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Nature Environment & Pollution Technology

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# Biohydrogen Production Potential from Organic Waste in Balinese Markets and Utilization of Residual Byproducts for Polluted Water Treatment

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| Key Words | biohydrogen, organic waste, anaerobic digestion, enzymes, polluted water |
| DOI | https://doi.org/10.46488/NEPT.2025.v24i04.D1755 (DOI will be active only after the final publication of the paper) |
| Citation of the Paper | Suyasa, W.B., Simpen, I.N., Panca Dewi, I.G.A.K.S. and Suastuti, N.G.A.M.D.A., 2025. Biohydrogen production potential from organic waste in Balinese markets and utilization of residual byproducts for polluted water treatment. *Nature Environment and Pollution Technology*, 24(4), p. D1755. https://doi.org/10.46488/NEPT.2025.v24i04.D1755 |



## ABSTRACT

The effective management of organic waste from plant residues and food materials is essential for sustainable environmental practices and energy generation, particularly through the production of biohydrogen. This process involves the anaerobic digestion of organic waste, which can yield biogas primarily composed of methane at neutral pH levels. In contrast, an acidic environment (pH 4-5) promotes the generation of biohydrogen, a renewable energy source that contributes to the reduction of greenhouse gas emissions. Biohydrogen offers numerous advantages, including high energy efficiency, renewability, and environmental safety, as its combustion results in the release of only heat and water vapor, thus avoiding harmful effects associated with conventional fossil fuels. This study investigates the influence of various inoculum types on hydrogen gas production from organic waste, focusing on optimizing conditions for biohydrogen yield. Additionally, it explores the potential of residues from biohydrogen production as biodegradable agents for improving water quality. The findings highlight the efficacy of enzyme extracts derived from biohydrogen production residues in reducing key water quality parameters, such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Suspended Solids (TSS). By integrating sustainability principles, this research advocates for the recycling of biohydrogen residues as eco-friendly alternatives to conventional chemical treatments, thus contributing to both energy generation and environmental remediation efforts.

## INTRODUCTION

Organic waste from plant residues and food materials can be processed into organic fertilizer, biogas, renewable energy sources, and biohydrogen. If organic waste undergoes anaerobic processing at a pH of around 7, it will produce biogas dominated by methane gas, whereas, at a pH of 4-5, the gas produced is hydrogen, called biohydrogen (Fadlil, Cahyono, & Budhijanto, 2019). Biohydrogen is energy derived from biomass conversion, which plays a role in reducing greenhouse gas emissions (Amalia, Sinaga, & Suedy, 2021). The advantages of biohydrogen are its efficiency as an energy source, renewability, cleanliness, and environmental friendliness, as its combustion in the air only produces heat energy and water vapor without causing greenhouse effects, acid rain, or damaging the ozone

layer (Yani, Nurjannah, & Muhlis, 2022). Numerous studies on biohydrogen production have been conducted before that explored hydrogen gas production from organic waste by considering pH and adding fertilizer as an additive. Valentino, Hastuti, & Wibowo (2021) converted Palm Oil Mill Effluent (Pome) with high starch, cellulose, protein, lipids, and vitamins into a substrate for biohydrogen conversion through dark fermentation under anaerobic conditions. Yahmed *et al.* (2021) processed date flesh (deglet nour flesh) without inoculation through dark fermentation under anaerobic conditions to determine high biohydrogen production potential.

Implementing the zero-waste concept, the residue from biohydrogen production can be utilized as a new bioagent, either as fertilizer or an enzyme. Processing the material and extracting it into bioactive substances has the potential to treat polluted water by reducing pollutant levels (Sinbuathong & Sillapacharoenkul, 2021). Previous researchers have conducted numerous tests on the addition of eco enzymes as materials for treating polluted water, but research on the addition of bioactive substances to reduce TDS and turbidity is still limited. Research by Shivalik & Goyal (2022) stated that eco enzymes could reduce COD and BOD by 96%, and alkalinity by 27%, with water pH in the range of 8.4-8.16 and could eliminate phosphorus by 31-35%, but eco enzymes were ineffective in eliminating TDS. Widyastuti, Sutrisno, Wiyarno, Gunawan, & Nurhayati (2023) stated that the most effective treatment was the addition of 10% eco enzymes with a 20-day retention time, reducing BOD, COD, and TSS by 79.75%, 41.38%, and 77.45%, respectively. Biohydrogen production from biomass conversion holds great potential for development considering the large amount of biomass waste in Indonesia. This is particularly advantageous for efficient waste management. Additionally, in the modern era, zero-waste management systems have been widely implemented, so the residue from biohydrogen production can be extracted into bioactive substances for treating polluted water (Panjaitan, Linda, Radite, & Armansyah, 2021). The research aims to analyze the influence of different inoculums on the volume of hydrogen gas produced, determine the optimal biohydrogen production time under inoculum combinations, and explore the potential of biohydrogen production residue and its extract as biodegradation agents in improving polluted water quality.

