Helium : Journal of Science and Applied Chemistry
https://journal.unpak.ac.id/index.php/he_jsac
Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

Combination of Coagulation and Fermentation Technology in Utilizing Liquid Tofu Waste into Liquid Organic Fertilizer for the Growth of Chinese Spinach *(Amaranthus dubius)* Plants

Nina Arlofa [a*], Dama Satya Kusuma [a], Dwiyan Arya Nugraha [a], Mochammad Rizal Laksana [a]

[a] Study Program of *Chemical Engineering, Faculty of Engineering, Serang Raya University, Jl. Raya Serang - Cilegon Km. 5 Taman Drangong , Serang , 42162, Indonesia*

[*] *Corresponding Author: nina.arlofa@unsera.ac.id*

Article history : *received: 25-03-2025; revised: 27-06-2025; accepted: 08-07-2025; published: 08-07-2025.*

ABSTRACT

Wastewater is known to contain organic compounds, such as proteins, fats, and carbohydrates, which can be utilized more effectively through technology, coagulation, and fermentation, allowing it to be used as an organic liquid fertilizer (OLF), a more environmentally friendly option. Research objectives are to determine the influence of powder seed sour Java as a coagulant on lower COD and BOD levels, the effect of adding EM4 to nutrients in waste liquid, and the impact of POC on the growth of spinach China (*Amaranthus dubius*). This research is conducted through the process of coagulation and flocculation using a solution seed powder of sour Java with varying concentrations of 500, 1000, 1500, 2000, and 2500 ppm. The concentration of 1500 ppm has resulted in the lowest COD and BOD levels, respectively, 250 ppm and 127 ppm. The solution with the lowest COD and BOD levels is fermented using EM4 with variations of 0, 0.5, 1, 1.5, and 2mL. The addition of EM4 is expected to accelerate the fermentation process and enhance the availability of beneficial nutrients, including N, P, and K, for plant growth and development. The total content of N, P, and K is highest, at 2.087%, following the addition of 1 mL of EM4 to 150 mL of the sample. POC with the addition of 1 mL of EM4 was applied to the spinach plant in China, resulting in a plant 25 cm tall, with nine leaves, and a total fresh weight of 39 grams.

Keywords: *Effective Microorganisms*, Fermentation, Coagulation, Waste Liquid Tofu.

1. INTRODUCTION

Tofu wastewater is one of the types of waste generated by the tofu industry, which is widely distributed across Indonesia. This wastewater contains organic compounds such as proteins, fats, and carbohydrates, which have potential for further utilization. However, it is often discharged directly into the environment without proper treatment. As a result, tofu wastewater can contaminate groundwater and the surrounding environment, negatively affecting environmental quality and human health. The high levels of Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in tofu wastewater indicate that this waste can significantly degrade water quality if discharged into water bodies without prior treatment [1]. Several studies have shown that BOD concentrations in tofu wastewater can exceed 5,000 mg/L, far above the safe threshold established for domestic sewage [2]. On the other hand, the high organic content in this wastewater makes it a potential raw material for liquid organic fertilizer, provided it is processed using appropriate methods such as fermentation and coagulation [3]. Therefore, the development of wastewater treatment technologies that can both reduce environmental pollution and produce valuable products for agriculture is highly relevant and urgently needed [4].

On the other hand, the demand for fertilizers to support agriculture continues to increase in line with population growth and the limited availability of

Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

productive agricultural land. The growing global food demand drives agrarian intensification, which in turn increases dependence on synthetic chemical fertilizers [5]. However, the long-term use of chemical fertilizers can lead to various negative impacts on the environment and human health, including the degradation of soil fertility, contamination of groundwater due to nitrate residues, and the emission of greenhouse gases such as nitrous oxide (N_2O) [6][7]. Therefore, the development of organic fertilizers has become a crucial strategy in achieving environmentally friendly and sustainable agricultural systems. Organic fertilizers offer several advantages, including improving soil structure, enhancing cation exchange capacity, and providing nutrients gradually and sustainably [8].

The utilization of organic waste, including tofu wastewater, as raw material for liquid organic fertilizer not only supports the principles of the circular economy but also adds value to previously underutilized waste. This initiative is also aligned with the green business approach, which integrates economic, social, and ecological aspects [9]. In addition to contributing to food security, the development of organic fertilizer products from waste can open new economic opportunities for communities, particularly in rural areas with high access to agricultural industries [10].

The integration of coagulation and fermentation technologies can serve as an innovative solution for processing tofu wastewater into a beneficial product, such as liquid organic fertilizer. Coagulation is a crucial initial step that aims to reduce the content of suspended solids and complex organic compounds in wastewater by adding specific coagulants, which promote flocculation and sedimentation [11]. The use of coagulants, such as natural extracts like moringa seed (Moringa oleifera) and inorganic coagulants like alum (aluminum sulfate), has been proven effective in lowering BOD, COD, and Total Suspended Solids (TSS) levels in tofu wastewater [12]. Following the coagulation process, the filtered waste becomes easier to process during the fermentation stage.

Fermentation plays a key role in breaking down complex organic compounds into simpler forms through the activity of microorganisms such as *Lactobacillus sp.* or *Saccharomyces cerevisiae*, thereby increasing the availability of essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K), as well as micronutrients like magnesium (Mg), zinc (Zn), and iron (Fe) [13]. The combination of these two processes not only accelerates the degradation of organic matter but also produces a more stable and high-value end product in the form of liquid organic fertilizer, which is highly beneficial for plant growth [14]. This technology is considered more efficient and environmentally friendly compared to conventional methods and holds significant potential for widespread application at both household and small-to-medium industrial scales [15].

