

Comparison of the nutrient composition of sludge under aerobic and anaerobic mineralization from African catfish, *Clarias gariepinus* (Actinopterygii: Siluriformes: Clariidae), reared in an intensive recirculating aquaculture system

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Abstract

One of the major challenges in an intensive recirculating aquaculture system (RAS) is the sustainable management of fish sludge. The sludge contains a significant amount of nutrients that can be utilized by hydroponically grown crops in an integrated system called aquaponics. While this system has promising results, techniques to maximize nutrient recovery still need to be developed. African catfish, *Clarias gariepinus* (Burchell, 1822), can be stocked at very high densities, therefore it produces a substantial amount of sludge. In this study, sludge from African catfish RAS was subjected to different mineralization treatments (T1: anaerobic, T2: aerobic, and T3: aerobic with carbon addition) for nutrient recovery. The supernatant in T3 after mineralization had a statistically significant difference ($P > 0.05$) in their concentrations of N ($2700 \text{ mg} \cdot \text{L}^{-1}$), P ($100 \text{ mg} \cdot \text{L}^{-1}$), K ($720 \text{ mg} \cdot \text{L}^{-1}$), Ca ($12\,115.6 \text{ mg} \cdot \text{L}^{-1}$), and Mg ($3391.9 \text{ mg} \cdot \text{L}^{-1}$) after 15 days, among the other mineralization methods and untreated sludge. It was then followed by the nutrient recovery performance of T2 and lastly, T1. Moreover, the low pH and warm temperature were observed to improve the solubilization of the nutrients, resulting in a higher nutrient recovery in T3. Hence, among the three mineralization treatments, T3 had the most potential to recover maximum nutrients from African catfish sludge to be used as organic fertilizer for hydroponically grown crops.

Keywords

aerobic mineralization, anaerobic mineralization, catfish aquaculture, decoupled aquaponics, nutrients, organic fertilizer

Introduction

Aquaculture plays a vital role in ensuring the world's food security by providing a stable supply of fish commodities as wild fish stocks steadily decline. However, traditional aquaculture is faced with sustainability issues such as the eutrophication and pollution of the receiving water bodies

due to the nutrient-rich effluent from fish farms (Cao et al. 2007). Recent advancements in aquaculture veers toward zero water discharge, giving rise to recirculating aquaculture systems (RAS). RAS is designed to accommodate intensive stocking densities while facilitating the reuse of water within the system using a series of water treatment components (Espinal and Matulić 2019).

Catfish (order Siluriformes) are among the top fish groups being cultured in Asian countries like the Philippines. Catfish culture is an important sector in the aquaculture industry. The production volume in the Philippines increased over time, amounting to 10 849.49 metric tons with a value of Php 1.18 billion (US \$20.23 million) yearly contribution in 2022 (PSA 2023). Moreover, the widespread production of catfish can be attributed to an established protocol from breeding up to grow-out culture (Setiadi et al. 2019). The African catfish, *Clarias gariepinus* (Burchell, 1822), was introduced in the Philippines in the early 1990s to aid with the catfish market, with native species declining in number. Since then, the African catfish has been preferred to be cultured. However, catfish are heavy waste producers that, along with the intensification of the stocking density, pose many problems in water quality management. Thus, a more sustainable solution to address nutrient overload and catfish health management must be developed.

The aquaponics system is the integration of raising freshwater aquatic organisms in RAS and cultivating plants in a soilless medium called hydroponics (Delaide et al. 2019). The major role players of aquaponics include cultured fish, plants, and microorganisms, which maintain an ecological balance in the system (Suhl et al. 2016). Nutrients from RAS are derived from fish feces and uneaten feeds that settle at the tank bottom as solid wastes, which are then converted by the microbial community present in the biological filter to make the nutrients available and provide plants with necessary mineral elements that are essential for growth, and thus reducing high nutrient emission in the receiving environments. Moreover, aquaponically-derived nutrients can reduce the reliance of hydroponic crops on inorganic fertilizers that are potentially harmful to the environment. The clean water then moves back to the fish tank for the fish to reuse.

