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Biochemical Dynamics during EM4-Assisted Fermentation of Water Hyacinth (*Eichhornia crassipes*) and Nutrient Characterization of a Potential Liquid Biofertilizer

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Abstract

Water hyacinth (*Eichhornia crassipes*) is an invasive aquatic plant that poses ecological challenges but also represents a potential resource for biomass valorisation. This study investigated the early-stage fermentation of water hyacinth using Effective Microorganisms 4 (EM4) at biomass to water ratios of 1:4 and 1:5 over seven days. Glucose concentration, oxidation-reduction potential (ORP), pH, and temperature were monitored, while citric acid and gluconic acid concentrations were measured on days 2, 4, and 6. The final liquid product from the 1:4 treatment was characterized for nitrogen, phosphorus, potassium, and organic carbon. Measured glucose concentrations decreased from 3,491.25 to 1,091.25 mg L^{-1} in the 1:4 treatment and from 3,228.75 to 953.75 mg L^{-1} in the 1:5 treatment, corresponding to decreases of 68.74% and 70.46%, respectively. ORP declined from positive to negative values in both treatments, while pH and temperature fluctuated throughout fermentation. Citric acid was the predominant measured organic acid, reaching its highest concentration on day 4 in the 1:4 treatment. Gluconic acid concentrations also peaked on day 4 in both treatments. The final product contained 1.35% nitrogen, 0.36% total P_2O_5, 1.53% K_2O, and 26.63% organic carbon. These findings demonstrate measurable changes in glucose concentration and fermentation conditions alongside the presence of nutrients in the final product. The results support further investigation of water hyacinth fermentation as a potential biomass valorisation pathway, although nutrient recovery efficiency, product safety, and agronomic effectiveness require additional validation.

Keywords: Biomass valorisation, EM4 fermentation, Glucose depletion, Liquid biofertilizer, Organic acids, Water hyacinth

1. Introduction

Water hyacinth (*Eichhornia crassipes*), also known as Pontederia crassipes, is an invasive aquatic plant that poses persistent challenges to tropical and subtropical freshwater ecosystems. Its rapid growth, prolific seed production, and clonal propagation facilitate the formation of dense floating mats, particularly in nutrient-enriched waters affected by agricultural runoff and urban pollution [1], [2], [3], [4], [5]. These infestations can reduce light penetration, alter water quality, disrupt aquatic food webs, and contribute to oxygen depletion. Water hyacinth also obstructs navigation and fishing activities, interferes with water use, and increases the burden of freshwater resource management, potentially affecting the livelihoods of communities dependent on aquatic resources [2], [6], [7], [8]. Although mechanical, chemical, and biological control methods are available, sustained management remains challenging because of rapid regrowth, operational costs, and potential environmental impacts [9], [10]. Consequently, strategies that integrate infestation management with the productive utilization of harvested biomass are increasingly relevant to sustainable freshwater resource management.

Beyond its ecological impacts, water hyacinth is a potentially valuable renewable feedstock because of its rapid biomass accumulation and diverse organic composition. Its biomass contains cellulose, hemicellulose, soluble carbohydrates, and minerals, although their proportions vary with environmental conditions, plant

maturity, geographical location, and analytical methods [11], [12], [13]. While soluble sugars may be readily accessible to microorganisms, structural carbohydrates generally require hydrolysis to improve their availability for fermentation [14], [15]. Previous studies have explored water hyacinth for biofuels, biogas, biochar, activated carbon, compost, and soil amendments [16], [17], [18], [19]. Its mineral constituents also offer opportunities for nutrient recovery; however, the potential accumulation of heavy metals must be considered when evaluating biomass for agricultural use [20], [21]. Collectively, these characteristics support water hyacinth valorization as a circular bioeconomy strategy that connects invasive biomass management with resource recovery and value-added product development.

Fermentation offers a potential route for converting water hyacinth biomass into liquid products for agricultural applications, including liquid organic fertilizers. Effective Microorganisms 4 (EM4) is used as a microbial inoculant in organic waste processing and liquid fertilizer production, although fermentation outcomes depend on substrate characteristics, inoculum conditions, and the physicochemical environment [22], [23], [24]. Soluble sugars provide accessible carbon and energy for microbial metabolism, and changes in their concentrations can indicate the progression of substrate utilization. Nevertheless, sugar depletion does not necessarily correspond to equivalent product formation because consumed carbohydrates may be converted into microbial biomass, organic acids, and other metabolites. Organic acid production may influence fermentation acidity and potentially affect nutrient solubilization, but these effects require experimental verification. Similarly, nitrogen, phosphorus, and potassium in the original biomass may be retained, redistributed, or lost during processing. Therefore, the assessment of fermented biomass requires consideration of both process dynamics and final product composition rather than relying solely on fermentation duration or apparent decomposition [25].

Process monitoring provides a basis for interpreting microbial fermentation and evaluating the relationships between substrate changes and product characteristics. Glucose concentration can be used to track changes in soluble carbohydrate availability, whereas oxidation-reduction potential (ORP), pH, and temperature provide complementary information about the physicochemical conditions of the fermentation medium. ORP can help characterize changes in redox conditions, although its interpretation depends on microbial activity, substrate composition, and measurement conditions [26]. Changes in pH may accompany organic acid accumulation and other biochemical transformations, while organic acid profiling provides additional information about the composition of the fermentation liquid [27], [28]. Despite the availability of these analytical approaches, interpreting individual measurements in isolation may provide an incomplete understanding of fermentation performance. In particular, an integrated assessment linking sugar concentration, redox dynamics, acidity, organic acid profiles, and final nutrient composition can help characterize the process without assuming specific metabolic pathways that have not been directly measured. Comparing different biomass-to-water ratios can further provide preliminary information about how substrate dilution relates to the observed fermentation characteristics.

Accordingly, this study investigated the seven-day fermentation of water hyacinth using EM4 at two biomass-to-water ratios, 1:4 and 1:5. The study evaluated daily changes in temperature, ORP, pH, and glucose-related absorbance, together with citric acid and gluconic acid concentrations measured on days 2, 4, and 6. The final liquid product was additionally characterized in terms of nitrogen, total phosphorus expressed as P_2O_5, potassium expressed as K_2O, and organic carbon. By integrating these measurements, the study aimed to characterize early-stage fermentation dynamics and relate the observed process changes to the nutrient composition of the resulting liquid product. The findings provide preliminary experimental evidence relevant to the valorization of invasive aquatic biomass and the development of potential liquid biofertilizer products. This approach aligns particularly with Sustainable Development Goal (SDG) 12, concerning responsible consumption and production, through the potential recovery of value from underutilized biomass. It also has a prospective connection to SDG 15 through the productive utilization of biomass obtained from invasive plant management. However, demonstrating fertilizer effectiveness, environmental benefits, or optimal fermentation conditions requires further validation beyond the process and product characterization in this study.