Chinese spinach (*Amaranthus dubius*) was selected as the subject of this study because it is a horticultural plant that grows easily, adapts well to tropical environments, and has a relatively short harvest cycle—approximately 25–30 days after planting [16]. Moreover, Chinese spinach is highly nutritious, containing significant amounts of iron, calcium, vitamins A and C, making it an essential source of healthy food for the community [17]. As a leafy vegetable, spinach is exceptionally responsive to the availability of vital nutrients, especially nitrogen and potassium, which play key roles in chlorophyll formation, leaf development, and biomass production [18].

The use of liquid organic fertilizer produced from tofu wastewater through coagulation and fermentation processes is expected to supply both macro- and micronutrients required by spinach more naturally and sustainably. Several studies have demonstrated that the application of liquid organic fertilizers derived from organic waste can significantly enhance the growth of spinach plants, as indicated by increased height, leaf number, and fresh weight [19]. Thus, utilizing tofu wastewater as liquid fertilizer not only holds the potential to improve agricultural yields but also offers an ecological solution to reduce environmental pollution caused by the direct disposal of untreated tofu waste [20].

Nutrients are essential minerals required by plants in specific amounts to support physiological and biochemical processes, ultimately determining growth success and crop yield. Macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) play crucial roles in plant metabolism and are often the primary indicators in fertilizer formulations [21]. Nitrogen (N)

OPEN ACCESS

Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

is a key component of chlorophyll and amino acids, directly involved in photosynthesis and vegetative growth. Adequate nitrogen availability promotes the development of dense, dark green foliage and strong stem growth [22]. A nitrogen deficiency can lead to stunted plant growth, leaf yellowing, and low yields. Phosphorus (P) functions in energy transfer through adenosine triphosphate (ATP) compounds and plays a vital role in root development, flowering, and fruit maturation. Providing sufficient phosphorus during the early stages of growth is essential for accelerating root development and enhancing plant resilience to environmental stress [23]. Potassium (K), on the other hand, primarily acts as an enzyme activator involved in protein synthesis and carbohydrate formation. Potassium also regulates osmotic pressure and water transport, thereby improving plant water-use efficiency and drought resistance [24].

Although potassium does not form part of the structural components of plant cells, its availability in sufficient quantities is critical due to its broad influence on plant metabolism. Therefore, liquid organic fertilizers that provide a balanced composition of these essential nutrients—including formulations derived from tofu wastewater through coagulation and fermentation processes—hold significant potential to support optimal growth in crops such as Chinese spinach (*Amaranthus dubius*) [25].

Previous studies have demonstrated the potential use of tofu wastewater as a raw material for producing liquid organic fertilizers. However, several limitations remain. One of the main challenges is the high values of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD), indicating that the organic content in the wastewater has not been fully degraded biologically [26]. This condition not only affects the quality of the resulting fertilizer but also poses a risk of environmental pollution if applied directly to the soil. Moreover, most prior research did not include coagulation and flocculation treatments as initial steps to reduce suspended solids and the organic load in the wastewater [27].

The use of Effective Microorganisms 4 (EM4) as a fermentation agent has been explored in some studies. Yet, the optimal concentration for achieving total nitrogen (N), phosphorus (P), and potassium (K) contents that meet the required standards has not been thoroughly evaluated. According to the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261/KPTS/SR.310/M/4/2019, the total N, P, and K content in liquid organic fertilizer should range between 2% and 6% [28]. In a previous study utilizing 1 liter of EM4 and 150 liters of tofu wastewater, the resulting concentrations of N, P, and K were only 0.008%, 0.048%, and 0.000274%, respectively—well below the standard thresholds [29].

This research aims to assess the effectiveness of integrating coagulation and fermentation technologies in converting tofu wastewater into liquid organic fertilizer. Specifically, it seeks to determine the optimal concentration of tamarind extract for reducing COD and BOD levels, as well as to determine the optimal EM4 concentration required to achieve the standard total N, P, and K content. Additionally, the study will evaluate the effect of the produced fertilizer on the growth of Chinese spinach (*Amaranthus dubius*). The results are expected to contribute positively to the sustainable management of tofu wastewater and its utilization as an environmentally friendly organic fertilizer.

2. METHODS

This research focuses on producing liquid organic fertilizer from tofu liquid waste for the growth of Chinese spinach (Amaranthus dubius) using coagulation and fermentation technology. COD and BOD levels are measured before and after the coagulation process, which aims to reduce COD and BOD levels in tofu liquid waste. The fermentation process is carried out anaerobically to break down organic matter and enhance nutrient content.

To ensure that the nutrients produced in the liquid organic fertilizer comply with the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261/KPTS/SR.310/M/4/2019 on Minimum Technical Requirements for Organic Fertilizers, Biofertilizers, and Soil Conditioners, the NPK content (%) is analyzed. The resulting liquid organic fertilizer will then be applied to Chinese spinach (Amaranthus dubius).

The results of this research are expected to provide an alternative method for reducing the use of chemical fertilizers in the cultivation of Chinese spinach (Amaranthus dubius).

2.1. Tools and Materials

The tools used in this research included a magnetic stirrer, a 600 mL beverage packaging bottle, a 3/16” hose, a mortar and pestle, heat-resistant gloves, an analytical balance, a COD meter, a BOD meter, a BOD bottle, a distillation apparatus set, and a UV-Vis spectrophotometer. The glassware used comprised 1 L, 500 mL, 250 mL, and 100 mL beakers; a 250 mL Erlenmeyer flask; a 10 mL measuring pipette; 20 mL and 50 mL volumetric pipettes; 100 mL, 250 mL, and 500 mL volumetric flasks; a ball pipette; and a funnel.

