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# Comparative Analysis of Organic Substrate Efficiency in Bio-hydrogen Production through Dark Fermentation

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## Abstract

This study examines the impact of substrate type, composition, and addition of gelatin solid waste (GSW) on bio-hydrogen production from organic waste by dark fermentation, a process known for its ability to sustainably convert organic materials into hydrogen without the need for light energy. Peels from fruits (FPs), vegetables (VPs), and a combination of the two (MFVPs) were used in a series of batch experiments to find the best substrate producing the highest bio-hydrogen. According to the results, the most efficient combination of 25% pea, 25% tomato, 25% banana, and 25% orange peels produced the highest HY of (73.16 ± 9.5 ml/g COD), VHP of (2.48 ± 0.33 L/L), and HC of (64.7 ± 3.7%), respectively. Then, an additional batch of experiments was conducted to investigate the effect of GSW addition on $H_2$ production and fermentation efficiency. Therefore, different concentrations of GSW (1, 2, 4, 6, 8, and 10 g) were added to this optimal mixture, and the findings showed that the optimal dose of GSW was 2 g; this dose considerably shortened the time to peak hydrogen production from 28 to 18 hrs. and boosted cumulative hydrogen production (CHP) by almost 60%. These results highlight that substrate diversification and nutrient supplementation significantly increased organic waste fermentation and biohydrogen production. The broader implications of this study include potential applications in industrial waste management and renewable energy sectors, where optimizing fermentation processes could reduce waste while providing a cleaner energy source

**Keywords:** Dark fermentation; Sole and multiple fermentation; Bio-hydrogen; Fruit peels; Vegetable peels; Gelatin solid waste.

## 1. Introduction

Growing global concerns about climate change, environmental deterioration, and the depletion of fossil fuel resources have made the demand for alternative sustainable and renewable energy sources a crucial priority[1]. In contrast, bio-hydrogen has garnered significant attention as a clean, renewable energy source because it is a highly energetic, sustainable, and eco-friendly renewable fuel[2]. Additionally, it releases zero carbon emissions during combustion, making it an excellent alternative to traditional fossil fuels[3]. Recently, the main challenge has been optimizing efficient, sustainable, and low-cost biohydrogen processes[4]. Compared to other technologies used for hydrogen production, dark fermentation (DF) has shown an impressive, promising approach [5].

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Dark fermentation (DF) is a biological process in which microorganisms convert organic substrates into hydrogen gas and other by-products such as volatile fatty acids, CO2, and trace gases in the absence of oxygen[6] . The mechanism of DF involves the breakdown of carbohydrates in the substrate by anaerobic bacteria, which release hydrogen as a metabolic by-product during this process [4]. alongside other by-products such as volatile fatty acids, and $CO_2$ in the absence of oxygen. The mechanism of DF involves the breakdown of carbohydrates in the substrate by anaerobic bacteria, which release hydrogen as a metabolic by-product during this process [7]. DF has several advantages, such as its simplicity, the utilization of inexpensive and abundant feedstocks, operation under mild conditions, and the absence of complex catalysts. However, substrate type, characteristics, and composition (i. e., carbohydrate, protein, and fat content), as well as its biodegradability, are crucial as they significantly influence DF efficiency and bio-hydrogen production yields[8]. As a result, a deep understanding of different substrates' effects on the efficiency and output of the process is needed[9].

Previous studies have often focused on investigating the sole effect of operating parameters and variations in experimental conditions, such as pH, temperature, organic loading rate (OLR) and hydraulic retention time (HRT)[10]. On the other hand, limited investigations into substrate performance comparisons were conducted across studies. Thus, to address this gap, comprehensive research must examine the potential effect of sole and multiple substrates on bio-hydrogen production under controlled conditions[11], [12].

Fruit and vegetable peels are desirable substrates for bio-hydrogen production efficiently using DF because of their high organic content (i.e., carbohydrates), high biodegradability, affordability, and availability when compared to other organic substrates[11]. Additionally, these substrates offer an effective, sustainable, economical, and eco-friendly approach to waste management [13]. Similarly, gelatin solid waste, which is primarily composed of proteins, is an excellent substrate for DF and previous studies found that it achieved remarkable bio-hydrogen production rates [14]. GSW is a well-known nutrient and buffering material for bio-hydrogen production[15]. It increases the process efficiency and hydrogen production, especially when added in the case of fermenting readily degradable and carbohydrate-rich materials like MFVPs. GSW maximizes hydrogen production while maintaining process stability through its natural buffer capacity, eliminating the need for any external alkali source. Combining the aforementioned two substrates (fruit and vegetable peels with gelatin solid waste) is suggested by this study as an innovative approach to substrate optimization. This mixture in dark fermentation can provide a balanced nutrient environment for the carbohydrate-rich peels, which can enhance microbial diversity and activity, leading to improved bio-hydrogen yields and production rates.