2. Method

2.1. Biomass preparation and initial glucose characterization

Water hyacinth was collected from Lake Toba, Parparean Village, Porsea District, Toba Regency, North Sumatra, Indonesia. The collected biomass was chopped and homogenized into a slurry without adding water. Before fermentation, the prepared biomass was analyzed for its initial glucose concentration using the 3,5-dinitrosalicylic acid (DNS) method. Glucose concentration was determined based on the absorbance of the prepared sample using the DNS analytical procedure. Figure 1 shows flowchart of the EM4 assisted fermentation process of water hyacinth, showing biomass preparation, mixing with water and EM4,

fermentation at biomass-to-water ratios of 1:4 and 1:5, and subsequent physicochemical, glucose-related, organic acid, and nutrient analyses.

The initial glucose concentration of the fresh water hyacinth biomass was 12,153.75 mg L^{-1}. This value was used to characterize the initial fermentable sugar content of the prepared biomass. The analytical result represents the glucose concentration measured using the specified sample preparation and analytical procedure rather than the total carbohydrate content of the biomass.

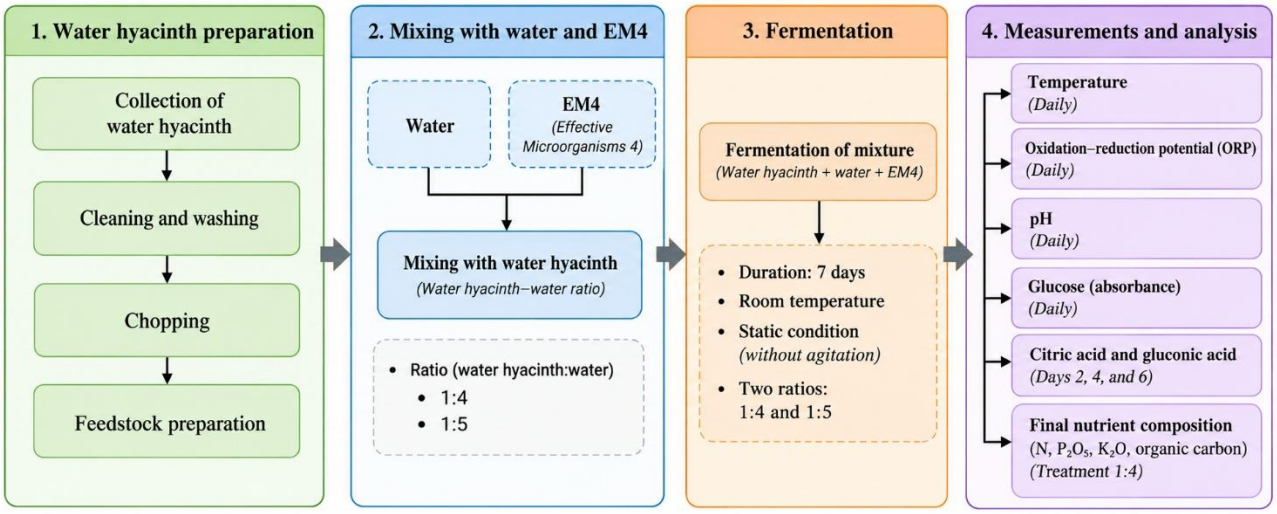


Figure 1. Illustration of experiment design

2.2. Fermentation setup and operating conditions

Fermentation experiments were conducted in 2 L glass beakers containing 1,800 mL of fermentation medium. Water hyacinth biomass was chopped and homogenized into a slurry before being mixed with water at two slurry-to-water volume ratios, 1:4 and 1:5. These treatments were used to examine fermentation characteristics under different biomass dilution conditions. The experiments were conducted at room temperature (approximately 23–24 °C).

EM4 was added at 10% of the fermentation medium volume. Fermentation proceeded without external aeration. The medium was intermittently mixed at 80 rpm using alternating 12 h mixing and 12 h resting periods throughout the seven-day fermentation period. Changes in glucose concentration and physicochemical parameters were monitored for both treatments to characterize the fermentation process.

2.3. Monitoring of glucose, pH, oxidation-reduction potential, and temperature

Fermentation was monitored daily for seven days. The measured parameters included glucose-related absorbance, pH, oxidation-reduction potential (ORP), and fermentation-medium temperature. Measurements were performed to characterize changes in substrate concentration and physicochemical conditions throughout the fermentation period. The pH, ORP, and temperature of the fermentation medium were monitored using digital measurement equipment (*Lutron YK-2001PHA - Taipei, Taiwan*). Measurements were obtained under the respective fermentation conditions and recorded for subsequent comparison between treatments.

Glucose concentration was assessed using the 3,5-dinitrosalicylic acid (DNS) method. Sample absorbance was measured spectrophotometrically, and glucose concentration was determined using the corresponding calibration curve. The relative decrease in measured glucose concentration was calculated using equation 1.

$$D_t = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

where (D_t) is the percentage decrease in glucose concentration at time (t), (C_0) is the initial glucose concentration measured on day 1, and (C_t) is the glucose concentration at time (t). Thus, the calculated decrease represents the change relative to the first fermentation-day measurement rather than the overall conversion of glucose initially present in the raw biomass. This calculation describes changes in measured glucose concentration and does not independently quantify microbial glucose consumption.

2.4. Determination of citric acid and gluconic acid

Citric acid and gluconic acid concentrations were determined using colorimetric methods with UV–Vis spectrophotometry. Fermentation samples were analyzed on days 2, 4, and 6. Separate calibration curves were prepared for each analyte, and concentrations were calculated from the corresponding linear regression equations. Citric acid and gluconic acid concentrations were determined using colorimetric methods with UV–Vis spectrophotometry on fermentation samples collected on days 2, 4, and 6. For citric acid, eight standard concentrations were prepared from a 10 mg/mL stock solution by dilution with distilled water to a final volume of 10 mL. Absorbance was measured at 407 nm, and the concentration was determined using a calibration curve [29], [30], [31]. For gluconic acid, standard solutions were reacted with $FeSO_4$, phosphate-buffered saline (PBS), and indigo carmine before absorbance measurement at 610 nm. Sample concentrations were calculated from the corresponding calibration curve [32].

2.5. Characterization of the final liquid product

The final liquid product was analyzed for nitrogen, total phosphorus, potassium, and organic carbon. Nitrogen was determined using a volumetric titration method, while total phosphorus was measured by spectrophotometry. Potassium was analyzed using atomic absorption spectrophotometry (AAS), and organic carbon was determined gravimetrically. The results were expressed as nitrogen (N), phosphorus pentoxide (P_2O_5), potassium oxide (K_2O), and organic carbon, respectively. All results were expressed as percentages on a dry-weight basis *(% w/w, dry basis*).