Materials used are Analytical-grade sodium hydroxide (NaOH, pellets ≥98%; 20% and 40% w/v) and potassium hydroxide (KOH, pellets ≥85%), which were obtained from Merck (Germany). Effective Microorganism 4 (EM4) was sourced from PT Songgolangit Persada (Indonesia), while tamarind extract and unrefined brown sugar were purchased from a certified food-grade supplier in Serang. Universal pH indicator (pH 1–14) and phenolphthalein (1% in ethanol) were obtained from Merck.

Tofu wastewater was collected from a local tofu industry in Serang. Filter paper (Whatman No. 1) was used for filtration. COD analysis used reagent vials containing $K_2Cr_2O_7$ (Hach, USA) and H_2SO_4 (95–98%, Merck), with Ag_2SO_4 and $HgSO_4$ added as needed (Merck). BOD analysis employed nutrient solutions prepared with $MgSO_4$, $CaCl_2$, $FeCl_3$, and phosphate buffer (Merck).

Nitric acid (HNO_3, 65%) and perchloric acid ($HClO_4$, 70%) were obtained from Merck. Phosphate and nutrient tests used molybdovanadate reagent (Hach), ammonium oxalate ($(NH_4)_2C_2O_4$, 4%, Sigma-Aldrich), and potassium oxide (K_2O, Merck). Ammonium and surfactant analyses employed STPB (Sigma-Aldrich), titan yellow (Himedia), and CTMAB standard solution (Sigma-Aldrich). Formaldehyde (HCHO, 37–40%) and ammonium sulfate ($(NH_4)_2SO_4$, 0.25 N) were also from Sigma-Aldrich and Merck, respectively. All solutions were prepared using distilled water, unless otherwise specified.

2.2. Procedure Test

2.2.1 Preparation of Waste Liquid Tofu

The preparation of tofu liquid waste for organic fertilizer production began with the collection of wastewater generated from the tofu production process. A volume of 300 mL of the collected tofu liquid waste was transferred into a beaker for further processing.

2.3. Measurement of BOD and COD Before Coagulation

2.3.1. COD Measurement Before Coagulation

A 2 mL sample of tofu liquid waste was placed into a vial containing 2.5 mL of 0.25 N potassium dichromate ($K_2Cr_2O_7$)reagent. The mixture was then heated at 150 °C for 2 hours using the closed reflux method. After cooling to room temperature, the COD concentration was measured using a COD meter. This procedure follows the standard method described by the American Public Health Association [30].

2.3.2. BOD Measurement Before Coagulation

For the BOD analysis, 95 mL of the tofu wastewater sample was transferred into an incubation bottle, followed by the addition of 5 mL of a standard nutrient solution consisting of phosphate buffer, magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), and ferric chloride ($FeCl_3$) to support microbial activity. A magnetic stirrer was inserted, and a sealed cup containing 1 g of potassium hydroxide (KOH) pellets was added to absorb CO_2. The bottle was sealed with a cap equipped with a hose connector and connected to a BOD meter. The system was then incubated at 20 °C for 5 days, after which the BOD reading was recorded. This method is based on the Standard Methods for the Examination of Water and Wastewater, 5210B [30].

2.4. Coagulation and Flocculation Procedure

A total of 10 mL of tamarind seed powder solution was added to the sample at varying concentrations (500, 1000, 1500, 2000, and 2500 ppm). The coagulation process was carried out for 1 hour at a stirring speed of 37.70 rad/s, followed by flocculation for 30 minutes at a reduced speed of 6.28 rad/s. The mixture was then allowed to stand for 1 hour to enable sedimentation, after which the supernatant was separated and passed through filter paper. This procedure was adapted from the conventional jar test method, using tamarind seed powder as a natural coagulant, as described by Ayangunna et al. (2016).

Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

They reported effective removal of organic contaminants using this approach [31].

2.5. Measurement of pH, COD, and BOD After Coagulation

A 0.1 M NaOH solution was added dropwise to the coagulated sample until the pH reached a neutral range (pH 4–9), as monitored using a universal pH indicator strip. The COD and BOD measurements after the coagulation process were conducted using the same procedures as described for the pre-coagulation analysis.

2.6. Fermentation

A total of 150 mL of the solution resulting from the coagulation and flocculation process was transferred into fermentation bottles. The EM4 activator was then added at various volumes (0, 0.5, 1, 1.5, and 2 mL) to each bottle. Separately, 80 g of brown sugar was dissolved in 100 mL of distilled water. From this solution, 10 mL was added to each treatment bottle as a carbon source. The mixtures were then homogenized thoroughly. Each fermentation bottle was connected to a water-filled gas discharge bottle using a 3/16-inch hose to allow gas release. The anaerobic fermentation process was allowed to proceed for 15 days under room temperature conditions, following the procedure modified from Leksono et al. (2024), who used EM4 and molasses in the production of liquid organic fertilizer from catfish waste [32].

2.7. NPK Content Determination in Liquid Organic Fertilizer

The NPK liquid organic fertilizer analysis was conducted following established analytical protocols:

2.7.1. Nitrogen (N) Determination (Kjeldahl Method)

A 5 g sample was digested in 25 mL of 98% H_2SO_4 and heated until white fumes appeared, then diluted to produce Solution A. This was alkalized, steam-distilled, and titrated against 0.25 N NaOH in the presence of Conway indicator. A blank test without a sample was also performed. This method follows the classical Kjeldahl digestion–distillation–titration technique [33].

2.7.2. Phosphorus (P) Determination:

A 0.5 g sample underwent acid digestion with HNO_3 and $HClO_4$, diluted, and reacted with molybdovanadate reagent. After 10 minutes of color development, absorbance was measured at 420 nm via UV-Vis spectrophotometry. This procedure aligns with the Hach molybdovanadate method (method 8114) for total phosphorus, which is recommended for fertilizer and wastewater testing [34], [35].

