrogen production rate, 94.10 mL H_2/h, was observed with 2 g GSW, a 3.4-fold increase over mono-fermentation. Interestingly, the lag phase decreased from 5.3 hours (in mono-fermentation) to 3.0 hours with 10 g GSW. The decrease in hydrogen production at higher concentrations of gelatinous solid waste (GSW) may be attributed to substrate inhibition, which is commonly observed in fermentative hydrogen production. At elevated concentrations of organic substrates or supplements like GSW, microbial metabolism may become disrupted due to the overload of nutrients or the accumulation of inhibitory by-products, such as volatile fatty acids or ammonia. These factors can inhibit the activity of hydrogen-producing bacteria, thereby reducing overall hydrogen yields. For instance, excess nutrients, particularly metal ions or nitrogen sources like ammonium, can lead to inhibitory effects on microbial growth and enzyme function, ultimately decreasing hydrogen output [27]

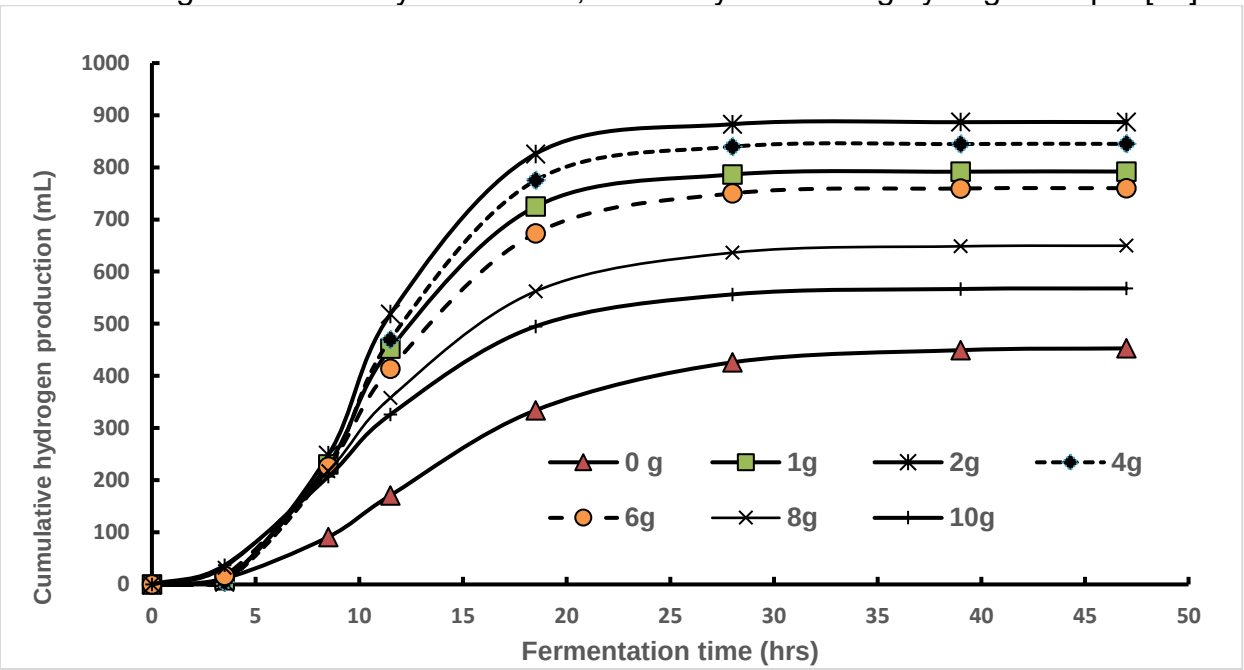

Fig. 1. CHP from co-fermentation of MFPVW and GSW

These results align with recent studies, which also show that co-fermentation of organic waste with supplemental substrates such as GSW or other nitrogen-rich materials can significantly boost hydrogen yield [28]. Furthermore, the modification of fermentation parameters, such as the C/N ratio and pH, is widely recognized in literature as key to optimizing biohydrogen production.