2.6. Data processing and interpretation

The experimental data were organized according to fermentation time and biomass-to-water ratio. Daily measurements of glucose concentration, pH, ORP, and temperature were used to characterize temporal changes during the seven-day fermentation period. Citric acid and gluconic acid measurements from days 2, 4, and 6 were used to describe changes in the concentrations of the two measured organic acids.

Glucose concentration depletion was calculated relative to the initial concentration of the corresponding analytical series, provided that the measurements were obtained on a comparable analytical basis. The two fermentation treatments were compared descriptively to identify differences in their measured physicochemical parameters and organic acid profiles. Final nitrogen, P_2O_5, K_2O, and organic carbon concentrations were summarized to characterize the resulting liquid product.

Interpretation focused on the relationships between measured glucose concentration, redox conditions, pH, temperature, organic acid concentrations, and final nutrient composition. Because these parameters do not independently establish microbial metabolic pathways, substrate conversion yields, or agronomic effectiveness, conclusions were restricted to the observed process characteristics and product composition. No statistical analysis was performed, as there was no experimental replication, and the data structure did not support valid statistical inference.

3. Results and discussion

3.1. Glucose depletion and substrate utilization during fermentation

Glucose concentration decreased substantially during the seven-day fermentation of water hyacinth biomass in both treatments (Figure 2). In the 1:4 water hyacinth-to-water ratio, the measured glucose concentration declined from 3,491.25 mg L^{-1} on day 1 to 1,091.25 mg L^{-1} on day 7, corresponding to a 68.74% decrease relative to the first measurement. Similarly, the 1:5 ratio exhibited a decrease from 3,228.75 to 953.75 mg L^{-1}, equivalent to a 70.46% reduction. These results indicate a substantial decline in measured glucose concentration during the fermentation period. Such changes are consistent with the utilization of soluble carbohydrates during microbial fermentation, in which available sugars can support microbial growth, maintenance, and metabolic product formation. However, the observed concentration decrease should not be interpreted exclusively as microbial glucose consumption because substrate release, dilution, sampling, and other changes in the fermentation medium may also influence the measured concentration. Glucose depletion is therefore a useful indicator of changes in substrate availability, but additional measurements are required to quantify actual substrate consumption and conversion efficiency [33], [34].

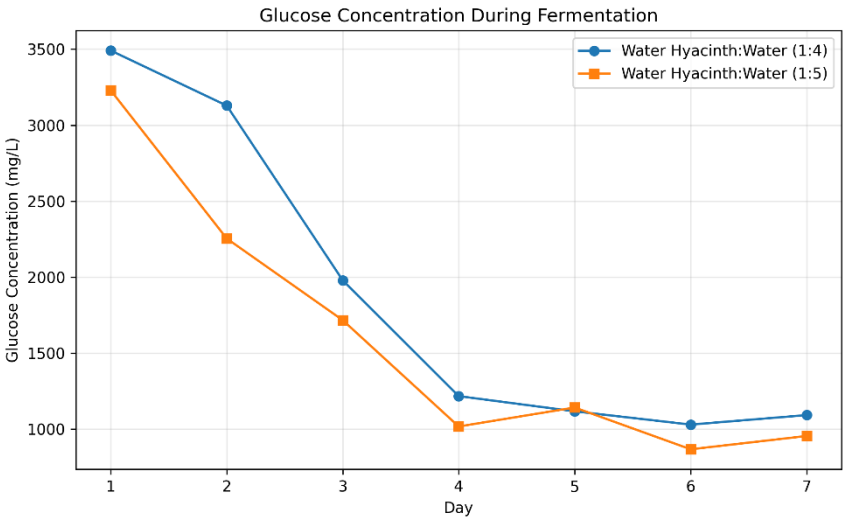


Figure 2. Glucose concentration during seven-day fermentation of water hyacinth at water hyacinth-to-water ratios of 1:4 and 1:5.

The two biomass-to-water ratios exhibited broadly similar decreasing trends, although differences were observed in the magnitude and timing of concentration changes. The 1:4 treatment maintained a higher measured glucose concentration than the 1:5 treatment during most of the observation period, consistent with its higher concentration at the first measurement. The largest decrease in the 1:4 treatment occurred between days 2 and 3, when glucose concentration declined from 3,128.75 to 1,978.75 mg L^{-1}. In the 1:5 treatment, a pronounced decrease occurred between days 1 and 2, from 3,228.75 to 2,253.75 mg L^{-1}. These patterns suggest that the temporal changes in measured glucose differed between the two treatments. The lower concentrations generally observed in the 1:5 treatment may partly reflect the greater dilution associated with the higher water proportion. Dilution can decrease the initial concentration of soluble substrates and may also alter the concentrations of other dissolved constituents. Consequently, the lower glucose concentration in the 1:5 treatment does not necessarily indicate greater microbial uptake or higher fermentation efficiency. Comparisons of substrate utilization between treatments require consideration of the initial substrate inventory, working volume, and changes in soluble sugar availability throughout fermentation. Substrate distribution and mixing conditions can also influence microbial exposure to nutrients and subsequent carbon allocation [35].

A further feature of Figure 2 is the stabilization and slight increase in measured glucose concentration toward the end of fermentation. In the 1:4 treatment, the concentration decreased to 1,028.75 mg L^{-1} on day 6 before increasing to 1,091.25 mg L^{-1} on day 7. The 1:5 treatment showed a similar pattern, with an increase from 866.25 mg L^{-1} on day 6 to 953.75 mg L^{-1} on day 7. These increases may reflect the release of soluble sugars from the plant matrix, changes in the relative rates of sugar release and microbial uptake, or variability associated with sampling and colorimetric analysis. Water hyacinth contains structural carbohydrates, including cellulose and hemicellulose, that can serve as potential sources of fermentable sugars following hydrolysis. Nevertheless, the extent to which these carbohydrates become available depends on substrate accessibility and the hydrolytic activity occurring under the experimental conditions [36]. Because the present study did not directly quantify cellulose hydrolysis, total soluble sugars, or individual sugar-release rates, the observed increases cannot be attributed conclusively to the breakdown of structural carbohydrates. Instead, they indicate that glucose concentration during biomass fermentation may reflect the combined effects of sugar availability and disappearance rather than a strictly monotonic depletion process.