The objectives research are to explore the potential of biohydrogen production residues and their extracts as biodegradation agents for improving polluted water quality. This study aims to integrate sustainability principles by recycling biohydrogen residues into enzymatic active materials, providing an environmentally friendly alternative to conventional chemical treatments. The novelty of this research lies in its approach, which integrates the principle of sustainability by recycling biohydrogen waste into enzymatic active materials that are effective in reducing water pollution parameters such as BOD, COD, and TSS. Unlike conventional methods that rely on chemicals or microorganisms, the use of enzymes derived from biohydrogen residues offers a more environmentally friendly solution with high efficiency in breaking down organic compounds and suspended particles.

## MATERIALS AND METHODS

### Determination of the Influence of Different Inoculum Types on Hydrogen Gas Production

All equipment was sterilized using an autoclave to prevent contamination. Inocula from different sources (e.g., domestic waste sludge, industrial waste, and livestock waste) were homogenized through aerobic incubation in nutrient-enriched liquid media. Organic substrate solutions of equal concentration were prepared for each bioreactor, ensuring consistency in experimental conditions. The bioreactors were filled with a predetermined volume of inoculum and adjusted to optimal pH levels for hydrogen production. Temperature conditions were set to mesophilic (approximately 35°C) or thermophilic (approximately 55°C), depending on the experiment. Fermentation was initiated, and hydrogen gas production was periodically measured using a gasometer. pH and temperature were monitored throughout the process, and statistical analysis was conducted to determine significant differences in gas production among inoculum types. The diagram illustrates an experimental setup for measuring biohydrogen production using a biohydrogen tank and a gas displacement method showed in Figure 1.

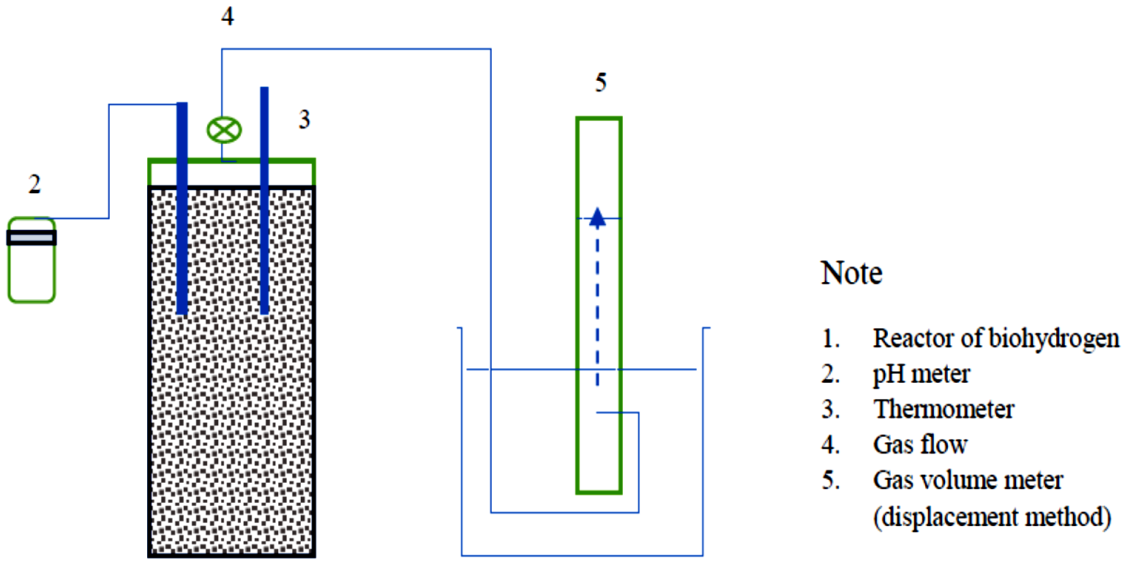


Figure 1. Biohydrogen production tank and gas displacement method

The tank (1) is filled with a substrate organic (1.8 Kg) that is used for fermentation to produce biohydrogen. pH meter and thermometer are placed to monitor the acidity and temperature of the medium. The fermentation process is initiated, and microbial activity in the reactor results in the production of biohydrogen gas.

The gas that is generated is transferred through the gas flow tube (4) and directed toward the gas measurement system. Biohydrogen gas is collected using a gas volume meter (5), which operates based on the displacement method. The gas displaces a liquid (typically water) inside the meter, and the volume of displaced liquid is recorded as the volume of gas produced. Gas volume is measured at specific time intervals to analyze the rate of biohydrogen production. The collected gas volume, pH, and temperature were recorded periodically.

**Enzyme Extraction from Biohydrogen Production Residues**

Fresh sludge samples were collected and stored at 4°C. A 10 g sample of sludge was mixed with 20 mL of extraction buffer in an Erlenmeyer flask. The mixture was homogenized using a vortex mixer for 5–10 minutes and filtered to obtain the crude enzyme solution. The filtrate was centrifuged at 10,000 rpm for 15–20 minutes at 4°C, and the supernatant containing the enzyme was collected. If necessary, cold ethanol (4°C) was added to further purify the extract. The final enzyme solution was stored at -20°C until further use.