Table 2. Gompertz Kinetic Analysis of Hydrogen Production with different concentrations of Gelatinous Solid Waste in Fermentation

Batches	Gompertz kinetics			
Concentrations of GSW	P (ml)	R_{max} (ml/h)	λ h	R^2
0g	454.00	27.7	5.3	0.990
1g	792.00	76.7	5.5	0.991
2g	886.00	94.1	5.9	0.997
4g	848.00	85.0	5.9	0.994
6g	760.00	64.4	5.0	0.990
8g	650.00	48.3	4.0	0.993
10g	568.00	41.7	3.5	0.997

3.2 Hydrogen Yield, COD and Carbohydrates removal efficiencies

Fig 2. illustrates COD removal, carbohydrate removal efficiencies and the hydrogen yield production. The COD removal and carbohydrate degradation efficiency also improved with GSW addition. In the mono-fermentation of MFPVW, the COD and carbohydrate removals were 39% and 32% respectively, with a hydrogen yield of 74.56 mL/g COD removed. However, with co-fermentation at a GSW-to-MFPVW ratio of 50:1, the COD removal increased to 57%, carbohydrate removal reached 53%, and hydrogen yield improved to 94.69 mL/g COD removed. These findings are in line with recent literature, where co-fermentation strategies are shown to enhance substrate utilization and biohydrogen production [29].

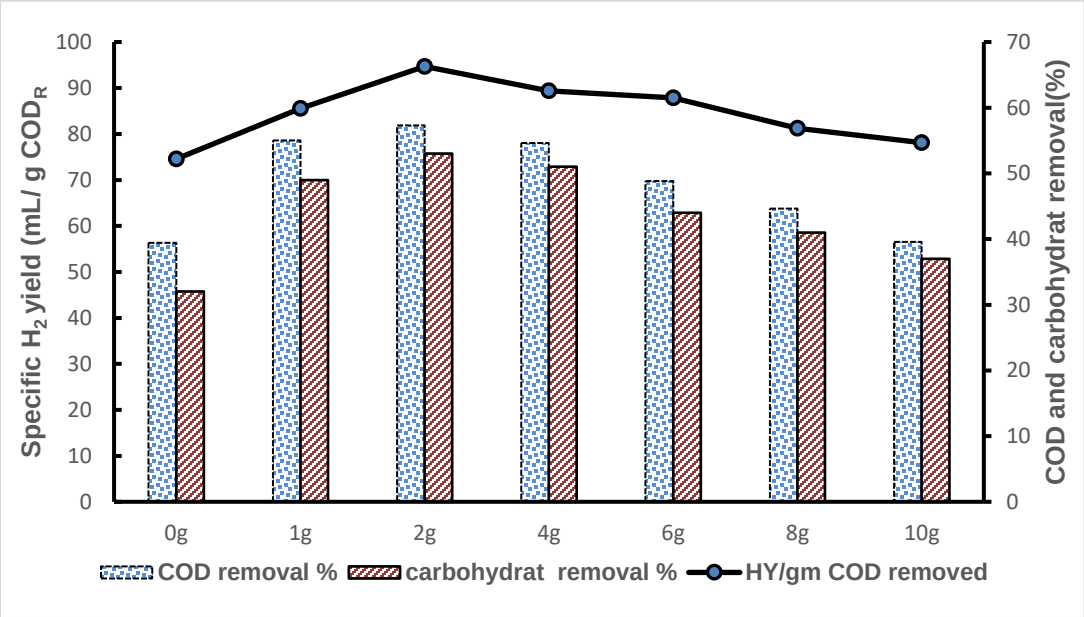

Fig 2. HY, COD and carbohydrates removal efficiencies

Moreover, research on co-digestion of biomass like Chlorella sp. and sugarcane leaves with anaerobic sludge has also demonstrated enhanced hydrogen production, where optimizing nutrient ratios like C/N played a crucial role in preventing process inhibition [30]

The results from this study, combined with the comparison to recent research, indicate that co-fermentation of MFPVW with GSW can significantly enhance hydrogen production and process efficiency. The optimal addition of 2 g GSW per 100 g of MFPVW was found to provide the best performance, with considerable improvements in both hydrogen yield and COD removal efficiency.