From a biomass valorization perspective, the observed glucose concentrations support the potential of water hyacinth as a feedstock for microbial conversion. The initial glucose concentration measured in the prepared fresh biomass was 12,153.75 mg L^{-1}, whereas the first fermentation measurements were 3,491.25 mg L^{-1} and 3,228.75 mg L^{-1} for the 1:4 and 1:5 treatments, respectively. These values should not be compared as equivalent concentrations unless the sample preparation, extraction, dilution, and analytical bases are confirmed to be comparable. The difference highlights the importance of distinguishing the glucose concentration measured in the initial biomass preparation from that measured in the fermentation medium. Moreover, glucose is only one component of the carbohydrate fraction, and its concentration does not represent the total fermentable carbon available in water hyacinth. Microbial conversion can distribute consumed carbon among biomass, organic acids, and other metabolites, meaning that glucose depletion alone cannot establish the yield of the desired product [33], [37]. In this study, the measured concentrations of citric acid and gluconic acid provide

complementary information on the composition of the fermentation liquid. Integrating these measurements with glucose dynamics can support a more comprehensive interpretation of early-stage fermentation, although quantitative conversion yields would require a consistent substrate balance and product mass balance. Overall, the results demonstrate that water hyacinth fermentation was accompanied by substantial changes in measured glucose concentration and provide a basis for further evaluating its use as a renewable feedstock for potential liquid biofertilizer production.

3.2. Dynamics of redox potential, pH, and temperature during fermentation

Temperature, oxidation-reduction potential (ORP), and pH exhibited distinct temporal patterns during the seven-day fermentation of water hyacinth at biomass-to-water ratios of 1:4 and 1:5 (Figure 3). These parameters provide complementary information about the physicochemical conditions of the fermentation medium and can help characterize changes occurring during substrate conversion. Temperature reflects the thermal environment in which microbial reactions take place, ORP indicates the prevailing oxidation-reduction conditions, and pH describes the acid-base state of the medium. Their combined interpretation is particularly relevant to heterogeneous biomass fermentation, where substrate availability and microbial activity may change over time. Nevertheless, these measurements are indirect indicators of biochemical processes and cannot independently establish specific metabolic pathways or microbial activities [38], [39], [40].

As shown in Figure 3(a), temperature varied between 23.0 and 26.0 °C across the two treatments. In the 1:4 treatment, temperature initially decreased from 24.5 °C on day 1 to 23.9 °C on day 3, increased to 25.4 °C on day 4, and subsequently fluctuated between 24.0 and 25.1 °C. The 1:5 treatment exhibited a wider fluctuation, with temperature decreasing from 24.5 °C on day 1 to 23.0 °C on day 3, increasing to 26.0 °C on day 4, and reaching the same maximum on day 6. Both treatments recorded 24.0 °C on day 5. These variations indicate that the measured temperature was not constant throughout fermentation, although the overall range remained relatively narrow. Temperature influences microbial growth, enzyme activity, and reaction kinetics, but the observed fluctuations cannot be attributed exclusively to microbial heat generation. Because the surrounding room temperature was maintained at approximately 23–24 °C, differences between the medium and ambient conditions, together with measurement variability and heat exchange, may have contributed to the recorded patterns. Without simultaneous ambient temperature measurements for each sampling point, the contribution of biological heat production cannot be quantified [39].

The ORP profiles in Figure 3(c) showed a pronounced decline in both treatments. In the 1:4 treatment, ORP increased initially from 112 mV on day 1 to 141 mV on day 2, before decreasing to 35 mV on day 3 and approximately 2 mV on day 4. Negative values were recorded from day 5 onward, reaching -37 mV on day 7. The 1:5 treatment followed a similar overall trajectory, declining from 148 mV on day 1 to 134 mV on day 2, 76 mV on day 3, and 19 mV on day 4. ORP approached zero on day 5 and subsequently decreased to -29 mV on day 7. The transition from positive to negative values indicates that the measured medium became progressively more reducing under both fermentation conditions. This pattern is consistent with the development of more reducing conditions in an organic-rich medium without external aeration. Microbial oxygen consumption, the accumulation of reduced compounds, and changes in the chemical composition of the medium may contribute to such behavior, although these mechanisms were not measured directly. ORP has been used to characterize changes in microbial fermentation environments and their relationships with metabolic activity, but its interpretation depends on the chemical matrix and measurement conditions [41], [42]. Accordingly, the negative ORP values in this study suggest a shift in redox conditions rather than proving that a specific anaerobic pathway dominated the fermentation process. The absence of external aeration and the use of alternating mixing and resting periods are relevant operational factors when interpreting this transition.