In traditional aquaponics design, the aquaculture and hydroponics components are coupled in a single-loop system. However, this setup results in lesser production yield in fish and plants compared to RAS and hydroponics, respectively, due to the different water parameter requirements of each production unit (Goddek et al. 2015). For instance, the optimum pH for fish and heterotrophic bacteria in the aquaculture component is higher than what is required for most hydroponic crops. In effect, this compromises the availability of nutrients for the plants, resulting in slower growth and longer production cycles. To address these drawbacks, there has been a shift in recent designs towards the decoupling of the compartments such that there is an independent control over each unit. The decoupled aquaponics system has an additional compartment for RAS sludge mineralization to recover particle-bound nutrients to be used as an inorganic nutrient source for plant growth (Goddek et al. 2016).

Sludge mineralization in aquaponics functions as a solid waste reducer and nutrient solubilization enhancer. This process maximizes the nutrient content in the liquid effluent through sludge digestion processes and increases

solid organic matter reduction by discharging after activation (Delaide et al. 2018). Several previous studies demonstrated sludge mineralization performance using aerobic and anaerobic methods to reduce solid organic waste in the systems (Goddek et al. 2018) and to evaluate nutrient recovery (Rakocy et al. 2007; Goddek et al. 2016; Panana et al. 2021), with both activation conditions proven to show promising results of on-site mineralization (Delaide et al. 2019).

Despite its high-density tolerance, fast growth rate, and resilience to fluctuating conditions, the African catfish, *C. gariepinus*, in aquaponics is not commonly used or studied. This study sought to evaluate the inorganic macronutrients produced in the mineralization of the fish sludge from African catfish, *C. gariepinus*, RAS when subjected to aerobic and anaerobic mineralization treatments.

Materials and methods

Decoupled aquaponics system. The experiment was conducted in the Freshwater Aquaculture Station of the University of the Philippines Visayas (FAS-UPV), Miagao, Iloilo, Philippines. The decoupled aquaponics system was composed of a catfish RAS unit, a lettuce hydroponic unit, and the experimental mineralization units. The RAS component consisted of a circular fish tank with a diameter of three meters, a height of 1.2 m, and a water depth of 0.8 m. A total of 500 individuals of African catfish, *C. gariepinus*, with mean body weight of 120 g, were stocked in the tank at a high density ($10 \text{ kg} \cdot \text{m}^{-3}$). The fish were fed at 3% body weight daily using commercial catfish feeds containing 34% crude protein. The fish tank water was recirculated through a settling tank ($1 \times 1 \text{ m}$), a biofilter tank ($1 \times 1 \text{ m}$), and a sump ($1 \times 1 \text{ m}$). The biofilter tank contained layers of bio media placed in fishnets and stackable trays. Water recirculation occurred continuously over 24 h, resulting in a total water exchange volume of 400% per day. The hydroponics had three elevated plant boxes ($1 \times 3 \text{ m}$) with lettuce *Lactuca sativa* in a deep-water culture method with a water depth of 25 cm. Individual plants were placed in each polystyrene cup at 15-cm intervals and held by a stationary raft secured by wire at a fixed level to allow aerial roots to develop. Each plant box contained 150 pieces of lettuce plants. The water was pumped into a separate hydroponics sump for each plant box, which was recirculated within individual units using a pump.

Sludge collection and mineralization treatments. The sludge was collected from the settling tank of the decoupled aquaponics system, where it was allowed to accumulate for seven days. After this period, the sludge was siphoned and allowed to settle for 24 h to concentrate the solids, after which the excess water was removed. This process yielded 10.5 L of concentrated sludge, which was then divided into three mineralization treatments, with each treatment receiving 3.5 L.