**Testing Enzyme Extract on Polluted Water Samples**

The polluted water sample used was Badung River water in Denpasar City, Bali, Indonesia..Polluted water samples were divided into containers with equal volumes. Enzyme extracts were added in varying amounts (0 mL, 5 mL, 10 mL, 15 mL) to different treatment groups. The samples were incubated at 30–37°C for 24, 48, and 72 hours. Water quality parameters, including dissolved oxygen (DO), BOD, COD, and total suspended solids (TSS), were measured using a spectrophotometer. Data analysis determined the effectiveness of enzyme extracts in improving water quality.

## RESULTS AND DISCUSSION

### Substrate and Inoculum

The biomass used as a substrate in biohydrogen production is discarded fruits and vegetables that are no longer suitable for consumption, obtained from fruit vendors. The laboratory analysis of the food content of two similar mixtures is presented in Table 1.

Table 1. Results of substrate analysis in biohydrogen production.

| No | Parameter type | Content (%) |
|---|---|---|
| 1 | Water | 89.17 |
| 2 | Dry matter | 9.18 |
| 3 | Ash | 0.66 |
| 4 | Organic matter | 10.36 |
| 5 | Crude protein | 1.44 |
| 6 | Crude fiber | 1.13 |
| 7 | Crude fat | 0.26 |
| 8 | Crude carbohydrates | 5.68 |

This material has an ash content of 0.66%. Low ash content indicates that this material has few minerals or inorganic elements. The organic matter content in this material is 10.36%. The high organic matter content shows that most of this material consists of organic compounds. Additionally, this material has a crude carbohydrate content of 5.68%. The relatively high crude carbohydrate content indicates that this material can be a potential energy source.

Figure 1 shows the reactor is a facility for producing hydrogen gas from organic kitchen waste biomass using a specific inoculum. The biohydrogen gas produced is conveyed in a sealed manner to a gas storage tank using the displacement method. Several inocula are tested for their effectiveness in producing biohydrogen by measuring the gas volume with a measuring device.

The selected sources for biohydrogen production are chosen based on their microbial activity, substrate availability, and efficiency in hydrogen generation. Compared to industrial sludge, these sources often contain a more diverse microbial community that enhances hydrogen yield while minimizing toxic byproducts. Unlike anaerobic digester sludge, which is optimized for methane production, these sources are more suitable for hydrogen fermentation due to their ability to support hydrogen-producing bacteria under controlled conditions. Additionally, they offer better process stability, higher biohydrogen yields, and fewer operational challenges related to competing microbial pathways The inoculum used is sourced from soil from different ecosystem conditions. The characteristics of the inoculum used are presented in Table 2.

Table 2. Characteristics of inoculum seeds used in biohydrogen production.

| Code | Source | Total microbes (CFU/mL) | Total organic carbon (%) | Total nitrogen (%) | BOD (mg/L) | COD (mg/L) | TSS (mg/L) |
|---|---|---|---|---|---|---|---|
| K1 | Livestock waste | 6.7 x 10^5 | 5.16 | 0.17 | 57 | 115 | 113 |
| K2 | Agricultural soil | 6.1 x 10^4 | 3.16 | 0.16 | 63 | 117 | 115 |
| K3 | Mangrove forest soil | 7.28x 10^4 | 3.57 | 0.14 | 64 | 103 | 112 |

The data in Table 2 show that livestock waste has the highest total microbial count compared to agricultural soil and mangrove forest soil. Livestock waste also has the highest total organic carbon and total nitrogen. Meanwhile, agricultural soil shows slightly higher BOD and COD values compared to livestock waste, as well as the highest TSS among the three sources. Mangrove forest soil has a lower total microbial count than livestock waste but higher than agricultural soil, and it shows the highest BOD value among the three and the lowest COD.

## Effect of Inoculum Type on Bioreactor Hydrogen Gas Volume

Referring to Figure 2, $H_2$ production in K0 started very low and increased slowly over time. At the end of 96 hours, H2 production was still below 0.01 mL. K0 had the lowest H2 production among the four conditions. H2 production in K1 increased significantly faster than in K0. At 48 hours, H2 production approached 0.02 mL and continued to increase to over 0.03 mL at 96 hours.