2.7.3. Potassium (K) Determination:

A 2.5 g sample was extracted with ammonium oxalate, alkalized, and complexed with STPB and Titan yellow indicator. Titration was performed using standard CTMAB solution, consistent with recognized fertilizer testing protocols [36].

2.8. Liquid Organic Fertilizer

The fermentation solution, which had undergone an anaerobic process for 15 days, was transformed into organic liquid fertilizer. This fertilizer was subsequently applied to Chinese spinach plants.

2.9. Application on Chinese Spinach Plants

The liquid organic fertilizer, produced using five different EM4 concentrations (0, 0.5, 1, 1.5, and 2 mL), was applied to Chinese spinach plants. The application was carried out by sprinkling 25 mL of the fertilizer daily onto each plant treatment for 21 consecutive days.

2.10. Chinese Spinach Plant

After 21 days of treatment, observations were conducted on Chinese spinach plants subjected to varying concentrations of EM4. Three growth parameters were evaluated: plant height (in cm), number of leaves (strands), and fresh weight (in grams).

3. RESULTS AND DISCUSSION

The combination of coagulation and fermentation technology can be utilized for processing tofu wastewater, which contains organic compounds such as proteins, fats, and carbohydrates, into liquid organic fertilizer. The reduction of COD and BOD values in tofu wastewater through the coagulation process is carried out using Java tamarind seed powder as a coagulant. Lowering COD and BOD values aims to produce fertilizer that does not degrade soil quality.

Java tamarind seed powder contains a high amount of protein. The protein in Java tamarind seed powder is expected to act as a natural polyelectrolyte, functioning similarly to synthetic coagulants [37]. Additionally, Java tamarind seed powder contains natural polysaccharides composed of D-galactose, D-glucose, and D-xylose, which serve as natural flocculants [38].

The variation in the concentration of Java tamarind seed powder used as a coagulant includes 500, 1000, 1500, 2000, and 2500 ppm. This aims to ensure that COD and BOD levels meet the appropriate standard limits. COD and BOD levels that exceed specifications may interfere with the fermentation process. The results of COD and BOD analysis before and after the coagulation and flocculation process are presented in Table 3.1.

Table 3.1 Results of COD and BOD Analysis of Waste Liquid Tofu Before and After Coagulation

Parameters	Result						Standard
	Before coagulation (ppm)	Concentration variation of tamarind seed powder solution (ppm)					
		500	1000	1500	2000	2500	
COD	877	284	277	250	258	264	Max 300 (ppm)
BOD	482	145	139	127	144	151	Max 150 (ppm)
pH (After adding NaOH 0,1M)	7	7	7	7	7	7	04-Sep

Description: Based on the wastewater quality standards in the Ministry of Environment Regulation No. 5/2014 (Indonesia) [39].

From Table 3.1 ("Results of COD and BOD Analysis of Waste Liquid Tofu Before and After Coagulation"), the lowest COD and BOD values—250 ppm and 127 ppm, respectively—were observed at a Java tamarind seed powder concentration of 1500 ppm. As indicated in the table, these values fall below the Indonesian regulatory limits of 300 ppm for COD and 150 ppm for BOD. Interestingly, further increases in coagulant dosage to 2000 ppm and 2500 ppm resulted in a slight rise in COD and BOD values. This phenomenon can be attributed to restabilization or particle re-dispersion, a known effect in coagulation processes where excessive coagulant dosage leads to charge reversal and destabilized flocs, thus decreasing removal efficiency [40], [41]. Moreover, the organic nature of tamarind seed powder might contribute additional organic matter at higher dosages, which can increase oxygen demand in the treated wastewater [42]. Therefore, 1500 ppm represents the optimum dosage, beyond which the treatment efficiency begins to decline.

We chose the 1500 ppm dosage because it achieved the highest removal efficiency. In contrast, the lower dose of 500 ppm, although compliant with standards, only achieved values of 284 ppm (COD) and 145 ppm (BOD), offering smaller safety margins. A one-way ANOVA was conducted to evaluate the effect of dosage on the removal efficiency of COD and BOD. The results showed statistically significant differences among dosage levels ($p < 0.05$), confirming that 1500 ppm represents an optimal yet safe dosage.

Furthermore, our findings are consistent with other studies on tamarind seed bio-coagulants. For example, Marendra et al. [43] reported that 500 ppm of tamarind seed extract removed over 50% of COD in tempe wastewater; however, the levels remained above regulatory thresholds. In contrast, Peni Pujiastuti et al. [44] achieved up to 95% removal of suspended solids at just 80–120 ppm, though improvements in COD/BOD were more modest. Our use of a higher 1500 ppm dosage ensures the robust removal of organic pollutants, aligning with these comparative results.

Furthermore, the fermentation process was carried out using EM4 on samples that had been coagulated with the addition of 1500 ppm Java tamarind seed powder coagulant. The variations of EM4 added were 0, 0.5, 1, 1.5, and 2 mL. The addition of EM4 is expected to accelerate the fermentation process and increase the beneficial nutrient content of N, P, and K, thereby supporting plant growth and development.

A brown sugar solution, prepared by dissolving 80 grams of brown sugar in 100 mL of distilled water, was added to each sample variation in a volume of 10 mL. Brown sugar serves as an additional nutrient for liquid organic fertilizer, while its glucose and fructose content act as a primary carbon source, supporting the initial growth of microorganisms during fermentation. This enables complex organic compounds in the

Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

medium to break down more quickly, optimizing the fermentation time [45].

The fermentation process was conducted anaerobically for 15 days in a sealed bottle, which was connected to a hose for gas release; the hose end was submerged in a water-filled bottle. After the fermentation process was completed, an analysis of N, P, and K content was conducted. The test results for N, P, and K content are presented in Table 3.2.