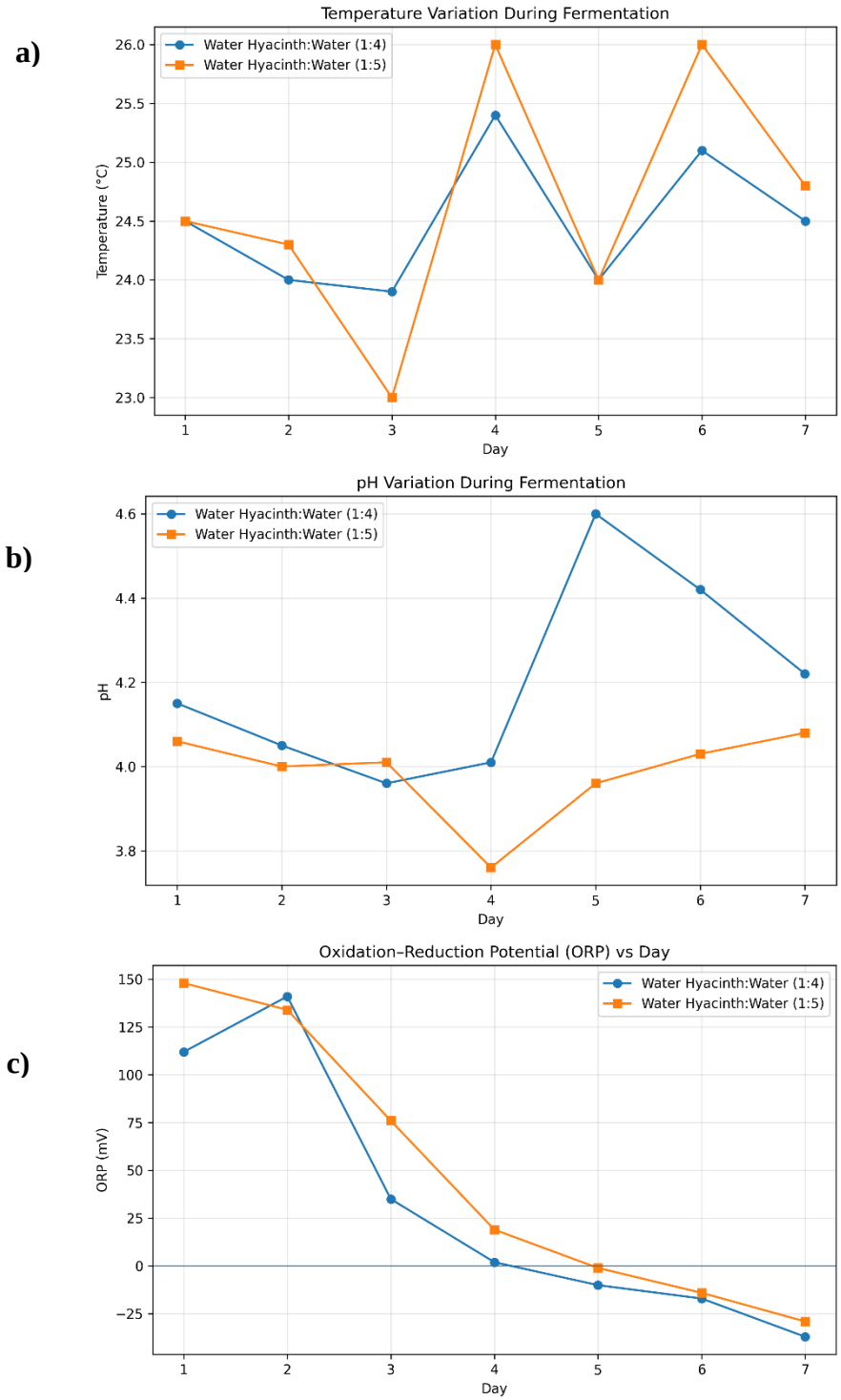


Figure 3. Changes in physicochemical parameters during seven-day fermentation of water hyacinth at water hyacinth-to-water ratios of 1:4 and 1:5: (a) temperature, (b) pH, and (c) oxidation-reduction potential (ORP).

The pH profiles in Figure 3(b) also showed treatment-dependent fluctuations. In the 1:4 treatment, pH decreased from 4.15 on day 1 to 3.96 on day 3, increased to 4.01 on day 4, and reached 4.60 on day 5. It then declined to 4.42 on day 6 and 4.22 on day 7. In contrast, the 1:5 treatment showed a decrease from pH 4.06 on day 1 to 4.00 on day 2, followed by a slight increase to 4.01 on day 3. The pH subsequently decreased to 3.76 on day 4 before increasing progressively to 4.08 on day 7. Thus, the 1:4 treatment exhibited its highest recorded pH on day 5, whereas the 1:5 treatment reached its lowest value on day 4. These results demonstrate that acid-base conditions evolved differently between the two treatments despite the overall acidic environment. Changes in pH during biomass fermentation may reflect the formation or consumption of organic acids, substrate degradation, and interactions with buffering constituents in the feedstock. However, pH variation

alone cannot identify the compounds responsible for changes in acidity [43], [44]. The measured citric acid and gluconic acid concentrations therefore provide complementary evidence for interpreting the pH trajectories. In particular, the decrease in pH to 3.76 in the 1:5 treatment on day 4 coincided with the sampling schedule for organic acid analysis, allowing comparison with the measured metabolite concentrations. Nevertheless, establishing a causal relationship would require additional information on other organic acids, alkalinity, and the buffering capacity of the fermentation medium.

Taken together, the physicochemical measurements indicate that fermentation was accompanied by changes in redox conditions and acidity, while temperature remained within a relatively narrow range. The progressive decline in ORP was broadly consistent with the absence of external aeration, whereas pH fluctuations suggest that acid-base conditions were influenced by processes beyond a simple continuous accumulation of acidic products. These patterns can be interpreted alongside the glucose concentration profiles presented in Figure 2 and the organic acid measurements discussed in Section 3.3. The concurrent decline in measured glucose and ORP is consistent with changes in substrate availability and redox conditions during fermentation; however, temporal coincidence does not establish a direct causal relationship. Similarly, the pH fluctuations cannot be explained solely by the measured citric acid and gluconic acid concentrations. Integrating these physicochemical indicators with substrate and metabolite measurements provides a more informative characterization of early-stage water hyacinth fermentation than any individual parameter alone. Further measurements, including dissolved oxygen, ambient temperature, additional fermentation metabolites, and microbial composition, would help clarify the mechanisms underlying these changes and support more detailed process evaluation for potential liquid biofertilizer production.

3.3. Citric acid and gluconic acid profiles during fermentation

The concentrations of citric acid and gluconic acid varied throughout the seven-day fermentation period in both water hyacinth-to-water treatments (Figure 4). Citric acid was the predominant measured organic acid, reaching concentrations substantially higher than those of gluconic acid at all three sampling points. In the 1:4 treatment, citric acid concentrations increased from 65.8 mg/mL on day 2 to 77.5 mg/mL on day 4, before decreasing to 60.9 mg/mL on day 6. A similar decreasing trend after day 2 was observed in the 1:5 treatment, where citric acid concentration declined from 60.9 mg/mL on day 2 to 59.0 mg/mL on day 4 and 55.4 mg/mL on day 6. These profiles indicate that the highest measured citric acid concentration occurred on day 4 in the 1:4 treatment, whereas the highest value in the 1:5 treatment was recorded on day 2. The subsequent decreases suggest that the measured concentrations did not increase continuously throughout fermentation. Organic acid concentrations reflect the balance between their formation, further microbial transformation, and other processes affecting their distribution in the fermentation medium. Therefore, the observed profiles provide evidence of changes in the measured organic acid composition but do not independently establish the underlying production or consumption rates [45], [46].

The differences between the two treatments suggest that the biomass-to-water ratio was associated with distinct organic acid concentration profiles. Citric acid concentrations were higher in the 1:4 treatment than in the 1:5 treatment at all sampling points, with the largest observed concentration occurring on day 4 in the 1:4 treatment. The higher biomass proportion in this treatment may have provided a greater concentration of organic constituents available for microbial conversion. However, this interpretation remains provisional because the concentrations of readily available sugars, other nutrients, and potential inhibitors were not independently quantified for each treatment. Furthermore, the effect of dilution cannot be separated from the effects of substrate loading using concentration measurements alone. The citric acid profile may be interpreted in the context of microbial carbon metabolism, because citrate formation is associated with central carbon metabolism and the tricarboxylic acid (TCA) cycle. In established citric acid production systems, particularly those involving *Aspergillus niger*, citrate accumulation depends on carbon availability, nutrient balance, microbial physiology, and operating conditions [47], [48], [49]. Nevertheless, the present study did not identify the microorganisms responsible for the measured citric acid or establish whether citrate accumulation followed a specific metabolic pathway. The results should therefore be interpreted as an observed citric acid concentration profile rather than evidence of a particular microbial mechanism.