This study aims mainly to enhance our understanding of substrate behavior and its effect on bio-hydrogen production through dark fermentation, by evaluating different substrates (i.e., fruit and vegetable peels) for sole, and multi-fermentation, as well as the capability of combining mixed fruit and vegetable peels with gelatin solid waste. Also, this study aims to identify the most promising substrate mixture for optimal bio-hydrogen production through a comparative analysis of these feedstocks in terms of bio-hydrogen yield, bio-hydrogen production rate, and organics removal efficiency.

## 2. Materials and methods

### *2.1. Experimental design and substrates*

Mixed fruit peels (MFPs), mixed vegetable peels (MVPs), and gelatin solid waste (GSW) were selected and tested to identify their bio-hydrogen production rates via the dark fermentation process. Every day, fruit peels [banana (B) and orange (O)] and vegetable peels [spinach (S), pea (P), and tomato (T)] were collected from Faragallah facility located in Alexandria, Egypt. This facility produces fruit juice and frozen or canned vegetables, generating approximately 20 tons of vegetable and fruit peels daily. A small quantity of these peels was ground using an electronic grinder. After that, the slurries were passed through a stainless-steel sieve with 2.0 mm holes. The produced filtrate was used for sole and mixed fermentation processes. As for the gelatin solid waste (GSW), it was collected from a gelatin industrial plant in Alexandria, chopped into tiny pieces, dried at 70°C for 4 hrs , and then disaggregated, sieved, and stored in powder form in a sealed vessel.

While the grinding and sieving steps were implemented, the substrates did not undergo any sterilization, which may raise concerns about contamination from indigenous microorganisms. However, given the anaerobic conditions of dark fermentation and the use of specialized hydrogen-producing bacteria (HPB), microbial competition was managed by maintaining strict environmental conditions. Nonetheless, further substrate pretreatment could be explored in future studies to assess its impact on bio-hydrogen yields.

The experimental design is shown in Table 1, with four consecutive batches of tests conducted to examine various substrate types and their combinations. Three batches of experiments were carried out in the first stage of this study to assess the effect of substrate composition and type on bio-hydrogen production. Vegetable peels were used in Batch 1, fruit peels were used in Batch 2, and a mix of the two was studied in Batch 3. These experiments helped identify the optimal combination of fruit and vegetable peels for bio-hydrogen production. To examine the influence of adding GSW powder, Batch 4 used the optimal mixture from Batch 3, and the GSW powder in a range of 1–10 g was investigated, keeping the same operating conditions from the previous experiments constant**.**

**Table (1):** The experimental design for sole and multi-fermentation batches using different substrates

| Experiment | Substrate Type | Substrate Composition | |
|---|---|---|---|
| Batch (1) | **Vegetable peels (VPs)**<br>[Spinach, Pea, and Tomato] | Sole vegetable peels (VPs) | 100% Spinach (S)<br>100% Pea (P)<br>100% Tomato (T) |
| | | Mixed vegetable peels (MVPs) | 50% S + 50% P<br>50% S + 50% T<br>50% P + 50% T |
| Batch (2) | **Fruit peels (FPs)**<br>[Banana and Orange] | Sole fruit peels (FPs) | 100% Banana (B)<br>100% Orange (O) |
| | | Mixed fruit peels (MFPs) | 50% B + 50% O |

| | | | |
|---|---|---|---|
| Batch (3) | **Mixed fruit and vegetable peels (MFVPs)**<br>[Spinach, Pea, Tomato, Banana, and Orange] | Mixed fruit and vegetable peels (MFVPs) | 25% S + 25% T + 25% B + 25% O<br>25% S + 25% P + 25% B + 25% O<br>25% S + 25% P + 25% T + 25% B<br>25% P + 25% T + 25% B + 25% O<br>25% P + 25% T + 25% S + 25% O<br>20% S + 20% P + 20% T + 20% B + 20% O |
| Batch (4) | **Mixed MFVPs and gelatin solid waste (GSW)** | Mixed MFVPs and GSW [optimal mixture resulted from batch 4] | Optimal MFVPs + 1g GSW<br>Optimal MFVPs + 2g GSW<br>Optimal MFVPs + 4g GSW<br>Optimal MFVPs + 6g GSW<br>Optimal MFVPs + 8g GSW<br>Optimal MFVPs + 10g GSW |

### *2.2. Mixed culture bacteria*

The inoculum was obtained from El-Agamy wastewater treatment plant in Alexandria, Egypt. The sludge was concentrated by settling for a full day, followed by the disposal of the supernatant and passing the residue through a sieve (no. 10) to remove coarse particles. Hydrogen-producing bacteria (HPB) were cultured using an anaerobic continuous stirred-tank reactor (CSTR) with a volume of 5 L, fed with glucose as a carbon source under the following operating conditions: HRT of two hours, temperature of 35°C, and pH of 5.5, for two months to eliminate methanogens and boost HPB activity. To suppress hydrogen-consuming bacteria, the HPB was pre-heated at 70°C for 30 minutes. The final pH, total suspended solids (TSS), and volatile suspended solids (VSS) of the sludge were 5.5, 30.6 g/L, and 25.3 g/L, respectively.