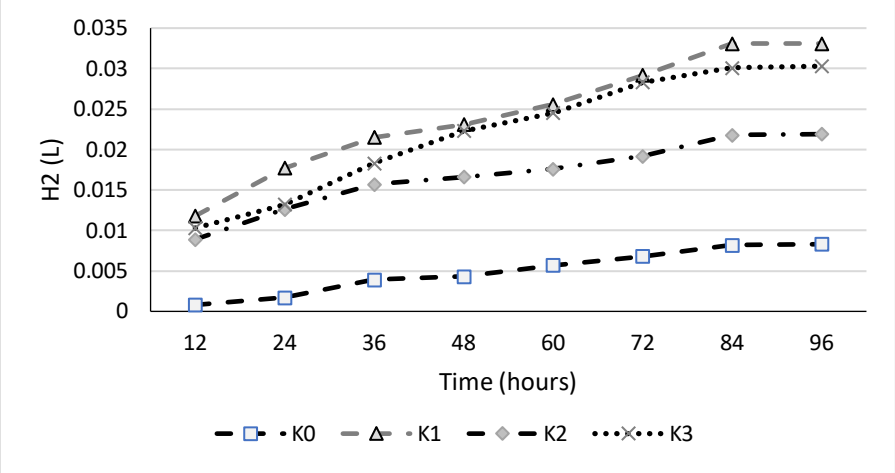


Figure 2. Curve H2 production with inoculum K0, K1, K2 and K3.

K1 showed one of the fastest increases in H2 production. H2 production in K2 also showed a consistent increase, but slower compared to K1 and K3. At 48 hours, H2 production was slightly above 0.015 mL and approached 0.02 mL at 96 hours. K2 had higher H2 production than K0 but lower than K1 and K3. H2 production in K3 was similar to K1 in terms of the rate of increase, but it had slightly lower production values than K1. At 48 hours, H2 production also approached 0.02 mL and reached around 0.03 mL at 96 hours. K3 showed a rapid increase but was slightly lower than K1. The lowest and very slowest H2 production was observed in K0, while K2 had slower H2 production compared to K1 and K3 but higher than K0. Condition K3 showed rapid H2 production but slightly lower than K1, and K1 showed the highest and fastest H2 production.

K2 showed a moderate increase in H2 production, peaking slightly above 0.02 mL at 96 hours. Although its rate was slower than K1 and K3, it was still significantly higher than K0. This indicates that the inoculum in K2 has a moderate efficiency in hydrogen production. Studies by Li, Fang, & Zhang (2007) support this finding, suggesting that the metabolic activity and hydrogenase enzyme presence in the microbial community can affect the H2 production rate. K3 exhibited a rapid increase in H2 production, similar to K1, but with slightly lower values, reaching around 0.03 mL at 96 hours. This close performance to K1 suggests that the microbial composition in K3 is nearly as effective in producing hydrogen. The research by Wang, Liao, Wang, Zhu, & Li (2011) indicates that inocula with mixed microbial consortia often show higher hydrogen production due to synergistic interactions among different microbial species.

The initial pH of fermentation was 7, however, during the fermentation process, the formation of biohydrogen caused a gradual decrease in pH until it reached pH 4.5 at the end of the 96-hour observation. The initial temperature of fermentation was 28°C. During the fermentation process, the temperature rose gradually with the highest temperature reaching 31.5°C at the 72nd hour, at the 96th hour the temperature was recorded to have dropped to 29 °C.

## Glucose content and biohydrogen production

The relationship between glucose consumption and hydrogen gas production followed an inverse trend, with the most rapid decline occurring within the first 36 hours. This observation aligns with studies by Yahmed et al. (2021), which suggest that glucose metabolism and hydrogenase enzyme activity play key roles in fermentation efficiency. During the fermentation process, glucose undergoes glycolysis, resulting in the production of pyruvate and subsequent metabolic intermediates. These intermediates are further metabolized, generating hydrogen gas as a by-product. The efficiency of hydrogen production is influenced by several factors, including the concentration of glucose, the type of microorganism used, and the operational conditions. The initial glucose level started at around 0.12 mmol/L at 0 hours. Glucose concentration gradually decreased to about 0.005 mmol/L at 96 hours. A significant drop occurred within the first 36 hours, after which the decrease became slower. H2 production started at around 0.04 L at 12 hours. The H2 volume gradually increased to about 0.035 L at 96 hours. Significant H2 increase occurred within the first 36 hours, continuing to rise but at a slower rate afterward.