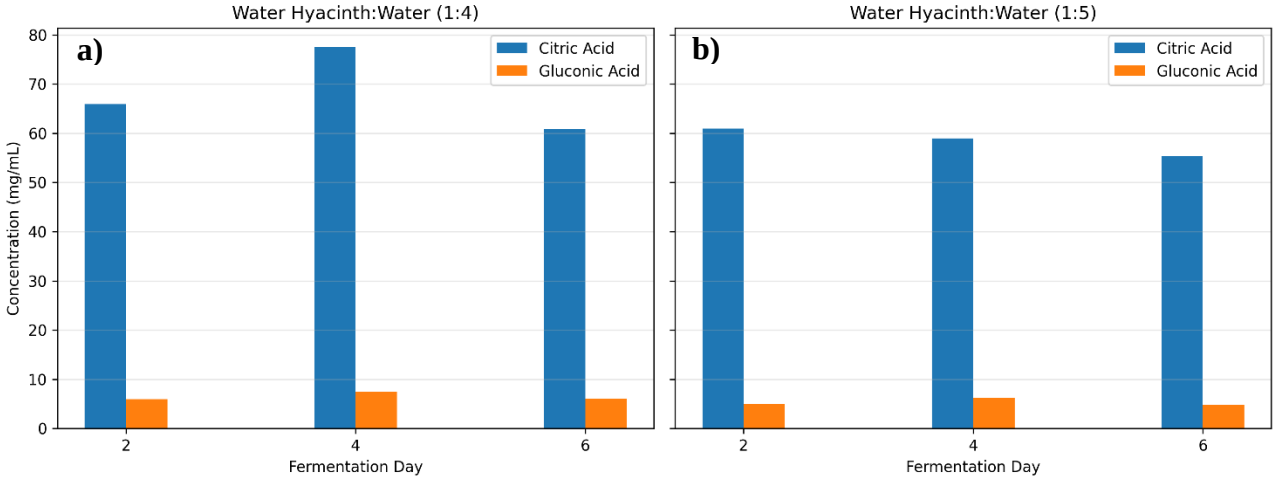


Figure 4. Citric acid and gluconic acid concentrations during water hyacinth fermentation at two biomass-to-water ratios: (a) 1:4 and (b) 1:5.

Gluconic acid concentrations were considerably lower than citric acid concentrations in both treatments. In the 1:4 treatment, the measured concentration increased from 6.0 mg/mL on day 2 to 7.5 mg/mL on day 4, before decreasing to 6.1 mg/mL on day 6. The 1:5 treatment exhibited a similar pattern, increasing from 5.0 mg/mL on day 2 to 6.3 mg/mL on day 4 and subsequently decreasing to 4.8 mg/mL on day 6. Thus, day 4 represented the maximum measured gluconic acid concentration in both treatments. The formation of gluconic acid generally involves the oxidation of glucose through glucose oxidase-dependent reactions in fungi or glucose dehydrogenase-associated pathways in certain bacteria [50], [51]. These pathways differ from the central metabolic route associated with citrate formation and may respond differently to substrate availability and environmental conditions. However, the microorganisms and enzymatic activities responsible for gluconic acid formation were not characterized in this study. Consequently, the observed gluconic acid profiles cannot be attributed to a specific organism or enzymatic pathway. The relatively low concentrations compared with citric acid indicate only that citric acid was the predominant measured acid among the two analytes under the tested conditions.

The organic acid profiles can also be considered alongside the glucose concentration and pH changes discussed in Sections 3.1 and 3.2. Both treatments exhibited substantial decreases in measured glucose concentration during the seven-day fermentation, while citric acid and gluconic acid concentrations reached their highest measured values on day 4 in the 1:4 treatment for citric acid and in both treatments for gluconic acid. These concurrent observations suggest that changes in soluble sugar availability occurred alongside changes in the concentrations of the two measured organic acids. Nevertheless, the data do not establish a direct quantitative relationship between glucose depletion and acid formation. Glucose may be allocated to microbial biomass, other metabolites, and maintenance processes, while organic acid concentrations may also be affected by subsequent microbial transformations and changes in the liquid phase. Similarly, the pH trajectories cannot be explained exclusively by the measured acids because the acidity of the medium also depends on other metabolites and the buffering characteristics of the biomass. Direct organic acid quantification is therefore essential for interpreting acidification, but a complete assessment of carbon conversion requires additional measurements, including a consistent initial substrate inventory and product mass balance [45], [50].

Overall, Figure 4 demonstrates that citric acid was the predominant measured organic acid in both treatments and that its concentration profile differed between the 1:4 and 1:5 ratios. Gluconic acid showed a consistent increase toward day 4 followed by a decrease on day 6, whereas citric acid reached its highest measured concentration on day 4 in the 1:4 treatment and on day 2 in the 1:5 treatment. These results provide preliminary evidence of changes in organic acid composition during the early-stage fermentation of water hyacinth. From a biomass valorization perspective, the detection of these organic acids contributes to the characterization of the liquid fermentation product. However, their presence alone does not demonstrate enhanced nutrient availability or confirm the agronomic effectiveness of the product. Further work incorporating microbial characterization, additional metabolite measurements, replicated experiments, and carbon balance analysis would be required to clarify the mechanisms governing organic acid formation and establish their quantitative relationship with substrate utilization.

3.4. Nutrient composition of the final liquid product

The nutrient composition of the final liquid product obtained from the 1:4 water hyacinth-to-water treatment is presented in Table 1. The product contained 1.35% nitrogen (N), 0.36% total phosphorus expressed as P_2O_5, 1.53% potassium expressed as K_2O, and 26.63% organic carbon (OC). Among the three reported macronutrients, K_2O exhibited the highest concentration, followed by N and P_2O_5. This profile indicates that the final product retained measurable quantities of the primary macronutrients commonly considered in fertilizer characterization. The presence of these nutrients supports further investigation of fermented water hyacinth as a potential source of agricultural inputs. However, the reported concentrations represent the measured composition of the final product and do not independently establish nutrient availability, plant uptake, or fertilizer effectiveness.

Table 1. Nutrient composition of the final liquid product obtained.

Parameter	Concentration (%)
Nitrogen (N)	1.35
Total phosphorus (P_2O_5)	0.36
Potassium (K_2O)	1.53
Organic carbon (OC)	26.63

Nitrogen is essential for plant growth, chlorophyll formation, and protein synthesis. The measured nitrogen concentration of 1.35% indicates that nitrogen was present in the final product, although its agronomic contribution depends on the proportions of organic and inorganic nitrogen and their availability following application. Similarly, the total P_2O_5 concentration of 0.36% indicates the presence of phosphorus-containing constituents that may contribute to plant nutrition. Phosphorus availability, however, depends on its chemical forms and the processes governing solubilization, precipitation, and retention. Organic acids detected during fermentation may influence these processes by modifying the chemical environment, but the present measurements do not establish a direct relationship between acid production and phosphorus availability. Potassium, reported as 1.53% K_2O, was the most abundant of the three reported macronutrients. Because potassium in plant tissues is commonly present in ionic forms, its transfer into a liquid product can be influenced by extraction conditions and partitioning between liquid and solid phases [52], [53]. Nevertheless, the specific contributions of fermentation to the release or retention of these nutrients cannot be quantified without corresponding initial-feedstock and residual-solid analyses.