### *2.3. Experimental setup*

Batch fermentation experiments were conducted using 200 mL batch reactors with a working volume of 150 mL. The reactors were seeded with 50 mL of HPB, and the remaining volume was filled with different volumetric ratios of fruit peels, vegetable peels, and gelatin solid waste, as shown in Table 1. The anaerobic condition was maintained by securely sealing the batches with aluminum caps and rubber stoppers. The pH of the media inside the batches was adjusted to 6.0 ± 0.2 using 1 N HCl and NaOH. The batches were incubated at 35 ± 2°C in mesophilic conditions, and each experiment was performed three times to ensure reproducibility.

### *2.4. Analytical methods*

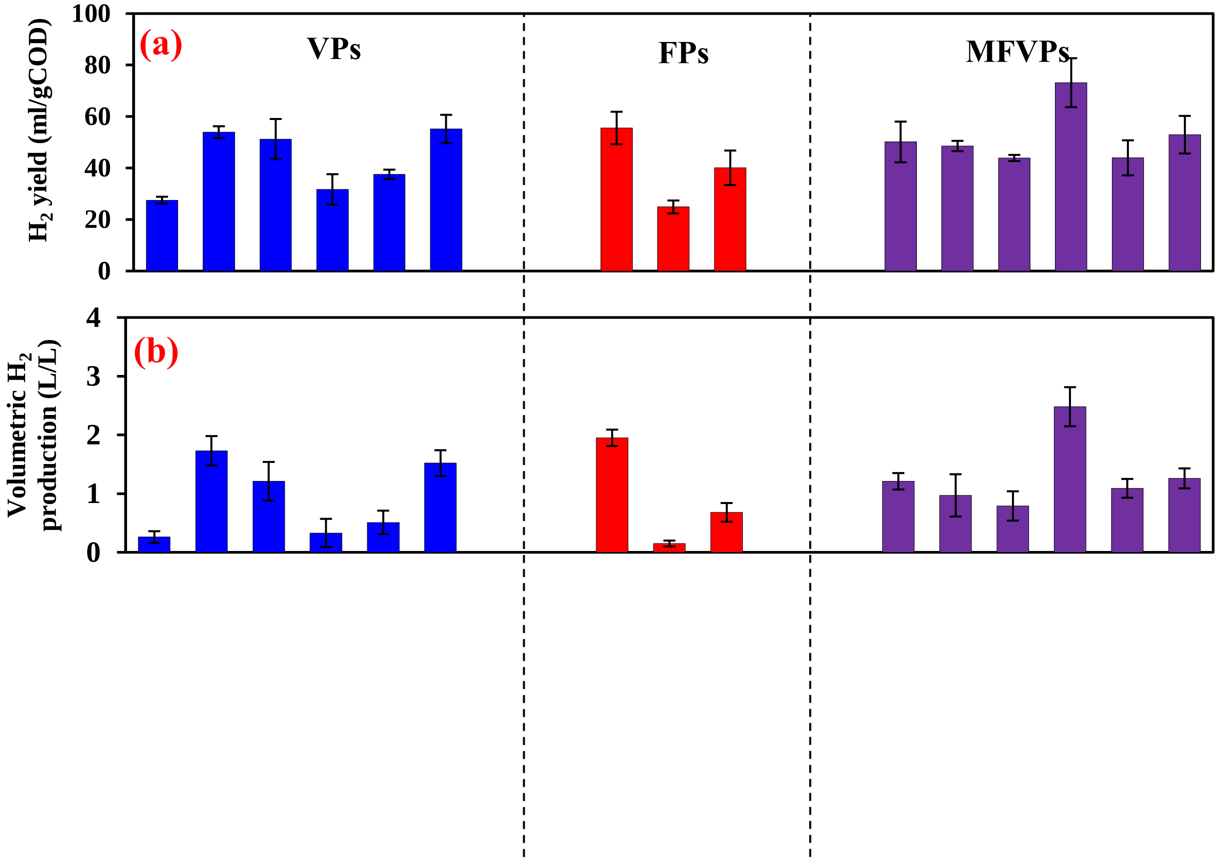
According to the American Public Health Association [16], total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN), chemical oxygen demand (COD), pH, carbohydrate, protein, and ammonia ($NH_4$-N) were measured. High-performance fluid chromatography was used to evaluate volatile fatty acids (VFAs) (LC-10AD, Shimadzu, Japan). The displacement method was used to measure the elicited gas. Additionally, a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a thermal conductivity detector was used to assess the bio-hydrogen content in the biogas output.

