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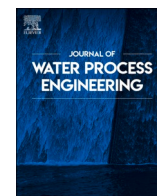
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Phytotreatment of tofu effluent using water lettuce (*Pistia stratiotes*) and potential of biogas production from resultant biomass

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ABSTRACT

Tofu effluent contains a high concentration of organic materials, nutrients, suspended solids and is also low in pH. This research was aimed at applying phytotreatment using floating plant species of *Pistia stratiotes* to polish tofu effluent before final discharge into water bodies while also producing biogas from the resultant biomass after treatment. A range-finding test (RFT) was conducted to determine the initial concentration to be treated and resulted in 10 % tofu effluent. Phytotreatment was conducted for a period of 14 days, focusing on the removal of organic matter and nutrient contents. After 14 days of treatment, *P. stratiotes* were able to remove total suspended solids (TSS) by 88 %, ammonia by 42.3 %, phosphate by 50 %, chemical oxygen demand (COD) by 84 %, and biological oxygen demand (BOD) by 95 %, significantly higher as compared to control. Phytotreatment was able to stabilize pH to a neutral value, and *P. stratiotes* were able to transfer oxygen from air to the rhizosphere area. The maximum daily production of biogas using the plant's biomass was higher as compared to the control; however, the overall biogas accumulation was significantly lower during the 45 days of observation. Further biomass pretreatment was suggested before digestion to obtain higher biogas production since the cellulose, hemicellulose, and lignin content inside the plant biomass were subjected to being hardly degraded by the anaerobic microorganisms.

1. Introduction

Tofu is one of the alternative foods rich in protein content [1,2]. Also known as bean curd, tofu originates from soybeans. It is produced via the coagulation of soy milk and then put under pressure on the curds to produce solid blocks. This type of food is currently in high demand for protein sources, especially for vegans and vegetarians [1]. With the increasing demand for this food, there are so many home industries producing tofu. On a home industry scale, a total of not <1000 L of effluent was produced per month from the tofu production processes

[3]. Most of the effluent originated from washing, soaking, boiling, and pressing processes, while other processes produced less amount of liquid waste [4,5]. Effluent from tofu production is categorized as a pollutant since it contains high organic content, high nutrient content, and is low in pH. Tofu effluent needs to be treated before it can be discharged safely to the open water bodies [6–8].

Previous research reported the use of granular activated carbon adsorption to treat tofu effluent resulted in the effluent having a concentration of chemical oxygen demand (COD) of 1148 mg/L, turbidity of 129 NTU, total suspended solids (TSS) of 210 mg/L, and a pH of 3.64

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Table 1
Initial characteristics of tofu effluent.

No	Parameter	Unit	Value	Effluent standard ^a	Analysis method
1	pH	–	4.60	6–9	pH meter
2	Turbidity	NTU	163	–	Turbidity meter
3	TSS	mg/L	248	Max 200	Gravimetric
4	DO	mg/L O ₂	0	Min 3	Winkler titration
5	COD	mg/L	2285	Max 300	Close reflux
6	BOD	mg/L	1462	Max 150	5-day BOD test
7	Nitrite	mg/L	0	Max 0.1	UV Spectro
8	Nitrate	NO ₂ -N mg/L	0.81	Max 10	UV Spectro
9	Ammonia	NO ₃ -N mg/L	52.12	Max 1	UV Spectro
10	Total Nitrogen	NH ₃ -N mg/L	398.32	Max 25	Kjeldahl
11	Phosphate	PO ₄ -P mg/L	141.07	Max 5	UV Spectro

^a Indonesian Government Effluent Standard for Industrial Activities No. 52014.

[5]. The same research suggested the use of polishing treatments like Fenton oxidation with a final effluent of 628 mg/L in COD, 27.6 mg/L in turbidity, 65 mg/L in TSS, and pH 7.38. Compared to Fenton oxidation, ultrafiltration membranes offered no better result. In another study, anaerobic digestion of tofu wastewater was able to remove 18.5 % COD, 27.2 % TSS, and 28.1 % TDS from initial values of 7027.5 mg/L, 6500 mg/L, and 3500 mg/L, respectively [2]. The use of an anaerobic baffled reactor successfully removed 95 % of COD from tofu effluent with an initial concentration of 12,400 mg/L, resulting in 620 mg/L COD in the final effluent [4].