The organic carbon concentration of 26.63% was substantially higher than the reported concentrations of the individual macronutrients. Organic carbon can originate from residual plant-derived compounds and microbial transformation products, reflecting the organic matter retained in the final product. Its presence is relevant to the characterization of biomass-derived fertilizers, but total organic carbon alone does not establish carbon stability, biodegradability, or improvements in soil properties. Furthermore, interpretation of the reported percentage requires confirmation of the analytical basis, particularly whether the result was determined on a wet-mass, dry-mass, or other basis. The nutrient profile should therefore be interpreted together with the product's physical and chemical properties rather than as a standalone measure of agricultural quality [54], [55].

The 1:4 treatment was selected for final nutrient characterization because it exhibited comparatively favorable results in the measured glucose and organic acid profiles. Although the two treatments showed broadly similar physicochemical trends, the 1:4 treatment presented higher measured concentrations of citric acid and gluconic acid at the corresponding sampling points and a higher citric acid maximum. This provides an experimental basis for selecting the treatment for further product characterization. However, because final N, P_2O_5, K_2O, and organic carbon measurements were available only for the 1:4 treatment, the nutrient composition cannot be directly compared between the two ratios. Accordingly, these results characterize the selected treatment rather than demonstrate that the 1:4 ratio produces a superior nutrient profile. Establishing comparative performance would require nutrient analyses of both treatments under equivalent analytical conditions.

Overall, the final product contained measurable nitrogen, phosphorus, potassium, and organic carbon, supporting its consideration as a candidate for further development as a liquid biofertilizer. Its practical suitability must nevertheless be assessed against the applicable Indonesian fertilizer quality requirements and supported by additional measurements of relevant physicochemical properties, nutrient forms, product stability, and potential contaminants. Agronomic validation through controlled plant-growth experiments

would also be necessary to determine whether the measured nutrient composition translates into a beneficial plant response. Thus, the present findings provide an initial chemical characterization of the fermented water hyacinth product rather than definitive evidence of regulatory compliance or fertilizer effectiveness.

3.5. Integrated Process Interpretation and Biomass Valorization Potential

The combined measurements of glucose concentration, oxidation-reduction potential (ORP), pH, temperature, organic acids, and nutrient composition provide an integrated perspective on EM4-assisted fermentation of water hyacinth. Over seven days, the measured glucose concentration decreased from 3,491.25 to 1,091.25 mg L^{-1} in the 1:4 treatment and from 3,228.75 to 953.75 mg L^{-1} in the 1:5 treatment, corresponding to decreases of 68.74% and 70.46%, respectively, relative to day 1. These changes occurred alongside a shift in ORP from positive to negative values, persistently acidic conditions, and fluctuations in temperature. Collectively, these observations characterize changes in substrate concentration and physicochemical conditions during fermentation. However, their temporal concurrence does not establish a direct causal relationship between glucose concentration changes and redox evolution.

The glucose concentration profiles did not decline continuously. In both treatments, a slight increase was observed on day 7 following lower concentrations on day 6. This pattern suggests that the measured glucose concentration may reflect the combined effects of sugar disappearance, the potential release of soluble sugars from the biomass, and variability associated with sampling or colorimetric analysis. Because these processes were not quantified separately, the observed concentration decrease cannot be interpreted as a direct measure of microbial glucose consumption or substrate conversion efficiency. Accordingly, the glucose data are interpreted descriptively rather than through a specific kinetic model. Replicated experiments, consistent measurements of soluble sugars, and further evaluation of the processes affecting sugar availability would be required for quantitative kinetic analysis.

The organic acid profiles provide complementary information on changes in the fermentation medium. Citric acid was the predominant measured organic acid in both treatments. Its highest measured concentration in the 1:4 treatment occurred on day 4, whereas the highest concentration in the 1:5 treatment was recorded on day 2. Gluconic acid reached its highest measured concentration on day 4 in both treatments. At the corresponding sampling points, the 1:4 treatment exhibited higher measured concentrations of both acids than the 1:5 treatment. Nevertheless, measurements were available only for days 2, 4, and 6, limiting interpretation of the temporal profiles and preventing determination of acid production or consumption rates. The measured acid concentrations also cannot be interpreted as product yields from glucose because other metabolites, residual carbohydrate fractions, and initial and final carbon inventories were not quantified. The results therefore characterize the measured organic acid composition without establishing specific microbial pathways or quantitative relationships between glucose changes and acid formation.

The final liquid product from the 1:4 treatment contained 1.35% nitrogen, 0.36% total phosphorus expressed as P_2O_5, 1.53% potassium expressed as K_2O, and 26.63% organic carbon. These results demonstrate that the characterized product contained measurable macronutrients and organic carbon, supporting further investigation of fermented water hyacinth as a potential liquid biofertilizer. However, nutrient concentrations alone do not establish nutrient recovery, nutrient bioavailability, or agronomic effectiveness. Because the final nutrient composition of the 1:5 treatment was not measured, the two treatments cannot be compared directly in terms of final nutrient composition. Interpretation of the reported percentages also depends on the analytical basis used for each parameter.

From a resource-valorization perspective, the findings illustrate the potential to investigate harvested water hyacinth as a feedstock for fermentation-derived agricultural products. Previous research has explored this biomass for bioethanol, biogas, hydrogen, organic acids, single-cell protein, and other value-added products [18], [56], [57], [58], [59]. The present study contributes to this field by combining measurements of glucose concentration, physicochemical conditions, organic acid profiles, and final-product nutrient composition during EM4-assisted fermentation. This approach is relevant to circular bioeconomy strategies because it investigates a potential route for recovering value from biomass associated with invasive plant management. Nevertheless, environmental benefits and practical feasibility remain to be established through assessment of biomass collection, processing requirements, residual waste management, product safety, and agronomic performance.

The findings should be interpreted in light of several limitations. The seven-day observation period and the use of only two biomass-to-water ratios limit evaluation of longer-term process behavior and operating-condition effects. The availability of independent biological replicates should be confirmed before inferential statistical comparisons are attempted. In addition, microbial community composition, dissolved oxygen, other sugars and metabolites, and nutrient partitioning between liquid and solid fractions were not determined.