3.3 Initial and Final pH Versus Total Ammonia Nitrogen at Different Concentrations of GSW

The results shown in Fig 3. illustrate the effect of adding GSW on the initial and final pH values, as well as the final ammonium concentration (NH_4^+) in each experiment. The initial pH values ranged from 5.53 at 0g of GSW, gradually increasing to 7.22 with the addition of 10g, indicating that GSW provides buffering capacity that raises the initial pH. The final pH decreased to a range between 4.33 and 6.12, with the most significant drop observed when no GSW was added (0g), while higher GSW additions helped maintain higher final pH levels. Regarding ammonium concentration, it increased significantly from 0.19 g/L without GSW to 1.44 g/L at 10g GSW, indicating that GSW enhances ammonium release during fermentation due to its nutrient content. The increased H_2 production by GSW addition was credited to the supply of buffering capacity. The optimum hydrogen yield obtained was 94.69 (ml H2/g COD removed) when initial pH, final pH, and final ammonium concentrations were 6.02,5.56 and 0.406 g/l, respectively.

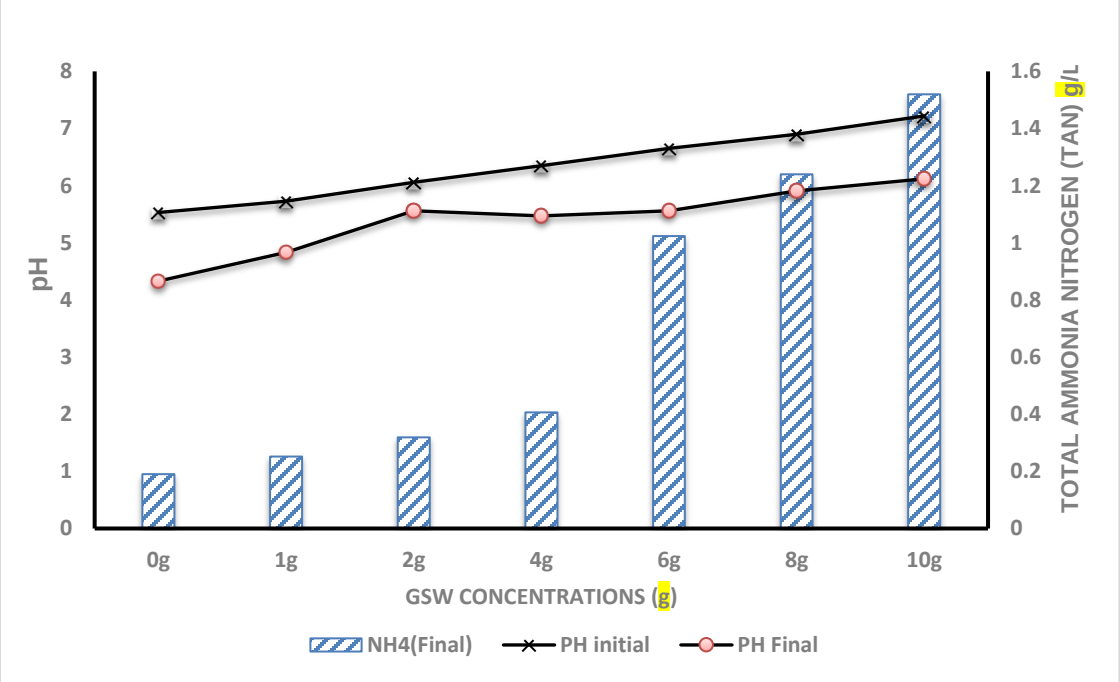

Fig 3. Initial and final pH versus total ammonia nitrogen at different concentrations of GSW

These findings align with recent studies, such as Lee et al. [31], which observed maximum hydrogen production at a pH of around 6.0, attributing stable hydrogen yield to the presence of metals like copper, iron, and magnesium in GSW. These metals act as both pH buffers and essential nutrients for microbial growth, consistent with the sharp rise in hydrogen production observed with GSW addition. Furthermore, the gradual increase in

ammonium concentration reflects enhanced microbial activity, as ammonium serves to buffer the pH and prevent excessive acidification, which could hinder fermentation, as noted by Lee et al., [31].