Table 3.2 Results of Analysis of N, P, and K Levels of Waste Liquid Tofu After Fermentation

Parameters	EM4 Variations (mL)					Total N, P, K Standard (%)
	0	0,5	1	1,5	2	
N (%)	0.273	0.787	0.831	0.833	1.104	-
P (%)	0.945	0.837	0.681	0.391	0.102	-
K (%)	0.342	0.352	0.574	0.651	0.664	-
Total N, P, K (%)	1.560	1.977	2.087	1.876	1.871	2-6%

Description: Quality standards refer to the Decree of the Minister of Agriculture of the Republic of Indonesia No. 261/KPTS/SR.310/M/4/2019 concerning Standards for Organic Fertilizer, Biofertilizer, and Soil Amendments [28].

From Table 3.2 ("Results of Analysis of N, P, and K Levels of Waste Liquid Tofu After Fermentation"), the total N, P, and K content increased with the addition of EM4 up to 1 mL, reaching a peak value of 2.087%, which falls within the quality standard range of 2–6%. To assess the statistical significance of the nutrient variations among the different EM4 treatments, a one-way ANOVA test was performed. The results showed that variations in total NPK content across EM4 volumes were statistically significant ($p < 0.05$), indicating that EM4 volume has a meaningful effect on nutrient enrichment. The EM4 dose of 1 mL was thus identified as the optimal level to maximize nutrient production during fermentation. These findings align with previous research highlighting the role of effective microorganisms in enhancing nitrogen transformation and nutrient mineralization in organic substrates [46].

The total NPK content peaked at an EM4 volume of 1.0 mL (2.0870%), then declined slightly at 1.5 mL and 2.0 mL, with values of 1.8760% and 1.8705%, respectively. This decrease in total NPK content at higher EM4 dosages can be attributed to substrate inhibition, where excessive microbial inoculum disrupts the balance of microbial metabolism, leading to nutrient competition and potential microbial stress [47]. Overpopulation of microbes can also accelerate nutrient turnover and volatilization, particularly nitrogen, which can be lost as ammonia during fermentation [48], [49]. Therefore, the 1.0 mL EM4 treatment represents an optimal microbial load that enhances fermentation efficiency without inducing nutrient loss.

The fermented sample was then applied to support the growth of Chinese spinach (Amaranthus *sp.*) planted in pots. Plant watering was conducted daily to achieve optimal results. Liquid organic fertilizer was used for a single watering at a dose of 25 mL per treatment, applied over 21 days [50]. Influence of parameters on the organic liquid fertilizer results study. The parameters measured were plant height, number of leaves, and fresh weight of spinach plants. The data on spinach plant growth are presented in Table 3.3.

Table 3.3 Data on Plant Height, Number of Leaves, and Fresh Weight of Plants With Watering Liquid Organic Fertilizer

Parameters	EM4 Variations (mL)				
	0	0.5	1	1.5	2
Plant Height (cm)	8.5	19	25	16	9.5
Number of Leaves	4	7	9	6	5
Fresh Weight of the Plant (gr)	9	27	39	19	14

The data on plant height, number of leaves, and fresh weight for each EM4 treatment are presented in Table 3.3 ("Data on Plant Height, Number of Leaves, and Fresh Weight of Plants with Watering Liquid Organic Fertilizer"). As presented in Table 3.3, the application of liquid organic fertilizer with EM4 positively influenced spinach growth parameters, with the most optimal performance observed at 1.0 mL EM4, resulting in the highest plant height (25 cm), leaf number (9), and fresh weight (39 g). However, a decline in all three parameters was observed when the EM4 dose was increased to 1.5 mL and 2.0 mL. This reduction may be attributed to microbial overactivity and nutrient competition, where excessive microbial load competes for available nutrients, reducing their bioavailability to plants [51]. Furthermore, high

Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

microbial populations can alter rhizosphere conditions, potentially increasing organic acid concentrations or triggering mild phytotoxicity, which may hinder plant uptake of nutrients or stress root systems [52], [53]. These findings suggest that although EM4 enhances microbial-driven nutrient release, there is a threshold beyond which the microbial activity becomes counterproductive to plant growth.

While planting spinach with organic liquid fertilizer without the addition of EM4, the result yielded the lowest height of 8.5 cm, the highest quantity of leaves (7), and the highest fresh weight of 9 grams. The results of watering organic liquid fertilizer with varying EM4 levels were based on the total content of N, P, and K as analyzed. A comparison of tall plants, number of leaves, and fresh weight of plants, as well as the total content of N, P, and K, against EM4 volume variations, is shown in Figures 3.3, 3.4, and 3.5.

.