Consequently, the data do not support a complete carbon balance or quantitative assessment of nutrient recovery. Potential heavy-metal accumulation also requires consideration because water hyacinth may take up contaminants from its growth environment [60]. Future work should prioritize replicated experiments, consistent analytical procedures, longer monitoring, nutrient characterization of both treatments, contaminant assessment, and controlled plant-growth tests. Overall, the results provide preliminary evidence of changes in measured glucose concentration, redox conditions, acidity, and organic acid profiles during EM4-assisted fermentation, while establishing a basis for further evaluation of the resulting liquid product as a potential biofertilizer.

3.6. Sustainability implications and contributions to the sustainable development goals

The valorization of water hyacinth (*Eichhornia crassipes*) provides an opportunity to connect invasive biomass management with resource recovery and sustainable development. Rapid biomass accumulation can create ecological and operational challenges in freshwater ecosystems, while the removal of these plants generates organic material that requires appropriate handling. Converting harvested biomass into useful products, including biofuels, biochemicals, carbon-based materials, and agricultural amendments, offers an alternative to disposal-oriented management [61], [62]. In this context, the present study investigated EM4-assisted fermentation as a potential pathway for converting water hyacinth into a liquid product containing measurable nutrients and organic carbon. The observed changes in glucose concentration, ORP, pH, and organic acid profiles provide preliminary information on the fermentation process, while the final product contained 1.35% nitrogen, 0.36% total P_2O_5, 1.53% K_2O, and 26.63% organic carbon. These findings establish a basis for considering the process within a circular bioeconomy framework, although the broader sustainability benefits remain to be demonstrated through further assessment.

The most direct conceptual connection is to Sustainable Development Goal (SDG) 12, Responsible Consumption and Production, particularly the efficient use of resources and the reduction of waste generation. Recovering value from harvested water hyacinth may reduce the amount of biomass requiring disposal while creating an alternative feedstock for agricultural applications. Fermentation is one possible conversion route alongside pyrolysis, composting, and other biomass-processing technologies [63], [64]. In the present study, the measured nutrient content of the final liquid product indicates the potential for further development as an agricultural input. However, the study did not quantify the total amount of biomass diverted from disposal, nutrient recovery efficiency, process energy requirements, or environmental impacts. Therefore, alignment with SDG 12 should be understood as a potential contribution through resource valorization rather than a demonstrated reduction in waste or environmental footprint. Comparative process assessment and, where appropriate, life-cycle assessment would be required to establish whether this fermentation pathway offers net environmental benefits relative to conventional biomass management [65].

The study also has a prospective relationship with SDG 15, Life on Land, through the broader management of invasive species and the protection of biodiversity. Harvesting water hyacinth can form part of management strategies intended to reduce excessive biomass accumulation and alleviate associated pressures on freshwater ecosystems. Integrating biomass utilization with removal activities may also create incentives for sustained management by generating products from harvested material [61], [62]. Nevertheless, the present experiment evaluated fermentation of collected biomass rather than the effectiveness of invasive-species control or ecosystem restoration. It therefore does not demonstrate reductions in water hyacinth coverage, improvements in water quality, or recovery of native biodiversity. Biomass valorization should complement integrated management strategies, including appropriate harvesting, prevention of reinvasion, and ecological monitoring, rather than being treated as a substitute for ecosystem management.

A further potential connection is SDG 2, Zero Hunger, particularly through the development of resource-efficient agricultural inputs. The presence of nitrogen, phosphorus, potassium, and organic carbon in the final product provides a basis for investigating its suitability as a liquid fertilizer or supplementary nutrient source. Valorization of aquatic biomass into agricultural products may support nutrient recycling and potentially reduce reliance on conventional inputs if the resulting product demonstrates suitable quality and agronomic performance [62], [63]. However, total nutrient concentrations alone do not establish nutrient availability or plant-growth benefits. Furthermore, water hyacinth can accumulate heavy metals and other contaminants from its growing environment, making contaminant analysis and ecotoxicological assessment important before agricultural application [21]. Future work should therefore include verification against applicable fertilizer quality standards, nutrient availability measurements, contaminant screening, and controlled plant-growth experiments to determine whether the product can contribute meaningfully to sustainable agricultural production.

Overall, this study illustrates a preliminary pathway for linking invasive aquatic biomass management with fermentation-based resource recovery. Its strongest sustainability relevance lies in the potential contribution to SDG 12 through the utilization of an underused biomass resource, with additional prospective links to SDG 15 through integrated invasive-species management and SDG 2 through the development of agricultural inputs. These contributions should not be interpreted as quantified environmental or socioeconomic impacts because biomass removal effectiveness, fertilizer performance, process economics, and life-cycle impacts were not assessed. Establishing the broader sustainability value of this approach will require connecting process performance with product safety, agronomic validation, and resource and energy balances. Such evidence would help determine whether EM4-assisted fermentation can become a technically reliable and environmentally appropriate component of sustainable water hyacinth management.

4. Conclusion

This study investigated the early-stage fermentation of water hyacinth (*Eichhornia crassipes*) using EM4 at biomass-to-water ratios of 1:4 and 1:5, focusing on glucose concentration, physicochemical dynamics, organic acid profiles, and final product composition. Both treatments exhibited substantial decreases in measured glucose concentration over seven days, reaching 1,091.25 mg L^{-1} and 953.75 mg L^{-1}, respectively. ORP progressively declined from positive to negative values, indicating a shift toward more reducing conditions, while pH and temperature exhibited temporal fluctuations. Citric acid was the predominant measured organic acid in both treatments. The 1:4 treatment reached its highest citric acid concentration on day 4, whereas the 1:5 treatment showed a declining profile throughout the sampling period. Both treatments exhibited maximum measured gluconic acid concentrations on day 4.

The final product obtained from the 1:4 treatment contained 1.35% nitrogen, 0.36% total P_2O_5, 1.53% K_2O, and 26.63% organic carbon, demonstrating the presence of measurable nutrients and organic carbon. These findings support the potential valorization of water hyacinth as a feedstock for developing liquid agricultural products and align conceptually with circular resource utilization under SDG 12. However, the observed concentration changes do not establish quantitative substrate conversion efficiency, nutrient recovery, or fertilizer effectiveness. Further research should incorporate biological replication, comprehensive carbon and nutrient balances, contaminant analysis, comparison of final nutrient composition between treatments, and controlled plant-growth experiments. Overall, this study provides preliminary process-level evidence supporting further investigation of EM4-assisted water hyacinth fermentation as a potential biomass valorization pathway.

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Conflict of interest

The author declare no conflict of interest.

Declaration of AI Use

During the preparation of this article, the author used Grammarly for improving grammar and enhancing the clarity and structure of English sentences.

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