## 3. Results and discussion

### *3.1. Effect of Substrate Type and Composition*

### 3.1.1. $H_2$ production

In the first batch of experiments, vegetable peels (VPs) were used as the substrate for sole and mixed fermentation. The findings indicated significant bio-hydrogen production through both individual and combined fermentation processes which can be attributed to the high carbohydrate content and the relatively easy biodegradability of vegetable peels [17]. However, as shown in **Fig. 1**, the fermentation of separated VPs generated higher values of volumetric hydrogen production (VHP), $H_2$ yield (HY), and $H_2$ content (HC) compared to Mixed VPs. Pea peels yielded the greatest levels of HY, VHP, and HC, measuring (53.94 ± 2.3 ml/gCOD), (1.73 ± 0.25 L/L), and (57.5 ± 7.5%), respectively. On the other hand, only one mix of VPs achieved close values. This mix was the combination of pea and tomato peels (50% P + 50% T), but it still had slightly lower values of VHP and HC compared to pea peels.



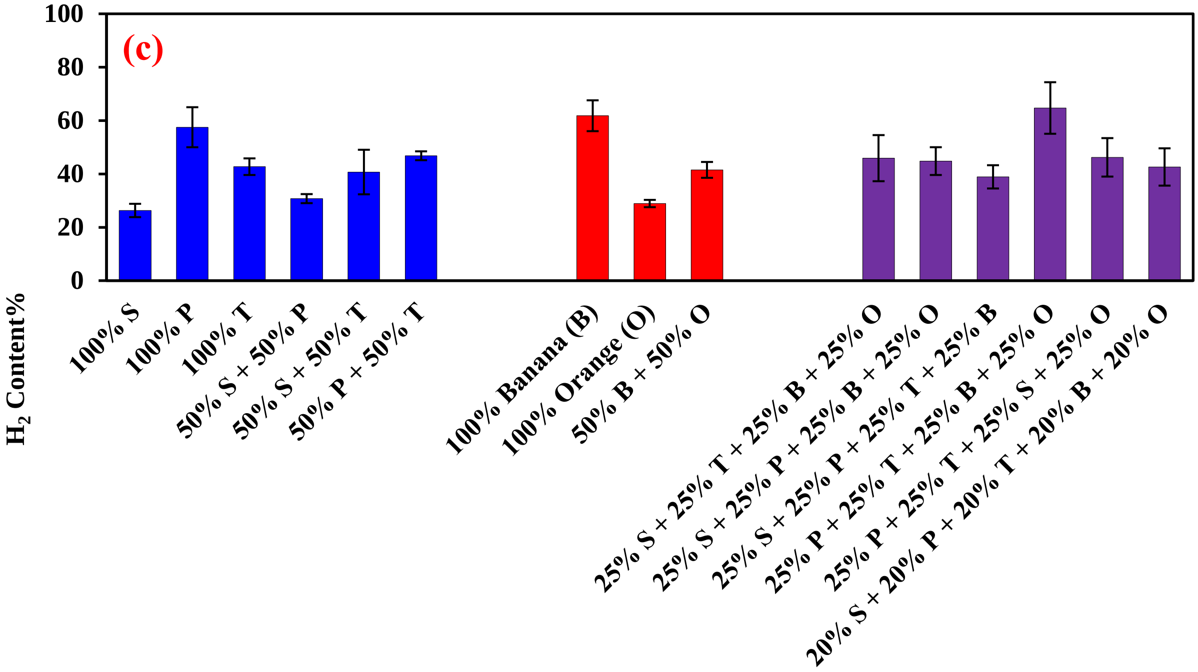


**Fig (1):** Effect of substrate type and composition on **(a)** $H_2$ yield, **(b)** Volumetric $H_2$ production, and **(c)** $H_2$ Content

The second batch used fruit peels (FPs) as the substrate. The highest HY, VHP, and HC were obtained from the sole fermentation of banana peels, while the lowest values were obtained from orange peels. When a mix of 50% banana peels and 50% orange peels (50% B + 50% O) was used, intermediate values were achieved compared to the results obtained from banana and orange peels separately. Banana peels yielded values of HY, VHP, and HC, measuring (55.57 ± 6.3 ml/gCOD), (1.95 ± 0.14 L/L), and (61.8 ± 5.78%), respectively. These high levels can be attributed that fruit peels generally contain higher amounts of simple sugars compared to vegetable peels [18].

In the third batch, a mix of vegetable and fruit peels was tested to identify the optimal combination for biohydrogen production (MFVPs). As demonstrated in **Fig. 1**, the results showed that the mixed fermentation of (25% pea + 25% tomato + 25% banana + 25% orange) had the highest HY of (73.16 ± 9.5 ml/gCOD), VHP of (2.48 ± 0.33 L/L), and HC of (64.7 ± 3.7%), respectively. This can be attributed mostly to the nutritional and trace element balances since the C/N was 25.73 ± 0.22. The combination of vegetable and fruit peels (MFVPs) appears to create a balanced substrate with sufficient initial fermentable sugars and sustained nutrient release. This optimization enhances both the rate and yield of hydrogen production. This synergistic effect may be attributed to the complementary nature of the substrates. Fruit peels facilitate quick fermentation, while vegetable peels ensure continuous hydrogen generation. This batch confirms that utilizing diverse substrates can improve microbial metabolism and overall biohydrogen production efficiency [19].