The 3.5 L sludge samples were transferred to 20 L buckets for each treatment: T1 (Anaerobic), T2 (Aerobic), and T3 (Aerobic + Molasses). Each sample was diluted to achieve a total solids (TS) concentration of $10 \text{ g} \cdot \text{kg}^{-1}$ (Khiari et al. 2019), resulting in a final volume of 17.5 L of diluted sludge per treatment. For T3, commercial molasses was added as a carbon source to assess its potential to accelerate nutrient mineralization. The amount of molasses added was calculated based on the carbon (33%) and nitrogen (5.2%) content of the sludge's dry weight (Strauch et al. 2018; Putra et al. 2019) and the carbon content of the molasses, which was 24% (w/w) (Samocho et al. 2007). To achieve the desired C/N ratio of 20:1, known to enhance nutrient mineralization in African catfish sludge (Rahmatullah et al. 2020), the formula used to compute the required amount of molasses was adapted and modified from Putra et al. (2019):

$$M = \frac{[C_R \times (\%N_S \times S)] - (\%C_S \times S)}{N_R \times \%C_M}$$

where M is the required molasses (w/w) [g], C_R is the carbon ratio, $\%N_S$ is the nitrogen percentage of the sludge, S is the weight (w/w) of the sludge [g], $\%C_S$ is the carbon percentage of the sludge, N_R is the nitrogen ratio, and $\%C_M$ is the carbon percentage of the molasses.

T1 was subjected to anaerobic conditions with no aeration and covered with an airtight lid. T1 was not mixed or agitated for the whole duration of the experiment. Meanwhile, T2 and T3 were subjected to aerated conditions with an airstone for each of the buckets added and rested on the bottom to provide vigorous aeration and mixing in the buckets for the duration of the experiment. The diagram of the experimental design is shown in Fig. 1.

Supernatant collection and water analysis. Prior to the start of the experiment, samples were obtained from the liquid fraction of the collected sludge to assess the initial nutrient composition. During the mineralization experiment, samples were collected on Day 5, Day 10, and Day 15. Super-

natant samples were collected using 500-mL polyethylene bottles and labeled accordingly. The samples were analyzed for total nitrogen, calcium, and magnesium at the Sugar Regulatory Administration (SRA) Agro-based Laboratory in Bacolod City, Negros Occidental. The Kjeldahl method, utilizing the Foss Tecator Digestion and Foss Kjeltac 8200 Auto Distillation Unit, was employed to determine total nitrogen (AOAC 1990). For calcium and magnesium, the EDTA (ethylenediamine-tetraacetic acid) titrimetric method was used. All analyses were conducted in triplicates.

Phosphorus and potassium analyses were performed at the Regional Organic Soils Laboratory of the Department of Agriculture, Western Visayas (ROSL DA-WV) in Jaro, Iloilo City. The vanadomolybdate method was employed for phosphorus determination, while the flame atomic absorption method was used to determine the potassium concentration. These samples were tested in duplicates. The generated data were analyzed and compared across treatments.

Changes in the concentrations of the nutrients before and after the mineralization treatments are computed as percent change (%) using the formula from Rakocy et al. (2007):

$$PC = 100 (C_{FN} - C_{IN}) \times C_{IN}^{-1}$$

where PC is the percent change [%], C_{FN} is the final concentration of nutrients [$\text{mg} \cdot \text{L}^{-1}$], and C_{IN} is the initial concentration of nutrients [$\text{mg} \cdot \text{L}^{-1}$].

Water parameters (pH and temperature) for each treatment were monitored daily on-site using a multifunction aquaponics pH meter.

Statistical analysis. The data gathered from the results of the analyses were expressed as means of replicates and were subjected to a one-way analysis of variance (ANOVA). The level of significance at 0.05 was employed. A post-hoc Tukey Test was used if the F-value was less than 0.05 to determine which treatments differed significantly from each other within and between groups. Statistical computations were processed using IBM SPSS version 26 and Microsoft Excel.