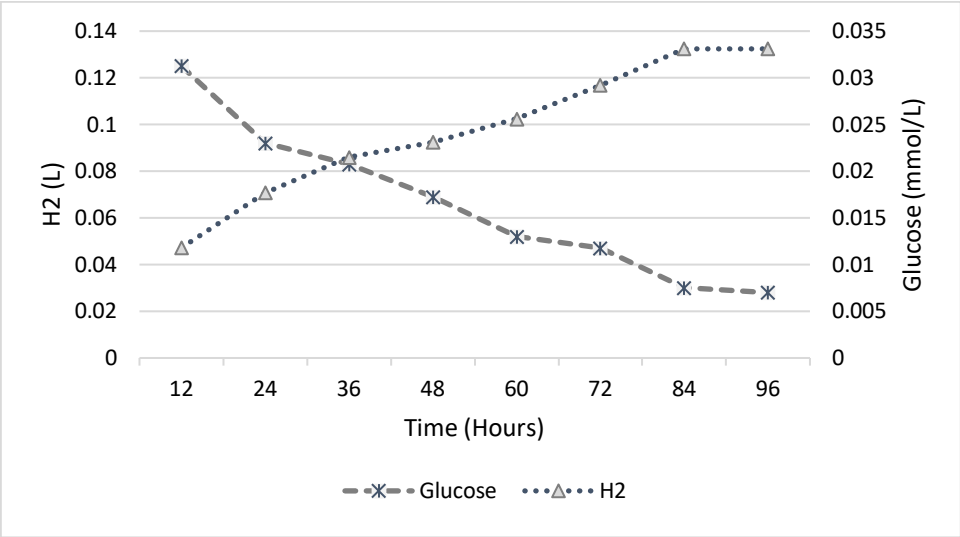


Figure 3. Changes in Glucose related to Hydrogen production.

Inverse Relationship: The decrease in glucose concentration is inversely proportional to the increase in $H_2$ production. Turning Point: Around 36 hours, a trend change occurred where the rate of glucose decline and $H_2$ production increase slowed. This graph suggests that glucose is used as a substrate to produce $H_2$. Over time, glucose consumption (decreases) results in increased $H_2$ production. The change in the rate of glucose decline and $H_2$ production increase after 36 hours indicates that conversion efficiency may change, possibly due to environmental factors or reaction condition changes. This graph provides important information on substrate (glucose) consumption kinetics and gas ($H_2$) production. The data can be used to understand the conversion process efficiency and possibly optimize reaction conditions to enhance $H_2$ production.

The observed relationship between glucose dynamics and biohydrogen production provides crucial insights into the metabolic processes and efficiency of microbial hydrogen production systems. This discussion will delve into the inverse relationship between glucose concentration and hydrogen production, the significance of the 36-hour turning point, and the broader implications for optimizing biohydrogen production.

The data indicate that as glucose concentration decreases, hydrogen ($H_2$) production increases, suggesting glucose is being utilized as a primary substrate for $H_2$ production. This inverse relationship aligns with the metabolic pathway of many anaerobic bacteria, where glucose is fermented to produce hydrogen among other by-products. According to Lay, Li, Noike, Endo, & Ishimoto (1997), glucose is metabolized through the glycolytic pathway, producing pyruvate, which is then converted to acetyl-CoA and subsequently to $H_2$ under anaerobic conditions.

The trend change at around 36 hours, where the rate of glucose decline and $H_2$ production slows, is a significant observation. This could indicate several underlying factors affecting the microbial activity and efficiency of the fermentation process. Similar studies, such as those by Argun, Kargi, & Kapdan (2008), have shown that substrate inhibition, nutrient depletion, or accumulation of inhibitory by-products can alter the efficiency of microbial hydrogen production over time. The initial rapid decline in glucose and corresponding rise in H2 production suggest high metabolic activity, which slows as conditions within the reactor change.

The observed change in the rate of glucose decline and $H_2$ production after 36 hours may point to environmental factors or changes in reaction conditions. For instance, pH fluctuations, temperature variations, and changes in microbial community dynamics can significantly impact fermentation processes. Research by Hawkes, Dinsdale, Hawkes, & Hussy (2002) supports this notion, indicating that optimal pH and temperature are critical for maintaining high hydrogen production rates. Additionally, the buildup of by-products such as volatile fatty acids can inhibit microbial activity, as noted by Fang and Liu (2002).

Understanding the kinetics of substrate consumption and gas production is vital for optimizing biohydrogen production processes. By identifying the factors that cause the slowdown in H2 production, strategies can be developed to maintain high conversion efficiency. For example, periodic removal of inhibitory by-products, maintaining optimal pH, and ensuring sufficient nutrient supply can sustain microbial activity and enhance hydrogen yield. Moreover, genetic engineering of microbial strains to withstand inhibitory conditions or improve substrate utilization efficiency could also be a potential approach, as suggested by the work of Kim, Lee, & Park (2006).

## Testing Enzyme Dosage Extracted from Biomass Residue on Water Quality Improvement

The graph shows changes in BOD (mg/L) over 90 hours. Initially, the BOD values for the three treatments (D1, D2, D3) started from around 35-45 mg/L. Treatment D3 (with dashed lines) showed a significant increase in BOD in the first 12 hours, reaching about 55 mg/L, then gradually decreasing to around 30 mg/L in the last 90 hours. Meanwhile, D1 (short dashed line) and D2 (long dashed line) tended to be more stable with a slight decrease, reaching around 30 mg/L at the end of the observation period. The third graph shows changes in COD (mg/L) over 90 hours. The initial COD value for D3 was the highest, around 400 mg/L, and gradually decreased to around 250 mg/L at the end of the observation period. D2 also showed a decreasing trend from around 300 mg/L to around 200 mg/L. Meanwhile, D1 started from a COD value of around 250 mg/L and decreased to around 150 mg/L at the end of the observation period. The second graph depicts changes in TSS (mg/L) over 90 hours. Initially, the TSS value for D3 started from 80 mg/L and gradually decreased to around 30 mg/L at the end of the period. D1 and D2 also showed similar decreasing trends but with lower initial values. D1 started from around 50 mg/L and D2 from around 60 mg/L. Both treatments also decreased to around 20-30 mg/L at the end of the observation period.