To summarize, the arrangement for the optimal type and composition of substrates that achieved the highest efficiency in hydrogen production was MFVPs of (25% pea + 25% tomato + 25% banana + 25% orange) > banana peels > pea peels.

### 3.1.2. Degradation Efficiency

The impact of substrate composition and type on COD (chemical oxygen demand) removal is depicted in **Fig. 2**. The obtained data showed that COD removal efficiency, a critical step in the synthesis of bio-hydrogen, is greatly impacted by substrate composition. The MFVPs substrate consisting of [25% pea, 25% tomato, 25% banana, and 25% orange peels] achieved the highest COD removal efficiency of 56.25%, which is consistent with the $H_2$ findings. This indicates a robust degradation of organic material because of the balanced nutrient profile that increased microbial activity. Bananas and pea peels are among the peels that provide a moderate level of COD removal, they obtained 32.41% and 30.94%, respectively. Orange peels, on the other hand, showed the lowest COD removal effectiveness of roughly 10% among the substrates examined. This could be because orange peels contain chemicals like limonene and essential oils that can limit microbial activity. Orange peels' complex structure and reduced biodegradability also make it harder for organic matter to break down during fermentation [20]. These findings suggest that a varied substrate composition improves the elimination of COD and the formation of bio-hydrogen while also being more effective at breaking down organic matter.

The breakdown of proteins, lipids, carbohydrates, and total organic carbon (TOC) was influenced by the type and composition of the substrate in a way that was consistent with the trends seen in $H_2$ production and COD removal efficiency, as illustrated in **Table (2)**. The most significant reductions in carbohydrates, proteins, and lipids were observed with the MFVPs substrate, which is composed of [25% pea, 25% tomato, 25% banana, and 25% orange peels]. This balanced nutrient profile is what supports a variety of microbial activities. Because orange peels contain chemicals that prevent microbial processes and because the organic material within the peels is less accessible, they showed the lowest rates of degradation for all components, especially carbohydrates. This tendency suggests that various substrates improve the breakdown of different organic components, leading to more effective degradation overall and biohydrogen creation [21].