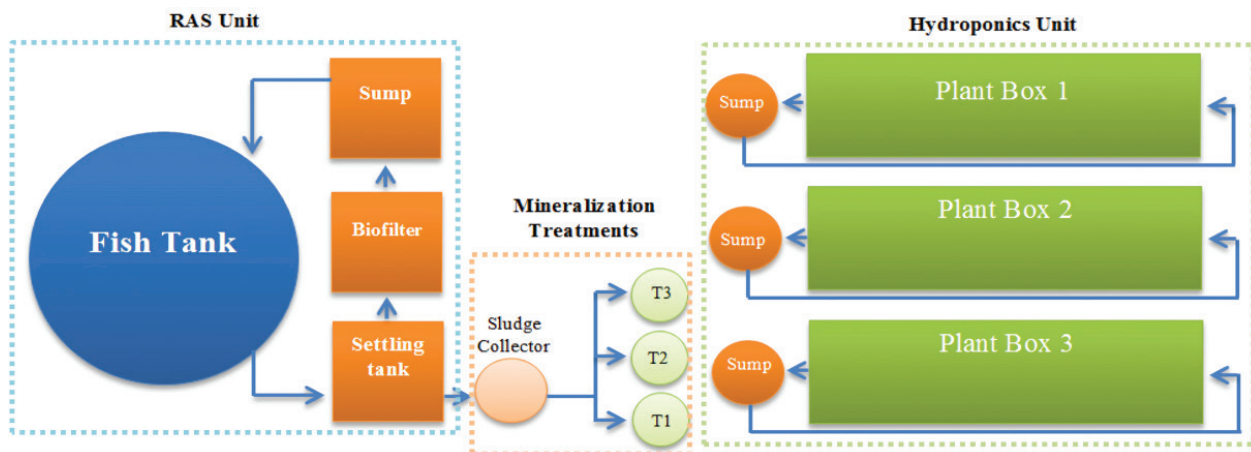


Figure 1. Diagram of the decoupled aquaponics system in FAS-UPV consisting of a RAS unit, hydroponics unit, and the experimental mineralization treatments.

Results

Temperature and pH parameters. During the experiment, the temperature (Fig. 2A) did not have major differences between the treatments, which ranged from 25.6 to 32.6°C. Meanwhile, the pH results (Fig. 2B) revealed that T3 had an acidic pH range of 3.36 to 5.91 compared to T1 and T2, with pH readings ranging from 5.8 to 7.39 and 5.97 to 7.42, respectively.

Sludge supernatant nutrient analysis. In this study, the catfish sludge water prior to mineralization had initial macroelement concentrations of 1060 mg · L⁻¹ for nitrogen, 20 mg · L⁻¹ for both phosphorus and potassium, 4846.2 mg · L⁻¹ for calcium, and less than 170 mg · L⁻¹ for magnesium. The performance of the three mineralization treatments, in terms of the concentrations of the five macronutrients essential for plants, namely nitrogen, phosphorus, potassium, calcium, and magnesium, over the 15-day mineralization period, is shown in Fig. 3.

For the total nitrogen, a sudden increase in the concentration was seen in T3 on Day 5 and a minimal increase in the following sampling days, with the maximum concentration at 2700 mg · L⁻¹. A notable increase was seen only after Day 15 in T2, with a final concentration of 2160 mg · L⁻¹, whereas for T1, the N concentration remained stagnant with very little increase (1072 mg · L⁻¹).

The phosphorus concentrations of the samples were tested as phosphate-phosphorus (PO₄³⁻-P) as it is the form that plants assimilate. P increased significantly only on Day 15 in T3, while for T1 and T2, the P concentrations decreased to less than 5 mg · L⁻¹ and remained the same for the rest of the mineralization period.

Compared to phosphorus, however, potassium concentration in the sludge supernatant was slightly higher after mineralization as it was easily soluble in water. From the initial K concentration of 20 mg · L⁻¹, the K concentration of samples in T1 only ranged from 20 to 30 mg · L⁻¹ and from 10 to 30 mg · L⁻¹ for T2. Interestingly, there was a huge increase in the K concentration of the sludge in T3. On Day 5, the K content was recorded at 670 mg · L⁻¹ and continued to increase on Day 10 reaching 830 mg · L⁻¹. However, on Day 15, the concentration slightly dropped to 720 mg · L⁻¹.

For calcium concentration after mineralization, T3 attained higher values than those of the samples T1 and T2.

The highest Ca concentration of 12 115.6 mg · L⁻¹ was recorded for T3 on Day 10 and Day 15. The Ca content for T2 also had a minimal increase on Day 5 with 7269.3 mg · L⁻¹ and remained relatively the same for the succeeding days. On the other hand, the Ca concentrations for T1 decreased to 2423.1 mg · L⁻¹, which was half of the initial concentration, and no improvements were observed during the treatment.