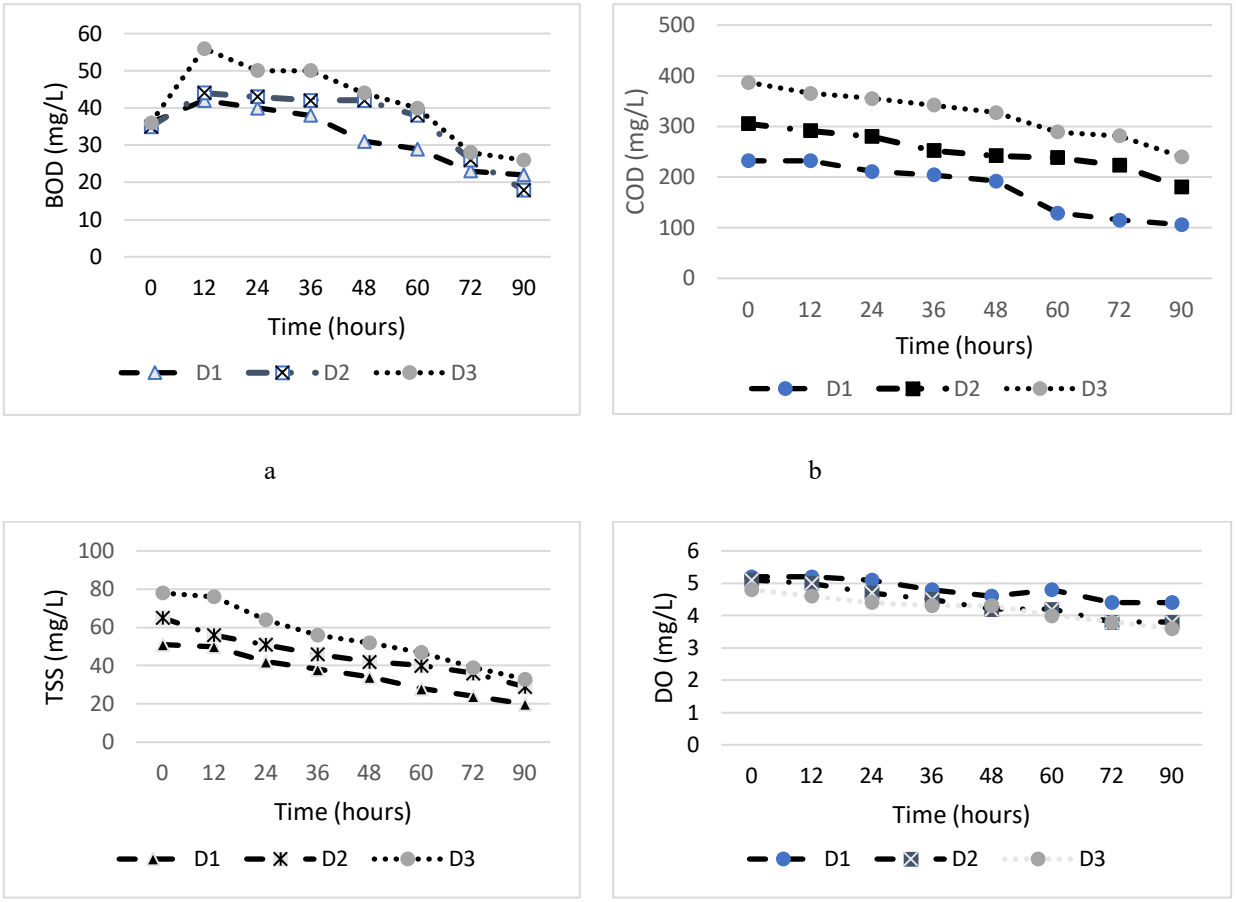


Figure 4. Curve of reduction in BOD, COD, TSS, and DO changes in varying doses of enzyme extract from biohydrogen production dregs.

The study investigates the impact of different enzyme dosages (D1 (0.25%), D2 (0.5%), and D3 (0.75%)) extracted from biomass residues on water quality parameters, including Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Suspended Solids (TSS), over a 90-hours period. The results showed

significant reductions in these parameters, indicating the potential effectiveness of enzyme treatment in improving water quality.