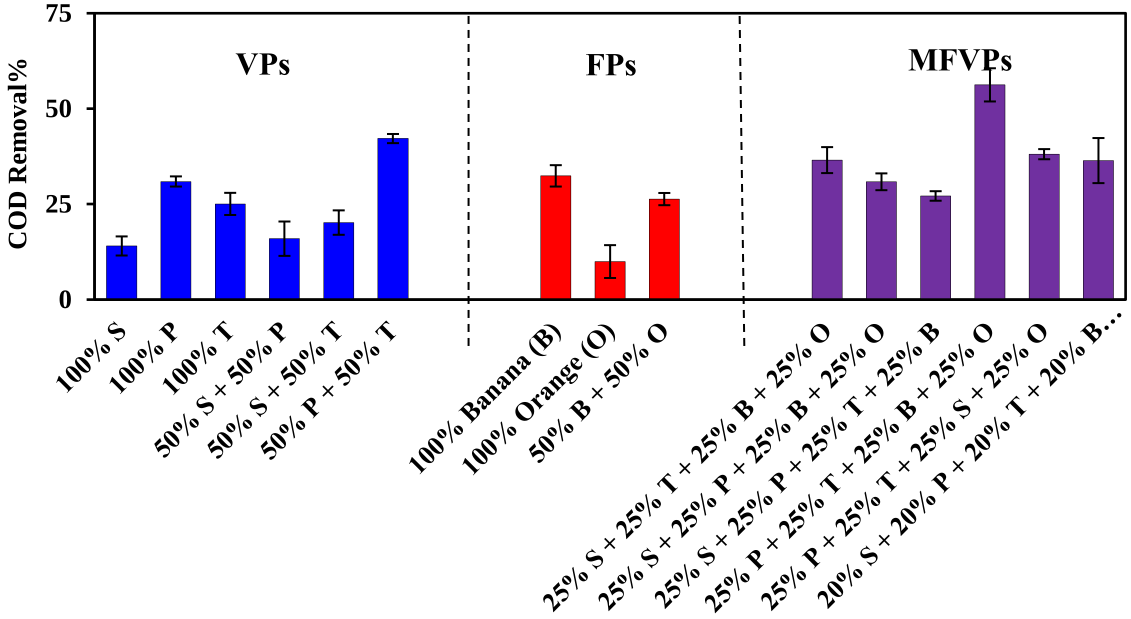


**Fig (2):** Effect of substrate type and composition on COD removal efficiency

**Table (2):** Effect of substrate type and composition on carbohydrates, protein, lipids, and TOC

| Substrate | Substrate Composition | Removal% | | | |
|---|---|---|---|---|---|
| | | Carbohydrates | Protein | Lipids | TOC |
| VPs | 100% Spinach (S) | 21.84 | 10.58 | 17.75 | 17.88 |
| | 100% Pea (P) | 57.23 | 26.34 | 35.65 | 41.27 |
| | 100% Tomato (T) | 34.41 | 21.39 | 28.82 | 29.43 |
| | 50% S + 50% P | 25.72 | 12.87 | 14.36 | 18.68 |
| | 50% S + 50% T | 24.83 | 20.24 | 24.08 | 23.03 |
| | 50% P + 50% T | 56.59 | 27.59 | 47.25 | 48.67 |
| FPs | 100% Banana (B) | 55.71 | 22.23 | 37.21 | 41.78 |
| | 100% Orange (O) | 12.23 | 15.02 | 24.89 | 15.69 |
| | 50% B + 50% O | 32.55 | 25.34 | 28.11 | 29.00 |
| MFVPs | 25% S + 25% T + 25% B + 25% O | 44.37 | 27.90 | 31.13 | 37.35 |
| | 25% S + 25% P + 25% B + 25% O | 44.29 | 20.07 | 35.06 | 36.74 |
| | 25% S + 25% P + 25% T + 25% B | 38.34 | 22.68 | 26.38 | 30.62 |
| | **25% P + 25% T + 25% B + 25% O** | **79.94** | **38.87** | **61.35** | **65.85** |
| | 25% P + 25% T + 25% S + 25% O | 55.67 | 33.10 | 50.37 | 48.05 |
| | 20% S + 20% P + 20% T + 20% B + 20% O | 49.28 | 32.40 | 35.35 | 40.35 |

### *3.2. Effect of GSW Addition*

In the fourth batch (details shown in **Table 1**), gelatin solid waste (GSW) powder was added to investigate its potential as a nutrient supplement and to boost substrate degradation and bio-$H_2$ generation. GSW powder can promote microbial growth and activity since it is high in proteins, carbohydrates, and other vital nutrients. This experiment aimed to determine whether adding different amounts of GSW (1, 2, 4, 6, 8, and 10 g) to the optimal substrate mixture found in the previous batches [optimal MFVPs of (25% pea, 25% tomato, 25% banana, and 25% orange)] could enhance microbial fermentation processes, leading to higher rates and yields of bio-$H_2$ production and better organic matter breakdown. Furthermore, the recommended dosage of GSW will be emphasized. The characteristics of the optimal MFVPs (resulting from the previous section) and GSW utilized in this batch are measured and presented in **Table (3)**. The impact of adding GSW to the process is discussed in the subsequent sections below.

**Table (3):** Characteristics of optimal mixed fruit and vegetable peels (optimal-MFVPs) and gelatin solid waste (GSW)

| Parameter | Unit | Optimal-MFVPs | 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 |
| 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 | - | 25.73 ± 0.22 | 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 |

### 3.2.1. $H_2$ production

The cumulative hydrogen production (CHP) from the fermentation of MFVPs with varying amounts of GSW added (1, 2, 4, 6, 8, and 10 g) via dry anaerobic digestion is shown in **Fig. (3 b)**. The data indicates that adding 2g of GSW to MFVPs increased bio-$H_2$ production to a peak CHP of 886 mL compared to 568 mL from MFVPs alone (**Fig. 3 a)**, which means CHP increased by 60% approximately. This improvement can be attributed to the nutrient-rich composition of GSW, which likely provided an optimal balance of proteins, carbohydrates, and other essential nutrients that stimulated microbial activity and fermentation efficiency [22]Moreover, the time required to reach the CHP peak was approximately 18 hours with the addition of 2 g-GSW, whereas it took 28 hours for MFVPs mono-fermentation. This suggests that the microbial processes were sped up by the presence of GSW, enabling faster substrate breakdown and faster hydrogen generation. This demonstrates how GSW can be used as a useful additive to maximize bio-hydrogen production.