Looking at the previously mentioned after-treatment effluent concentration, most of the treatment resulted in the COD content still higher than 300 mg/L (Indonesian Government Effluent Standard for Industrial Activities No. 52014). There is a need to polish the effluent further before final discharge into the water bodies [7,9,10]. Previous research mentioned the use of Fenton oxidation and ultrafiltration membranes to further polish the effluent after treatment [5]. However, concerns are raised due to the chemical residue and cost-related matters for operation and maintenance [11–13]. In the effort of overcoming these concerns, phytotreatment using floating plants offers possible treatment with considerably easier operation, limited chemical residues, and relatively lower operation and maintenance costs [14–16]. Previous research has proven that floating plants of *Eichhornia crassipes* were able to polish coffee effluent, resulting in 93.8 % removal of COD, 90.2 % of TSS, 95 % of NH₃-N, and 45.4 % of color [17]. This plant was also able to polish paper mill effluent with an overall removal of >70 % of TDS, BOD, COD, TKN, and P [18]. *Azolla pinnata* and *Lemna minor* showed a capability in polishing palm oil mill effluent with removal values of COD 66 % and 78 %, ammonia 95.5 % and 98 %, and phosphate 93.3 % and 86.7 %, respectively [19]. Other floating plant species, *Pistia stratiotes*, showed to be capable of polishing domestic wastewater with removal performances of 99.8 % in COD, 97.2 % in BOD, 46.4 % in ammonia, 100 % in nitrate and nitrite, and 80.4 % in phosphate [20] while also being able to bioaccumulate metals from paper mill effluent [21].

Knowing the potential of floating plants in polishing wastewater before final discharge into the water bodies, there is an opportunity to polish tofu effluent with this method. Although phytotreatment has been widely studied for its role in pollutant removal, the use of *P. stratiotes* in treating tofu effluent, along with an evaluation of its growth and potential of biogas production from the resultant biomass during treatment, remains unexplored. This research bridges this gap by analyzing the performance of *P. stratiotes* in polishing tofu effluent, focusing on the removal of organic contents (COD and BOD), nutrients (ammonia and

phosphate), and pH stabilization. This research also seeks an opportunity to produce alternative energy in the form of biogas using the resultant plant's biomass after the treatment. The presented results are expected to provide knowledge of an alternative polishing technology for tofu effluent and/or similar characteristics of wastewater while also contributing to the effort of sustainable development goals (SDGs) number 6 (clean water and sanitation), 12 (responsible consumption and production), and 14 (life below water).

2. Materials and methods

2.1. Source of wastewater and initial characterization

Tofu effluent used in this study was obtained from home industry in Surabaya, Indonesia (–07°15'00.9100", 112°45'29.0891"). Samples were taken from the final washing tank and transferred into plastic containers. Samples were transported at a controlled temperature of ±4 °C and directly analyzed once they arrived at the laboratory. Initial analysis of the tofu effluent characteristics was conducted at the Laboratory of Environmental Management, Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember, Surabaya, with the results tabulated in Table 1.

2.2. Plant propagation and range finding test (RFT)

Pistia stratiotes plants were obtained from the Campus C area, Airlangga University, Surabaya, Indonesia (–07°17'03.8819", 112°47'37.6898"). The obtained plants were then propagated under greenhouse conditions at the Department of Environmental Engineering, Faculty of Sciences and Technology, Airlangga University. Propagation was carried out to obtain a sufficient number of plants for range finding and phytotreatment tests. The plant was propagated in a 45 L container filled with tap water. The second generation of plants (indicated by new sprouts and branches connected to the mother plants) were used in the consecutive tests. Selected healthy plant criteria were having a minimum of 5 petals, green color (no yellowing or withering at all), and having a minimum petal span of 4–6 cm in diameter.

The range-finding test (RFT) was carried out in a plastic container filled with 2 L of tofu effluent (maximum depth of 15 cm). In this stage, a maximum concentration of 20 % tofu effluent was chosen to be tested, and a control reactor (filled with tap water) was used as a positive comparison [22,23]. In this stage, 5 %, 10 %, 15 %, and 20 % of tofu effluent concentration was obtained by diluting it with tap water. The decision to select the maximum concentration of 20 % was based on the previous literatures that stated that *P. stratiotes* can withstand COD concentrations up to 500 mg/L [20,24,25]. A total of 5 healthy plants were placed into the RFT container, and visual analysis was conducted on days 7 and 14. Selection of the tofu concentrations for the main phytotreatment test was carried out by analyzing the number of withered plants after 14 days of exposure.