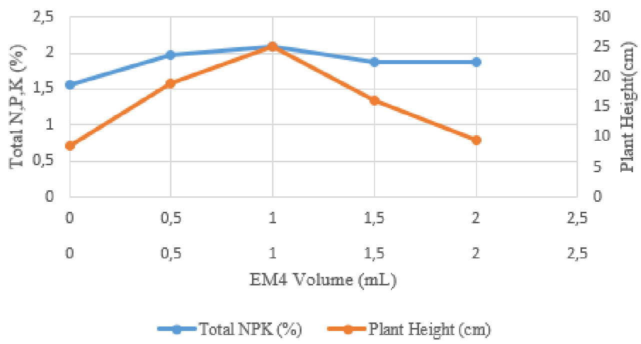

Figure 3.3 Comparison of Total NPK, Plant Height vs EM4 Volume

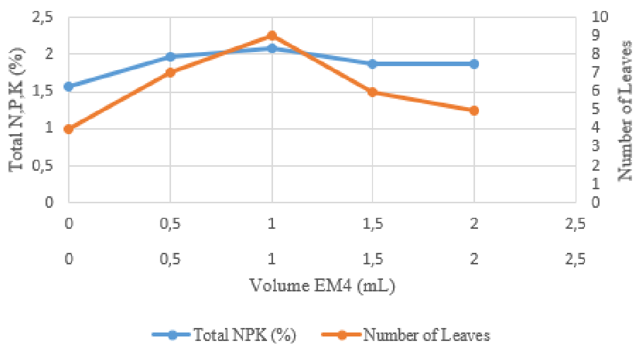

Figure 3.4 Comparison of Total NPK, Number of Leaves vs Volume of EM4

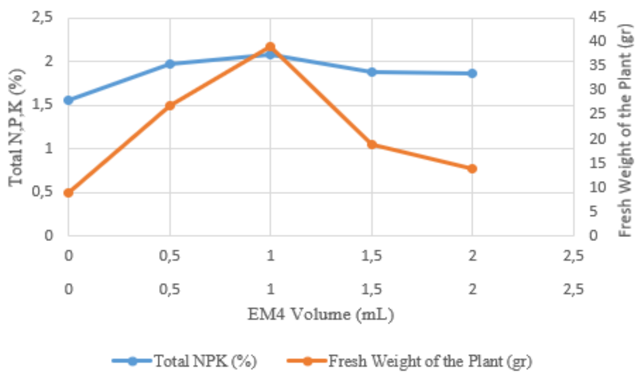

Figure 3.5 Comparison of Total NPK, Fresh Weight vs EM4 Volume

The three graphs illustrate a strong relationship between total NPK content and plant physiological responses, specifically plant height, leaf number, and fresh weight. The optimal EM4 dose of 1 mL produced the highest nutrient content (2.087%) and the best plant growth performance—25 cm height, nine leaves, and 39 g fresh weight. This result suggests that a moderate level of microbial inoculant enhances the availability of essential nutrients through fermentation, particularly nitrogen and phosphorus, which are critical for vegetative development, protein synthesis, and biomass accumulation [54], [55].

However, when the EM4 volume increased beyond 1 mL (to 1.5 and 2.0 mL), a decline in growth was observed, although NPK levels remained relatively stable. This phenomenon can be attributed to microbial saturation, oxygen depletion, or the accumulation of excess fermentation by-products, such as organic acids or ammonia, which can inhibit nutrient uptake or root respiration [56]. These results underscore the importance of determining the optimal microbial dosage, as excessive levels may reduce efficacy or cause phytotoxic effects despite sufficient nutrient content.

4. CONCLUSION

Based on the research conducted, it can be concluded that a concentration of 1500 ppm of sour Java yields the most optimum results, as indicated by the lowest significant values of COD and BOD. The addition of 1 mL of EM4 was found to be the optimal condition for enhancing the content of essential nutrients, namely nitrogen (N), phosphorus (P), and

Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

potassium (K). The N, P, and K contents obtained from the addition of 1 mL of EM4 were 0.8317%, 0.6810%, and 0.5743%, respectively. In comparison, previous research [1] using the same ratio between EM4 and waste volume (1:150) reported significantly lower values of 0.008% for N, 0.048% for P, and 0.000274% for K. Furthermore, the application of this liquid organic fertilizer to plants demonstrated a notable increase in plant growth parameters. Specifically, spinach plants treated with POC containing 1 mL of EM4 grew to a height of 25 cm, developed nine leaves, and reached a total fresh weight of 39 grams.

REFERENCE

[1] S. Nurhasanah, D. Wulandari, and A. Wahyudi, "The effect of aeration process on the reduction of BOD and COD in tofu wastewater," *J. Environ. Eng.*, vol. 27, no. 2, pp. 87–93, 2021.

[2] P. T. Nguyen, H. H. Ngo, W. Guo, and L. D. Nghiem, "Treatment of high-strength wastewater from tofu production by anaerobic membrane bioreactor," *Bioresour. Technol.*, vol. 282, pp. 368–374, 2019, doi: 10.1016/j.biortech.2019.02.047.

[3] Y. Astuti and A. S. Prasetyo, "Utilization of tofu wastewater into liquid organic fertilizer through fermentation process," *J. Indones. Agric. Sci.*, vol. 25, no. 1, pp. 14–21, 2020.

[4] S. B. Kurniawan, M. F. Imron, and A. Iskandar, "Recent advancements in the application of coagulation–flocculation process for industrial wastewater treatment," *Environ. Technol. Innov.*, vol. 25, 102020, 2022, doi: 10.1016/j.eti.2021.102020.

[5] FAO, *The State of the World's Land and Water Resources for Food and Agriculture – Systems at Breaking Point*, Rome: Food and Agriculture Organization of the United Nations, 2021.

[6] J. Chen, "The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility," in *Proc. Int. Workshop Sustained Management Soil-Rhizosphere System*, 2006, pp. 16–20.

[7] O. V. Kharissova, B. I. Kharisov, and C. M. Oliva González, "Green and sustainable agriculture: Use of natural fertilizers and biofertilizers," *Chemosphere*, vol. 285, 131398, 2021, doi: 10.1016/j.chemosphere.2021.131398.

[8] A. Sutanto, B. H. Prasetyo, and A. Hidayat, "Effect of organic fertilizer on physical and chemical properties of soil and corn yield," *J. Soil Land Resour.*, vol. 7, no. 2, pp. 591–600, 2020.

[9] R. M. Dangelico and D. Pujari, "Mainstreaming green product innovation: Why and how companies integrate environmental sustainability," *J. Bus. Ethics*, vol. 95, no. 3, pp. 471–486, 2010.