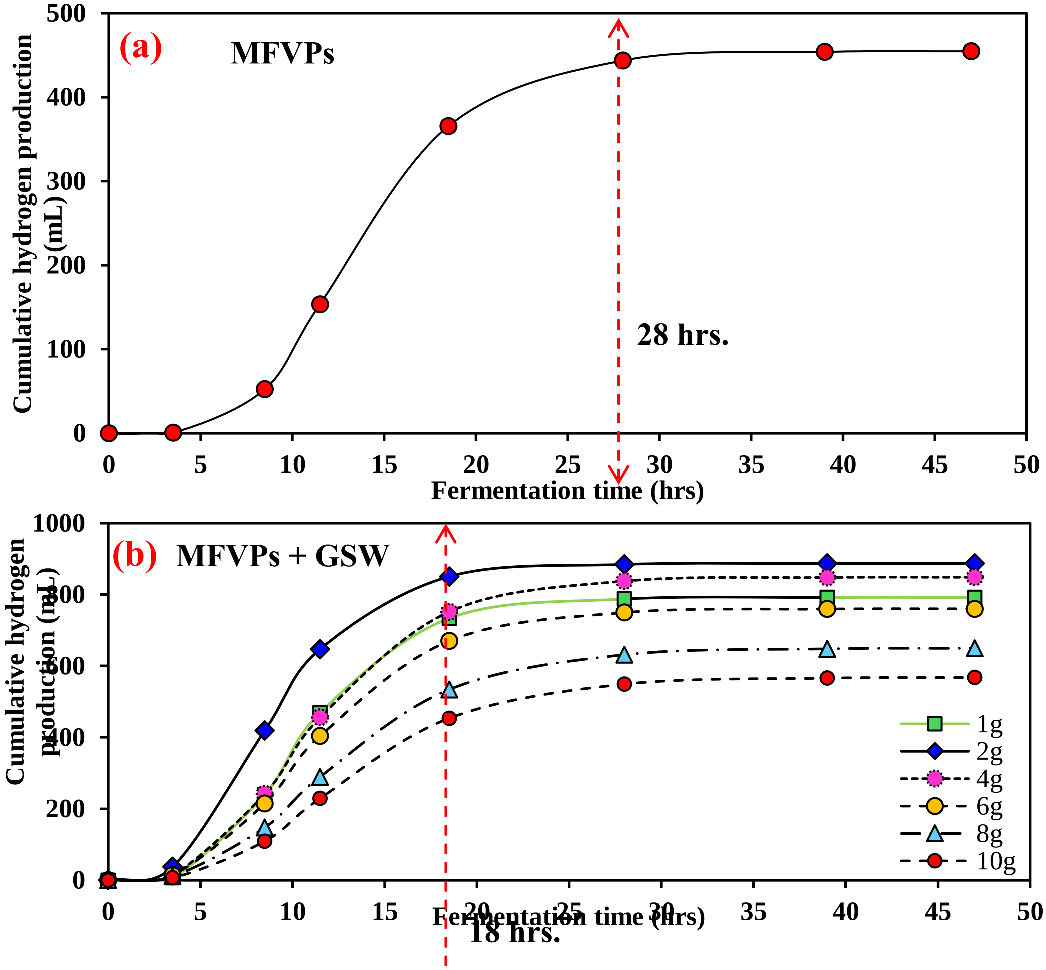


**Fig. (3):** Effect of adding different doses of gelatin solid waste (GSW) on cumulative bio-$H_2$ from the fermentation of mixed fruit and vegetable peels (MFVPs)

Similarly, the effect of adding different doses of GSW on $H_2$ yield, content, and volumetric production is presented in **Table (4)**. The results indicated that the optimal augmentation occurred with 2 g of GSW when applied to the MFVPs substrate. This dose achieved the highest levels of HY of (94.7 ml/gCOD), VHP of (3.26 L/L), and HC of (80.36%), respectively. However, a decrease in these values was seen as GSW concentrations were raised above 2 g. This indicates that higher concentrations of GSW may have inhibitory effects, possibly as a result of nutrient imbalances or the buildup of byproducts like ammonia, which can suppress microbial metabolism, even though small additions of GSW effectively stimulate microbial activity and improve fermentation efficiency. Thus, achieving the optimal GSW concentration is essential for optimizing hydrogen production without having a negative impact on the fermentation process [23].

**Table (4):** Effect of adding different doses of GSW on $H_2$ yield, content, and volumetric production

| Substrate Composition | | $H_2$ yield (ml/gCOD) | $H_2$ Content% | Volumetric $H_2$ production (L/L) |
|---|---|---|---|---|
| **MFVPs** | **Optimal Mixture** | 73.16 | 64.7 | 2.48 |
| **MFVPs + GSW** | 1 g | 85.58 | 72.12 | 2.68 |
| | **2 g** | **94.7** | **80.36** | **3.26** |
| | 4 g | 89.42 | 76.59 | 3.08 |
| | 6 g | 87.88 | 73.48 | 2.86 |
| | 8 g | 81.28 | 70.27 | 2.71 |
| | 10 g | 78.13 | 68.55 | 2.14 |