3.4 Effect of calcium (Ca^{+2}) concentrations on the cumulative hydrogen production

The results presented in Fig 4. demonstrate the effect of initial calcium concentrations on cumulative hydrogen production. GSW, which contains high levels of calcium ions (Ca^{2+}), was co-fermented with MFPVW. The findings reveal that hydrogen (H_2) production significantly increased from 454 mL to 886 mL as the Ca^{2+} concentration rose from 0.69 g/L to 5.98 g/L. These results align with the findings of Elsamadony & Tawfik, Elsamadony & Tawfik, (2015a), which indicated that calcium ions act as coagulants, aiding in biomass accumulation and stimulating microbial growth, ultimately enhancing the specific growth rate. Calcium is essential for the catalytic activity that drives these biological processes.
However, when the calcium concentration exceeded 5.98 g/L, hydrogen production dropped significantly to 568 mL. This observation is consistent with the results from J. Zhang et al. [32] who investigated the addition of lime mud from paper (LMP) to food waste (FW) in a dry anaerobic process. In their study, CHP increased by 54% when 3 g of LMP was added to a 200 g FW bioreactor. Nevertheless, they also observed that an overdose of LMP (33% Ca concentration) could inhibit hydrogen-producing bacteria.
Furthermore, Elsamadony & Tawfik, Elsamadony & Tawfik, (2015a) found that volumetric hydrogen production improved from 4.5 to 7.2 L H_2 when the calcium concentration increased from 1.8 g/L to 6.3 g/L. Like the current study, they also reported a decrease in hydrogen production when calcium concentrations exceeded 6.3 g/L.

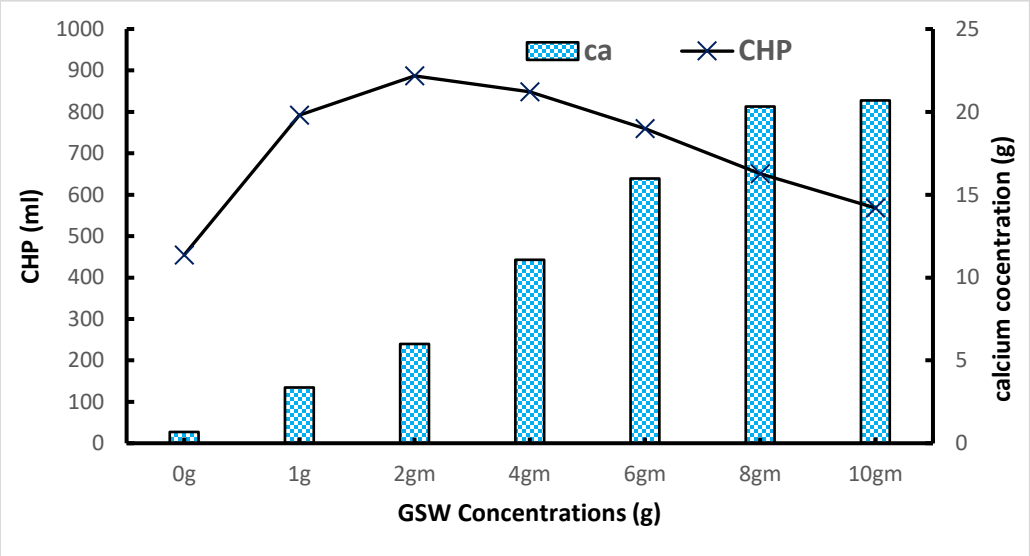

Fig 4. Effect of Ca^{+2} on cumulative hydrogen production

3.5 Soluble Metabolites Components

The soluble metabolites produced at the end of all batch fermentations primarily included volatile fatty acids (VFAs) such as acetate (HAc), butyrate (HBu), and propionate (HPr). Of these, butyrate and acetate are the preferred metabolites for enhancing hydrogen production and yield, as they are directly associated with higher hydrogen output. On the other hand, propionate is involved in hydrogen-consuming pathways, which leads to a reduction in hydrogen production [33]. The concentrations of these VFAs are influenced

by the type of substrate used in the fermentation process [23].