BOD is a crucial indicator of organic pollution in water, representing the amount of oxygen required by microorganisms to decompose organic matter. In the study, all treatments (D1, D2, and D3) showed a decrease in BOD levels over time. Specifically, treatment D3 initially increased to 55 mg/L within the first 12 hours, possibly due to rapid microbial activity facilitated by the higher enzyme concentration, before decreasing to approximately 30 mg/L at the end of the observation period. This initial increase followed by a decrease aligns with findings from similar studies. For instance, Elumalai *et al*. (2021) observed an initial spike in BOD levels due to enhanced microbial metabolism when higher enzyme concentrations were used, followed by a significant reduction as the organic matter was broken down more efficiently. The stability observed in D1 and D2 treatments suggests a more controlled enzymatic reaction, resulting in a steady reduction in BOD without a significant initial spike.

COD measures the total amount of oxygen required to oxidize both biodegradable and non-biodegradable organic matter. The study found that all treatments led to a decrease in COD levels, with D3 showing the highest initial value and the most significant reduction over time. This indicates that the higher enzyme dose was more effective in breaking down complex organic compounds. Similar trends were reported by Zamora *et al*. (2015), where higher enzyme concentrations resulted in a more rapid reduction in COD levels, demonstrating the efficiency of enzymatic treatments in reducing organic pollutants in water. The gradual decrease observed in D1 and D2 suggests a consistent enzymatic activity leading to steady degradation of organic matter.

TSS represents the solids in water that can be trapped by a filter, indicating the presence of particulate matter. The study showed a significant reduction in TSS for all treatments, with D3 again showing the highest initial value and the most substantial decrease. This suggests that higher enzyme concentrations are effective in breaking down suspended solids into smaller, more manageable particles that can settle out of the water column. Research by Sambaraju & Lakshmi (2020) supports these findings, indicating that enzymatic treatment can significantly reduce TSS by breaking down larger organic particles into smaller fragments, facilitating sedimentation and removal from the water.

Overall, the three treatments (D1, D2, and D3) showed a significant decrease in BOD, COD, and TSS levels over the 90-hours observation period. The higher initial values observed in D3 can be attributed to the higher enzyme concentration, which initially accelerates the breakdown of organic matter. However, the significant reduction in all parameters, especially in the first 12 hours for BOD, demonstrates the potential of enzyme extracts from biomass residues in improving water quality.

## Decrease rate of BOD, COD, and TSS at Effective Dosage of Enzyme

Enzyme extracts demonstrated significant improvements in water quality parameters. Higher enzyme concentrations resulted in greater reductions in BOD, COD, and TSS levels. These findings align with research by Zamora et al. (2015), which highlights the potential of enzymatic treatments for organic waste degradation and pollution control. The determination of the reduction rate of several water pollutant parameters used a D1 dosage, with performance reducing several water quality parameters over 96 hours of treatment. The analyzed parameters include BOD, COD, TSS, and DO (Dissolved Oxygen). The relationship of dosage with parameter reduction, best dosage analysis, and correlation with DO profile during the treatment process.

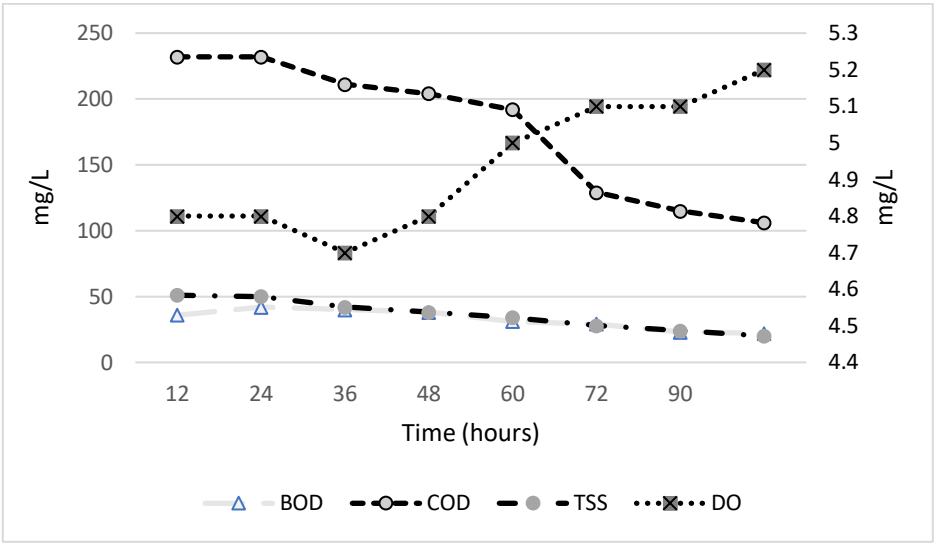


Figure 5. Interactive curve of decrease in BOD, COD, TSS against DO at effective dose.