Meanwhile, almost the same dynamics were observed for the magnesium concentration, where T3 obtained the highest concentrations. From the initial Mg level of less than 170 mg · L⁻¹, the Mg concentration in this treatment increased to 1695.9 mg · L⁻¹ on Days 5 and 10, which doubled on Day 15 to 3391.9 mg · L⁻¹. Meanwhile, for T1 and T2, there is no notable increase in the Mg levels where the concentration remained constant to less than 170 mg · L⁻¹.

Notable changes were observed in the concentrations of the nutrients of the catfish sludge supernatant after subjecting the sludge to different mineralization treatments (Fig. 4). The results showed that among the three treatments, the macronutrients N, P, K, Ca, and Mg from the catfish sludge were best mineralized in T3 with percentage-point changes of 155, 400, 3500, 150, and 1895, respectively.

Discussion

Catfish culture in RAS is an efficient method for stocking catfish at very high densities in a limited space without compromising the water quality (Strauch et al. 2018). Effective wastewater treatment in RAS enables the stocking of African catfish, *C. gariepinus*, at densities up to 200 kg · m⁻³ (Palm et al. 2019). However, this system does not eliminate the risk of increased nutrient loads, as adjustments in feeding occur as the catfish grow. This leads to challenges similar to those faced in traditional intensive systems (Strauch et al. 2018). To address this drawback, nutrients can be recovered and utilized for other purposes, such as integrating a hydroponic compartment to form an aquaponic system. This integration allows nutrient-rich water from the catfish RAS to serve as an organic fertilizer for plant production while purifying the water that returns to the fish tank (Oladimeji et al. 2020).

Traditional single-loop aquaponics designs have several limitations, including reduced independent control over the hydroponic and fish tank units. This compromises the

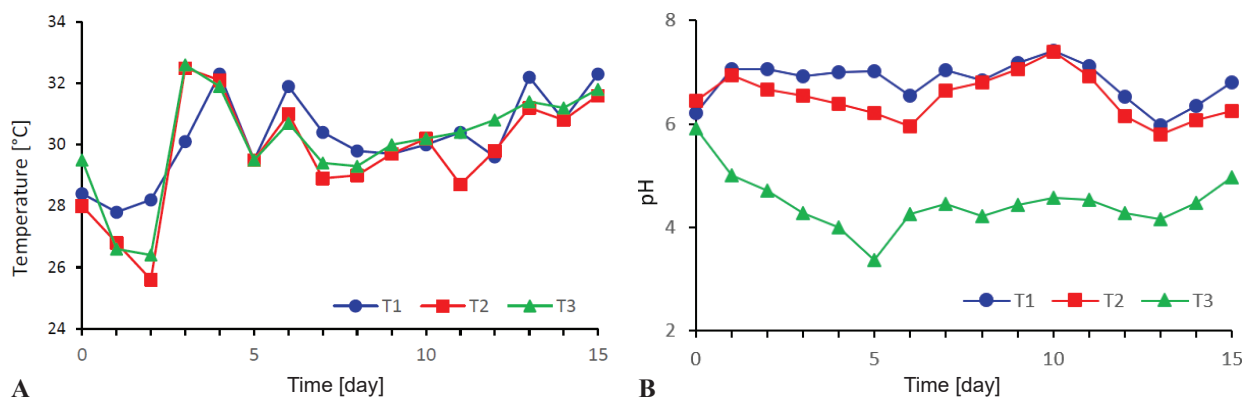


Figure 2. Variation of temperature and pH in the mineralization treatments over the 15-day experimental period. **A** temperature and **B** pH.

specific requirements for optimal growth for both plants and fish (Goddek et al. 2015). In contrast, the decoupled aquaponics system eliminates the loop by entirely separating each compartment. This separation can be achieved

by incorporating an additional unit known as a mineralization chamber, which processes fish waste into organic fertilizer. The decoupling allows for complete manipulation of the water parameters including pH, temperature,