[10] S. R. Dewi and S. Hadi, "Community empowerment in processing organic waste into liquid fertilizer for economic independence," *J. Community Serv.*, vol. 5, no. 1, pp. 14–21, 2019.

[11] G. T. Metcalf and H. Eddy, Wastewater Engineering: Treatment and Resource Recovery, 5th ed. New York, NY, USA: McGraw-Hill Education, 2014.

[12] D. Lestari and H. Susanto, "Effectiveness of natural and chemical coagulants in reducing tofu wastewater pollutants," *J. Environ. Eng.*, vol. 27, no. 1, pp. 33–41, 2021.

[13] R. N. Yanti, H. Purnomo, and A. Wahyudi, "Fermentation of tofu liquid waste using baker's yeast for liquid organic fertilizer production," *J. Agric. Technol.*, vol. 21, no. 2, pp. 85–93, 2020.

[14] K. Y. Kim, K. B. Lee, and C. O. Jeon, "Microbial communities and metabolic pathways involved in the degradation of organic matter in anaerobic digesters for wastewater treatment," *Sci. Rep.*., vol. 8, 1181, 2018, doi: 10.1038/s41598-018-19466-6.

[15] M. Ariani and S. Gunawan, "Processing of small-scale tofu industry liquid waste into liquid organic fertilizer," *J. Technol. Innov.*, vol. 10, no. 1, pp. 45–52, 2019.

[16] H. Supriyadi and B. E. Prasetyo, "Growth and yield of spinach (Amaranthus spp.) on several planting media types," *J. Hortic.*, vol. 28, no. 1, pp. 32–38, 2018.

[17] M. Ali and S. C. S. Tsou, "Combating micronutrient deficiencies through

Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

vegetables – A neglected food frontier in Asia," *Food Policy*, vol. 22, no. 1, pp. 17–38, 1997.
[18] D. P. Sari and S. Rahayu, "Growth response of spinach (Amaranthus sp.) to application of organic and inorganic fertilizers," *J. Trop. Agrotech.*, vol. 8, no. 1, pp. 45–52, 2020.
[19] G. Pramono and H. Sutikno, "Effect of liquid organic fertilizer from organic waste on growth and yield of red spinach (Amaranthus tricolor)," *J. Agrosci.*, vol. 19, no. 2, pp. 101–107, 2021.
[20] F. Wulandari and S. Yuliani, "Utilization of tofu liquid waste as environmentally friendly liquid organic fertilizer for vegetable crops," *J. Sustain. Environ. and Agric. Management*, vol. 6, no. 1, pp. 73–80, 2022.
[21] P. Marschner, *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed. London, UK: Academic Press, 2012.
[22] B. H. Purwanto and T. Haryanti, "The role of nitrogen in the growth and yield of horticultural crops," *J. Indonesian Horticult.*, vol. 9, no. 2, pp. 113–121, 2018.
[23] C. Grant, S. Bittman, M. Montreal, C. Plenchette, and C. Morel, "Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development," *Can. J. Plant Sci.*, vol. 85, no. 1, pp. 3–14, 2005.
[24] W. T. Pettigrew, "Potassium influences on yield and quality production for maize, wheat, soybean and cotton," *Physiol. Plant.*, vol. 133, no. 4, pp. 670–681, 2008, doi: 10.1111/j.1399-3054.2008.01073.x.
[25] W. H. Nugroho and B. Prasetyo, "Macronutrient content in liquid organic fertilizer from tofu wastewater and its effect on spinach growth," *J. Agric. Environ. Sci.*, vol. 12, no. 1, pp. 55–62, 2021.
[26] M. Yuliastri and Sutaryo, "Processing of tofu liquid waste into liquid organic fertilizer using EM4 and anaerobic fermentation," *J. Agric. Technol.*, vol. 21, no. 1, pp. 12–19, 2020.
[27] A. J. Adi and Z. Z. Noor, "Coagulation–flocculation treatment of wastewater in the petroleum industry using inorganic coagulants: A review," *J. Hazard. Mater.*, vol. 170, no. 1, pp. 530–541, 2009.
[28] Ministry of Agriculture, Republic of Indonesia, *Ministerial Decree No. 261/KPTS/SR.310/M/4/2019 on Organic Fertilizer, Biofertilizer, and Soil Conditioner Standards*, Jakarta, Indonesia: Ministry of Agriculture, 2019.
[29] D. Rachmawati and Hadiyanto, "Utilization of tofu liquid waste for production of liquid organic fertilizer with the addition of EM4," *J. Chem. Eng. Indones.*, vol. 16, no. 2, pp. 45–52, 2017.
[30] APHA, AWWA, and WEF, *Standard Methods for the Examination of Water and Wastewater*, 23rd ed., Washington, DC: American Public Health Association, 2017.
[31] R. R. Ayangunna, S. O. Giwa, and A. Giwa, "Coagulation-Flocculation Treatment of Industrial Wastewater Using Tamarind Seed Powder," *Int. J. ChemTech Res.*, vol. 9, no. 5, pp. 771–780, 2016.
[32] T. Leksono, R. Banjarnahor, N. Irasari, and S. W. Sidauruk, "Liquid organic fertilizer produced from innards waste of catfish (*Pangasius djambal*) by using fermentative microbes (EM4) and molasses," *IOP Conf. Ser.: Earth Environ. Sci.*, vol. 1328, no. 1, p. 012019, 2024, doi: 10.1088/1755-1315/1328/1/012019.
[33] J. Kjeldahl, "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern," *Z. Anal. Chem.*, vol. 22, no. 1, pp. 366–383, 1883.
[34] Hach Company, *Molybdovanadate Reagent for Phosphorus Determination (Method 8114)*, Loveland, CO: Hach, 2025.
[35] Hach, "Phosphorus, Total – Molybdovanadate with Acid Persulfate," application note, 2024. §
[36] M. Fawzy, "Fertilizer Analysis Protocol," *Scribd*, 2015. Available: Fertilizer Analysis Protocol.
[37] Hendrawati, "Use of tamarind seeds (Tamarindus indica L.) and winged bean seeds (Psophocarpus tetragonolobus L.) as natural coagulants in groundwater quality improvement," *Valensi*, vol. 3, no. 1, pp. 23–34, 2013.
[38] T. J. Galuh and Yusniar, "Effectiveness of alum and tamarind seed powder

OPEN ACCESS

Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

(Tamarindus indica) in reducing COD levels in laundry wastewater," Dept. Public Health, Diponegoro Univ., Semarang, Indonesia, 2015.