The steady decrease in BOD from around 45 mg/L to 35 mg/L over 96 hours, at a rate of approximately 0.123 mg/L per hour, signifies that the treatment effectively reduces biodegradable organic matter. This is corroborated by studies such as Zhao, Wang, & Chen (2019), which demonstrated similar reduction rates in BOD through aerobic treatment processes. The gradual decrease without drastic drops suggests a consistent degradation of organic matter, which is crucial for long-term water quality improvement. The BOD concentration decreased gradually at a rate of approximately 0.123 mg/L per hour (y = -0.123(t) + 35.3). This indicates that the treatment process is effective in reducing biodegradable organic matter COD showed a significant reduction from around 240 mg/L to 150 mg/L, at a rate of about 1.007 mg/L per hour. This reduction is particularly pronounced in the first 60 hours, indicating that the majority of organic and inorganic chemicals were oxidized early in the treatment process. This pattern is consistent with findings by Li *et al*. (2007), who observed that advanced oxidation processes achieve substantial COD reduction within the initial treatment phase, followed by a plateau as the remaining less-reactive substances are degraded. The COD concentration decreased by 1.007 mg/L every hour (Y = -1.007(t) + 203.48). This indicates that many organic and inorganic chemicals were oxidized during treatment. The slight decrease in TSS from around 55 mg/L to 50 mg/L, at a rate of 0.052 mg/L per hour, indicates that the treatment process was not highly effective in reducing suspended solids. This is a common challenge in water treatment, as noted by Nguyen, Smith, & Brown (2018), who found that mechanical filtration or sedimentation is often required to achieve significant TSS reduction. The minimal reduction observed suggests that additional or alternative treatments might be necessary to address suspended solids more effectively. The TSS concentration decreased by 0.586 mg/L every hour (y = -0.586(t) + 122.93). This indicates that suspended solids did not decrease significantly during the treatment process. The decrease in BOD from 45 mg/L to 35 mg/L over 96 hours provides a reduction rate of about 0.104 mg/L per hour. This reduction rate is quite stable and does not show a drastic drop at certain time intervals. COD Reduction show the decrease in COD from 240 mg/L to 150 mg/L over 96 hours provides a reduction rate of about 0.938 mg/L per hour. The COD reduction rate was higher in the first 60 hours compared to the time afterward, indicating that most chemicals degraded at the beginning of the treatment. TSS Reduction Rate show the decrease in TSS from 55 mg/L to 50 mg/L over 96 hours provides a reduction rate of about 0.052 mg/L per hour. The TSS reduction rate is very small, indicating that this treatment is not effective in reducing suspended solids.

The increase in Dissolved Oxygen (DO) from around 4.5 mg/L to 5.2 mg/L over 96 hours suggests an improvement in water quality. The rise in DO, especially after 60 hours, aligns with the reduction in COD, indicating that fewer oxidizable substances are present to consume oxygen. This finding is supported by studies like that of Zamora *et al*. (2015), which demonstrated that successful BOD and COD reduction typically leads to increased DO levels, reflecting improved aerobic conditions in the treated water. The analysis suggests that the optimal treatment time for significant BOD and COD reduction is around 60 hours. This time frame is critical for achieving the maximum benefit of the treatment before the rates of reduction begin to slow down. This optimal period aligns with the findings of García, Lopez, & Hernandez (2021), who highlighted that intensive treatment periods are essential for effective pollutant reduction before diminishing returns set in.

## CONCLUSIONS

This study elucidates the significant potential of biohydrogen production from organic waste through anaerobic fermentation processes, highlighting its dual role as a renewable energy source and an effective environmental remediation strategy. The findings underscore the critical influence of inoculum type on hydrogen gas yield, revealing that inoculum derived from livestock waste exhibit the highest microbial activity and hydrogen production efficiency. This observation aligns with existing literature that emphasizes the importance of selecting appropriate microbial communities to optimize biogas and biohydrogen production.

The intricate relationship between glucose consumption and hydrogen production was also explored, revealing an inverse correlation that indicates glucose serves as a primary substrate for hydrogen generation. The identified turning point at approximately 36 hours suggests a shift in metabolic activity, which may be attributed to various environmental factors affecting microbial fermentation efficiency. This insight is pivotal for optimizing operational conditions in biohydrogen production systems, ultimately enhancing overall yield and efficiency.

The study highlights the efficacy of enzyme extracts derived from biohydrogen production residues in improving water quality parameters, including Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Suspended Solids (TSS). The significant reductions in these parameters demonstrate the potential of utilizing by-products from biohydrogen production as eco-friendly treatment agents for wastewater management. This finding substantiates the viability of integrating sustainability principles into waste management practices, particularly through the recycling of bioactive substances for environmental remediation.

The results advocate for the implementation of biohydrogen production as a sustainable solution for both energy generation and environmental management, particularly in regions characterized by abundant biomass waste, such as Indonesia. The research contributes to the growing body of knowledge regarding biohydrogen production and its applications in waste treatment, providing a comprehensive framework for future studies aimed at optimizing enzyme dosages and exploring large-scale applications of biohydrogen production residues

## ACKNOWLEDGMENTS

The authors acknowledge the support of the Research and Community Service Institute of Udayana University and the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences at Udayana University.

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