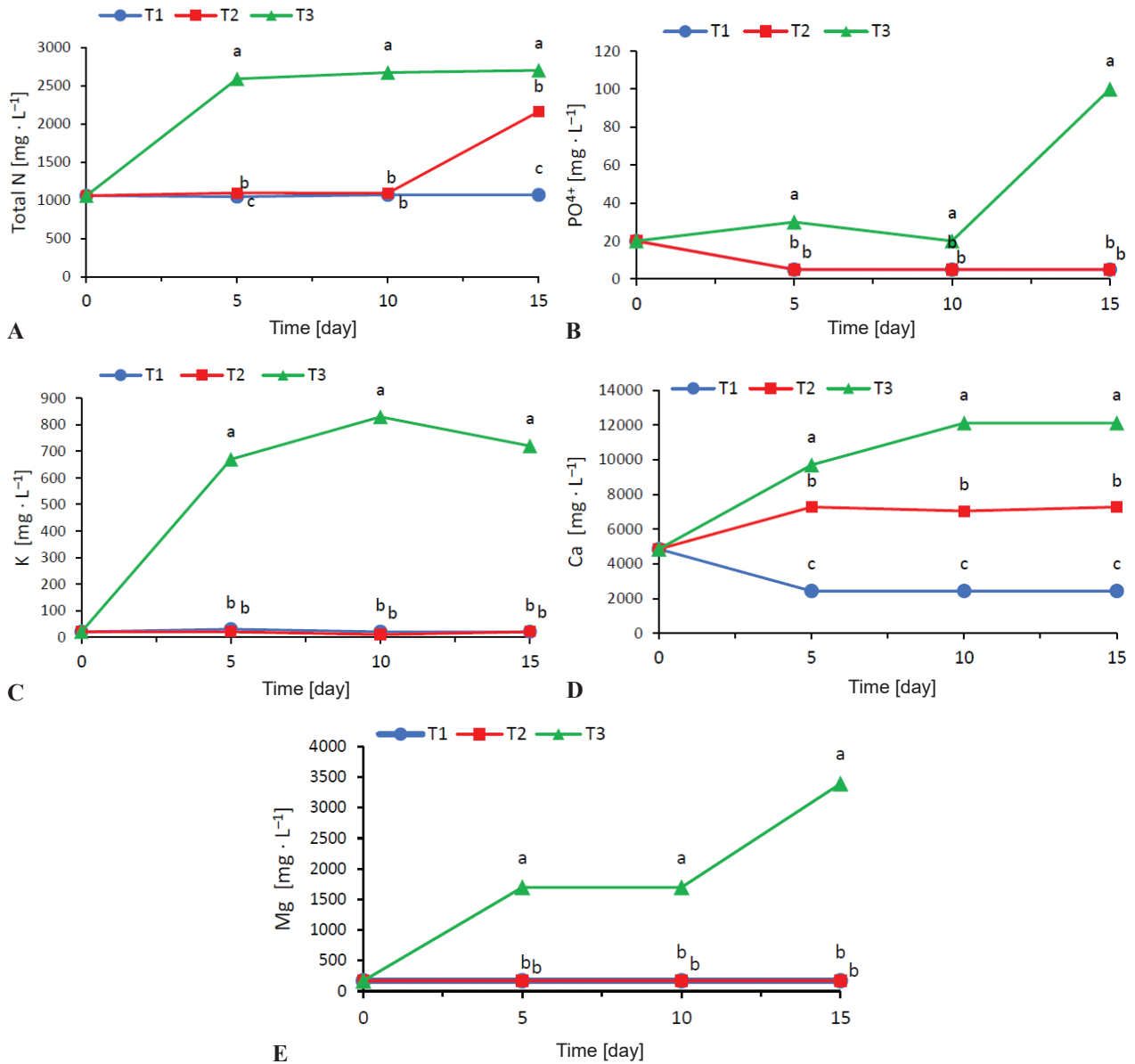


Figure 3. The concentrations of different nutrients over time under different mineralization treatments for 15 days. **A** nitrogen, **B** phosphorous, **C** potassium, **D** calcium, and **E** magnesium. Different lower-case letters indicate significant differences ($P < 0.05$).