### 3.2.2. Degradation Efficiency

A consistent trend was seen in the removal of COD, proteins, carbohydrates, lipids, and TOC in response to different dosages of GSW as presented in **Table (5)**. At lower concentrations, the removal efficiency of all components examined was significantly improved, especially with the addition of 2 g of GSW, the highest COD, proteins, and carbohydrates, were 65.5%, 45.4%, and 84.36%, respectively. This improvement can be ascribed to the extra nutrients that GSW offered, which probably encouraged microbial development and activity and improved the rate at which organic matter degraded. Nevertheless, the removal efficiencies started to decrease as the GSW dose rose over 2 grams. This implies that while a moderate dosage of GSW can enhance overall breakdown and optimize microbial metabolism, high doses may cause nutrient imbalances or the build-up of byproducts that limit microbial activity, including ammonia [24]. To maximize the breakdown of these essential organic components without introducing unfavorable effects that could impede the fermentation process, it is imperative to maintain an adequate GSW concentration. Moreover, acetate (HAc), butyrate (HBu), and propionate (HPr) were the predominant volatile fatty acids (VFAs) produced throughout all batches **(Fig. 4)**, according to the examination of soluble metabolites, suggesting their important involvement in the fermentation process. Notably, the concentrations of HAc and HBu, which peaked at 8.11 g/L and 5.81 g/L, respectively, were dramatically increased when 2 g of GSW was added to MFVPs. The high carbohydrate conversion rate of 84.36% indicates that this improvement is due to enhanced microbial activity and efficiency in converting carbohydrates. The increased concentrations of HAc and HBu imply that supplementing with GSW maximizes the metabolic pathways that prefer these VFAs, which are essential intermediates in the generation of hydrogen. It's interesting to note that adding GSW also produced a very low propionate (HPr) concentration (0.58 g/L). The initial pH of 6, which favors pathways that create acetate and butyrate over propionate, is probably the cause of this lower amount of HPr. Because different metabolic pathways compete with one another for hydrogen, high propionate levels are frequently linked to decreased hydrogen yields. This makes the lower HPr concentration favorable. The lowest quantities of HAc (3.1 g/L) and HBu (4.84 g/L) were obtained from the sole fermentation of MFVPs without GSW addition, on the other hand, suggesting less effective fermentation and VFA synthesis. This implies that the extra nutrients from GSW not only improve the synthesis of important VFAs but also change the metabolic balance to favor pathways that produce hydrogen. These results highlight the significance of maximizing the efficiency of biohydrogen generation and related metabolic processes by adjusting both substrate composition and nutrient supplementation.

**Table (5):** Effect of adding different doses of GSW on degradation efficiency in terms of COD, carbohydrates, protein, lipids, and TOC

| **Substrate** | **Substrate Composition** | **Removal%** | | | | |
|---|---|---|---|---|---|---|
| | | **COD** | **Carbohydrates** | **Protein** | **Lipids** | **TOC** |
| **MFVPs** | **Optimal Mixture** | 56.25 | 79.94 | 38.87 | 61.35 | 65.85 |
| **MFVPs + GSW** | 1 g | 60.5 | 80.64 | 40.5 | 64.25 | 66.34 |
| | **2 g** | **65.47** | **84.36** | **45.36** | **69.45** | **68.5** |
| | 4 g | 61.18 | 81.36 | 42.6 | 66.78 | 65.4 |
| | 6 g | 58.3 | 78.6 | 40.9 | 62.12 | 63.47 |
| | 8 g | 54 | 74.3 | 37.66 | 58.36 | 59.87 |
| | 10 g | 48 | 72.5 | 35.6 | 55.36 | 56.25 |

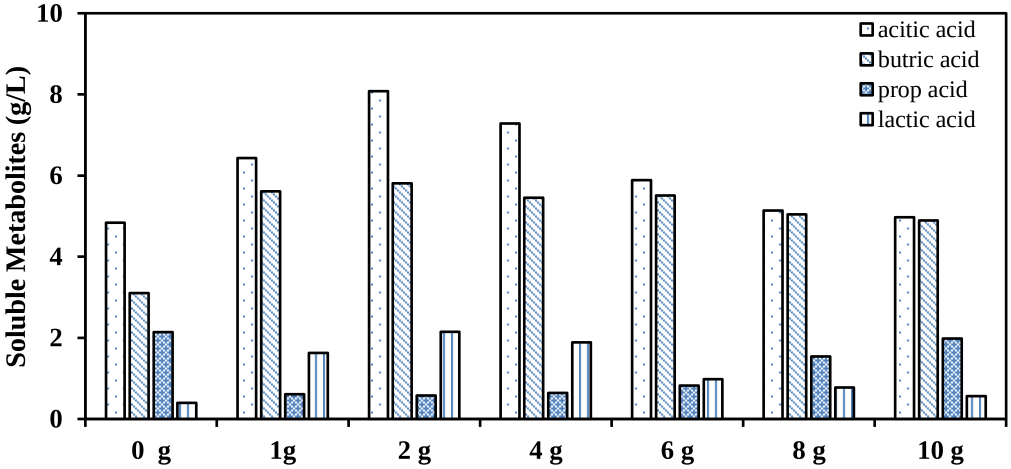


**Fig. (4):** Effect of adding different doses of GSW on the production of soluble metabolite concentrations

## Conclusion

In this study, the effects of substrate type and composition and nutrient supplementation on bio-hydrogen production from organic waste via dark fermentation were investigated. According to the findings, the optimal substrate combination of fruit and vegetable peels, consisting of 25% pea, 25% tomato, 25% banana, and 25% orange peels achieved maximum hydrogen yield as well as the highest efficacy in organics degradation. Additionally, the effect of adding gelatin solid waste (GSW) was examined by testing a range of 1-10 g of GSW added to the optimal MFVPs, and the results showed that a 2 g dose resulted in a 60% increase in CHP as well as an acceleration of the fermentation process. However, performance declined when GSW was raised above this optimal dose (from 2 to 10 g), demonstrating the necessity of supplementing with balanced nutrients.

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