[39] Ministry of Environment of the Republic of Indonesia, "Regulation No. 5 of 2014 on Wastewater Quality Standards," Jakarta, Indonesia, 2014.

[40] A. A. Ndabigengesere and K. S. Narasiah, "Quality of water treated by coagulation using Moringa oleifera seeds," *Water Research*, vol. 32, no. 3, pp. 781–791, Mar. 1998, doi: 10.1016/S0043-1354(97)00295-9.

[41] M. R. Teh, P. Wu, and P. Juan, "Optimal coagulant dosing for turbidity removal: Restabilization and charge reversal phenomena," *Journal of Environmental Management*, vol. 206, pp. 1004–1011, Jan. 2018, doi: 10.1016/j.jenvman.2017.11.009.

[42] M. A. Beltrán-Heredia and J. Sánchez-Martín, "Treatment of wastewater by Moringa oleifera extract: a review," *Environmental Technology Reviews*, vol. 3, no. 1, pp. 82–93, 2014, doi: 10.1080/21622515.2014.980011.

[43] S. M. P. Marendra et al., "Lab scale investigation on biocoagulant performance to remove organic from tempe liquid waste treatment," *IOP Conf. Ser.: Earth Environ. Sci.*, vol. 1381, p. 012038, 2024.

[44] P. Pujiastuti, Y. M. Wibowo, and N. Narimo, "The biocoagulation test of tamarind seed (Tamarindus indica) and winged bean in chemical laboratory wastewater," *AIP Conf. Proc.*, vol. 2638, p. 100002, 2022.

[45] Reski, "Training on the production of organic fertilizer from agricultural and household organic waste in Batulaya Village, Tinambung Regency, West Sulawesi," Agr. Technol. Educ., State Univ. of Makassar, 2023.

[46] M. Ronga, G. Parisi, L. Pentangelo, et al., "Effective microorganisms (EM) improve compost quality, microbial activity and lettuce yield," *Agronomy for Sustainable Development*, vol. 38, no. 2, pp. 1–10, 2018, doi: 10.1007/s13593-018-0502-3.

[47] M. Ronga, A. Parisi, G. Ilaria, et al., "Effect of microbial inoculants on tomato (Solanum lycopersicum L.) under organic farming conditions," *Agronomy*, vol. 9, no. 3, pp. 1–14, Mar. 2019, doi: 10.3390/agronomy9030143.

[48] S. Saha, R. Prakash, and R. D. Singh, "Biofertilizers: A potential tool for sustainable agriculture," *Environmental Technology & Innovation*, vol. 23, p. 101603, 2021, doi: 10.1016/j.eti.2021.101603.

[49] M. R. Glick, "Plant growth-promoting bacteria: mechanisms and applications," *Scientifica*, vol. 2012, Article ID 963401, pp. 1–15, 2012, doi: 10.6064/2012/963401.

[50] R. Arif, I. Ali, and H. Fauziyah, "Effect of watering intervals on the growth and development of spinach (Amaranthus spinosus)," *J. Biol. Sci.*, vol. 5, no. 2, pp. 123–130, 2017.

[51] T. R. Whiteley, R. J. Read, and M. R. Tredgett, "Over-inoculation effects in microbial fermentation systems," *Journal of Applied Microbiology*, vol. 121, no. 3, pp. 603–612, Sep. 2016, doi: 10.1111/jam.13199.

[52] J. C. van Zijderveld, J. Dijkstra, W. van Straalen, H. B. Perdok, and J. van Vuuren, "Effect of microbial inoculant levels on nitrogen efficiency in fermentative processes," *Animal Feed Science and Technology*, vol. 145, no. 1-4, pp. 102–117, Feb. 2008, doi: 10.1016/j.anifeedsci.2007.07.002.

[53] E. M. Jackson and C. L. Carter, "Effect of EM application on nutrient cycling and losses during compost fermentation," *Bioresource Technology*, vol. 124, pp. 287–292, Dec. 2012, doi: 10.1016/j.biortech.2012.08.011.

[54] N. R. Yuliani, A. R. Purnama, and S. Wahyuni, "The Effect of EM4 on Nutrient Availability and Growth of Organic Vegetables," *International Journal of Agricultural Research*, vol. 15, no. 4, pp. 176–183, 2021, doi: 10.3923/ijar. 2021.176.183.

Volume 5, Number 1, June 2025, Page 45 - 56
e-ISSN: 2776-4508 ; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

[55] R. Seneviratne, L. Indrasena, and K. Jayasinghearachchi, "Effective Microorganisms (EM) in organic agriculture: a review of past research," *Journal of Agricultural Sciences*, vol. 5, no. 1, pp. 1–8, 2010.

[56] Y. Zhang, J. Liu, X. Wang, and W. Zhao, "Microbial balance and phytotoxic risk during fermentation of organic fertilizer with EM," *Bioresource Technology*, vol. 234, pp. 160–167, 2017, doi: 10.1016/j.biortech.2017.03.061