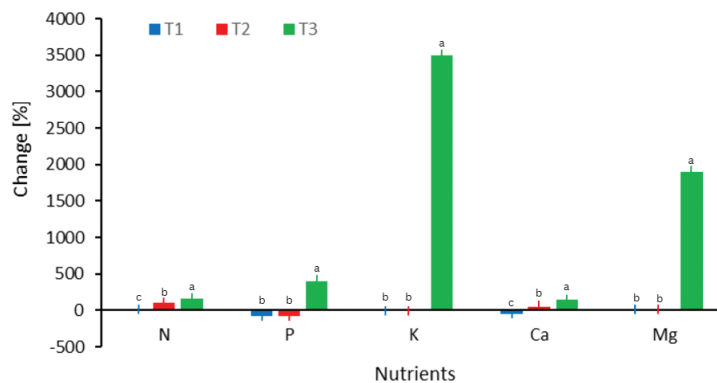


Figure 4. Changes in nutrient concentrations of sludge after subjecting to mineralization treatments for 15 days. Different lower-case letters indicate significant differences ($P < 0.05$).

and nutrient levels required to optimize each compartment without affecting the others (Lucas 2020).

Fish waste and uneaten feed, which contribute to sludge, contain significant amounts of macro and micronutrients essential for plant growth (Delaide et al. 2019). However, these nutrients are often bound in solid particles within the sludge and are not readily available for crops to assimilate, necessitating mineralization to recover these nutrients for use as organic fertilizer. In a decoupled aquaponics system, fish sludge, often discarded as waste, is treated in the mineralization chamber, which recovers nutrients bound in the sludge and reintroduces them into the hydroponics system as fertilizer (Pinho et al. 2021). Furthermore, the water directed to the hydroponics compartment does not recirculate back into the RAS, allowing nutrient concentration in the hydroponics unit for plant use (Lucas 2020).

Both aerobic and anaerobic mineralization methods show promise in recovering particle-bound nutrients, but each has distinct advantages and disadvantages (Delaide et al. 2019; Zhang et al. 2021). For this experiment, catfish sludge from the RAS underwent various on-site mineralization treatments to determine which method would enhance nutrient viability for hydroponically grown lettuce. The initial nutrient concentration in this study was consistent with the sludge water from an intensive African catfish RAS, as reported by Knaus et al. (2020). Applying the appropriate mineralization technique could significantly increase these concentrations, maximizing nutrient availability for the plants.

Noteworthy changes in nutrient concentration in the supernatant were observed after subjecting the catfish sludge to aerobic mineralization with carbon source addition (T3). This indicates that aerating the fish sludge and adding molasses effectively increased macronutrient availability within just 15 days of treatment. This duration is shorter than the prominent increases observed after 29 days in treatments involving simple aeration (Rakocy et al. 2007).

In this study, molasses served as a carbon source for heterotrophic microorganisms, thus accelerating bacterial growth in the system. Reported microorganisms present in aquaculture sludge include genera such as *Rhizobium*, *Flavobacterium*, *Acinetobacter*, *Aeromonas*, and *Pseudomonas* (see Sugita et al. 2005; Munguia-Fragozo et al. 2015). The growth of microbial populations, enhanced by a constant supply of oxygen and carbon sources, increases carbon dioxide production as a byproduct of respiration. Under aerobic mineralization conditions, heterotrophic bacteria break down organic matter, releasing bound nitrogen (N) into the supernatant (Panana et al. 2021). This study observed a marked increase in N concentration on Day 5 for T3, compared to the aerated treatment without a carbon source, which rose on Day 15. Unlike in a biofloc system, where produced bacterial biomass is consumed by cultured aquatic animals, the microbial biomass in the aerobic mineralization treatment with molasses continues to accumulate. This biomass, rich in protein, collectively known as single-celled protein (SCP), can contain up to 85% protein of its dry weight (Sillman et al. 2019). When these bacteria die, their protein becomes part of the organic matter, contributing to N availability in the supernatant.

Additionally, the increase in carbon dioxide from bacterial respiration forms carbonic acid, thereby lowering the pH in the system (Delaide et al. 2019). The drop in pH in T3 correlated with increased concentrations of macronutrients, highlighting the pH's role in solubilizing nutrients from insoluble mineral forms during mineralization. These nutrients became soluble when subjected to acidic conditions, thus increasing their concentrations in the supernatant (Delaide et al. 2019). However, in T2, nitrogen concentration also significantly increased on Day 15, despite a pH above 6.0. This suggests that N solubilization is more reliant on organic matter breakdown, particularly proteins, rather than solely on pH reduction (Panana et al. 2021).