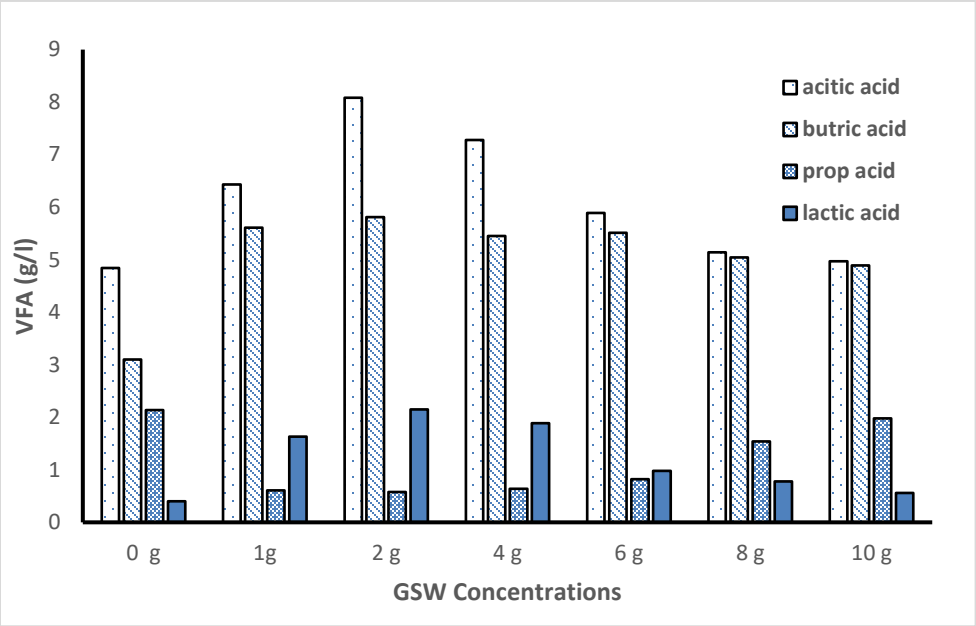

Fig 5. Soluble metabolites produced during fermentation

As shown in Fig. 5, during the mono-fermentation of MFPVW and when co-fermented with varying amounts of GSW (1, 2, 4, 6, 8, 10 g), the concentrations of acetate and butyrate reached their peaks at 8.0 g/L and 5.81 g/L, respectively, when 2 g of GSW was added. At the same point, HPr was at its lowest concentration (0.58 g/L). This is likely due to the initial pH of 6, which facilitated carbohydrate conversion, with 53% removal observed. In contrast, during the mono-fermentation of MFPVW without any GSW addition, the acetate and butyrate concentrations were significantly lower, at 4.84 g/L and 3.1 g/L, respectively, while propionate reached a higher concentration of 2.14 g/L. This is consistent with findings by [33], who reported that during mono-fermentation of cellulosic substrates, higher HPr production and a lower HAc/HBu ratio were observed.

Recent research confirms that hydrogen production during fermentation is closely linked to the volatile fatty acid (VFA) profiles, specifically the balance of acetate, butyrate, and propionate. Studies highlight that higher concentrations of acetate and butyrate promote increased hydrogen yield, whereas an excess of propionate can hinder the process. For example, in an anaerobic digestion process, researchers have found that the shift towards acetate-oxidizing and hydrogenotrophic pathways, when coupled with proper VFA management, leads to enhanced hydrogen production [34]. Similarly, the work of Hori et al., [35]. emphasizes the inhibitory effects of propionate accumulation on hydrogen production, while managing VFA ratios like acetate and butyrate can boost efficiency. These findings are supported by research demonstrating that controlling the fermentation environment, such as through co-substrate addition, can optimize VFA levels for improved hydrogen yield [36], [37].

4 CONCLUSIONS

This study demonstrates the significant potential of using GSW to enhance biohydrogen production through the co-fermentation of MFPVW. The results indicate that the optimal addition of 2 g of GSW per 100 g MFPVW achieves the highest hydrogen yield, coupled with increased COD removal and carbohydrate degradation. The introduction of GSW not only shortened fermentation time but also improved microbial stability and process efficiency. These findings provide valuable insights into biohydrogen production's potential to contribute to renewable energy solutions, while simultaneously addressing organic waste disposal challenges. Further research should focus on scaling up the process and investigating the economic feasibility of co-fermentation systems at an industrial level.

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Data availability The authors affirm that all data relating to the current research are comprehensively incorporated within this manuscript.
Ethical Approval The current study did not involve any human or animal subjects. Therefore, it was not subject to review by an ethics committee and did not require informed consent.

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