Numerous studies have demonstrated pH's role in leaching nutrients such as phosphorus, potassium, calcium, and magnesium from fish sludge (Jung and Lovitt 2011; Panana et al. 2021). These minerals often become trapped in solid organic molecules within the sludge and are released in their ionic forms after mineralization. However, to maintain their solubilized ionic states, pH must be reduced to an acidic range to prevent nutrient precipitation (Delaide et al. 2019). Macroelements P, Ca, and Mg are effectively solubilized at pH levels below 6.0, facilitating solubilization of bones from fishmeal and minerals that trap these nutrients, like calcium phosphate and struvite (Stewart et al. 2006; Conroy and Couturier 2010). This is further supported by the finding from Goddek et al. (2018), which indicates improved release of P, Ca, and other essential macronutrients when mineralization treatments are acidic. In contrast, Jung and Lovitt (2011) found that adding a carbon source enhanced the fermentation process by anaerobic heterotrophic bacteria, resulting in organic acid production, a drop in pH, and facilitating nutrient leaching. In the presently reported study, although there was a declining trend in the pH of the anaerobic sludge treatment, it did not drop below 6.0, possibly due to the limited presence of hetero-lactic bacteria in the sludge and stagnant conditions of the mineralization setup.

Among the analyzed nutrients, T3 exhibited substantial increases in potassium and magnesium concentrations, with percentage-point increments of 3500 and 1895, respectively. The primary source of potassium in an aquaponics system is fish feeds, which contribute only a minimal amount, resulting in low concentrations in the system (Delaide et al. 2019). Potassium is highly soluble and can be present in the liquid fraction of the sludge even before the mineralization treatments (Panana et al. 2021). The high K concentration in T3 can be attributed to the molasses added during treatment, as it is a source of potassium fertilizer (Otani et al. 2023). Magnesium typically originates from tap water and is also present in trace amounts in fish feeds (Delaide et al. 2019). Under neutral or higher pH conditions, Mg in the system can precipitate into struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$), whereas it can be effectively solubilized in acidic conditions (Zhang et al. 2016). The solubilization of struvite releases both Mg and P, explaining the similar trends for both nutrients in T3, where pH dropped as low as 3.36. Conversely, treatments T1 and T2, which maintained non-acidic pH

ranges, exhibited low concentrations or declines in P, K, Ca, and Mg. Higher pH levels facilitate the precipitation of these minerals into insoluble forms, thereby reducing their availability in the supernatant (Zhang et al. 2016; Delaide et al. 2019; Eck et al. 2019).

Overall, fish sludge mineralization through aeration with molasses addition represents a promising approach for nutrient recovery in a decoupled aquaponics system, potentially reducing reliance on inorganic nutrient solutions typically used in hydroponics. Contrary to previous literature suggesting Ca deficiency in coupled aquaponics systems (Seawright et al. 1998; Schmautz et al. 2017), the results indicate that aeration with molasses mineralization can enhance Ca concentrations to levels sufficient for hydroponic lettuce growth. The resulting concentrations of Ca and Mg reached the sufficiency range of 0.8% to 1.2% and 0.2% to 0.7%, respectively, as recommended for hydroponic lettuce growth (Pickens et al. 2022). However, the concentrations of other macronutrients, N, P, and K, remained lower than the optimal levels for hydroponically grown lettuce. This limitation reflects the inherent challenges in controlling nutrient composition derived from biological processes involved in mineralization, which may necessitate supplementary inorganic fertilizers to achieve optimal growth.

Conclusion

A significant amount of nutrients can be recovered from intensive African catfish RAS sludge when subjected to aerobic mineralization with the addition of a carbon source. The results revealed that African catfish sludge

treated with aeration and molasses achieved significantly higher concentrations of N, P, K, Ca, and Mg indicating its potential as an organic fertilizer for plants in aquaponics.

Integrating aeration and organic carbon sources into the mineralization of fish sludge in a decoupled aquaponics system offers a sustainable approach to nutrient recovery. This research provides valuable insights into optimizing nutrient availability for plant production while addressing waste management challenges in aquaculture systems.

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