2.3. Phytotreatment of tofu effluent

Phytotreatment of tofu effluent was carried out with the concentration selected in the RFT stage. A total of 5 L of the selected tofu effluent concentration was placed into plastic containers (maximum depth of 15 cm). A total of 10 healthy plants, weighing 10 g in total wet weight, were placed into the containers afterwards. The system was programmed to run in a batch floating treatment, and the amount of evaporated water was substituted with tap water every day [15,20]. Plant control (PC): only plants in tap water; effluent control (EC): only tofu effluent with no plants; and treatment reactors (T): plants in tofu effluent were used in this stage. Plant growth and water parameters were monitored throughout the tested period of 14 days.

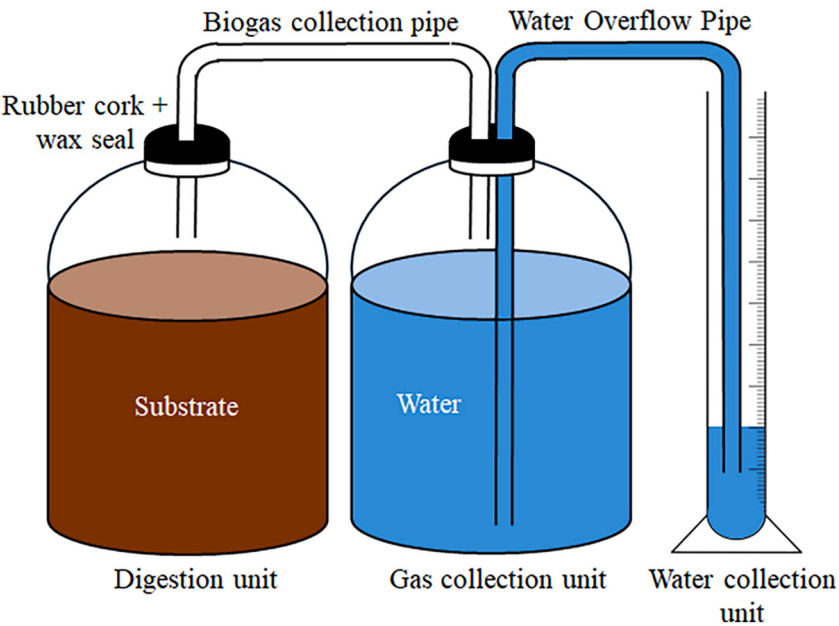


Fig. 1. Biogas production reactor diagram.

2.3.1. Analysis of wet and dry weights

Analysis of wet and dry weights was carried out to understand whether the use of tofu effluent as a living medium is affecting the growth of *P. stratiotes* or not [26,27]. For this parameter, wet and dry weights were taken on days 0, 3, 6, 10, and 14. Wet weight was analyzed by weighing 3 plant samples in each reactor after drying onto a paper filter for approximately 1 min. The dry weight of plants was measured by drying 3 plant samples in an oven for 24 h at the temperature of 105 °C following the gravimetric method.

2.3.2. Analysis of parameters

For each reactor, analysis of pH, total suspended solid (TSS), ammonia, phosphate, chemical oxygen demand (COD), biological oxygen demand (BOD), and dissolved oxygen (DO) was conducted on days 0, 3, 6, 10, and 14. All of the parameters were analyzed at the Laboratory of Environmental Management, Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember, Surabaya, following the protocol as mentioned in Table 1 previously.

Table 2
Plants observation during RFT.

Day	Tofu effluent concentration (%)				
	0	5	10	15	20
0					
7					
14					

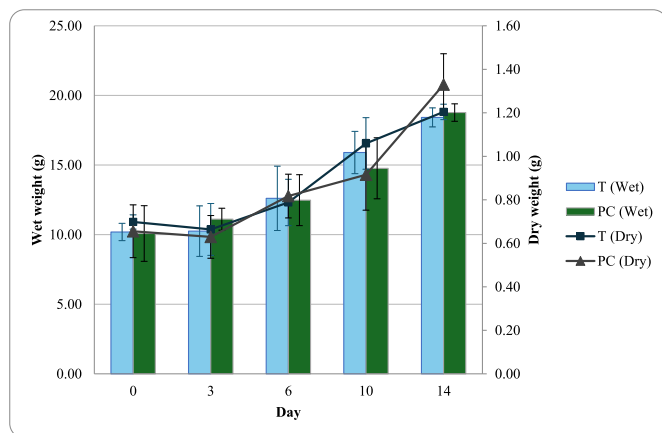


Fig. 2. Wet and dry weights of plants during phytotreatment (T: treatment and PC: plant control).

2.4. Biogas production and characterization

To test the biogas production by *P. stratiotes*' biomass, analysis of biogas production was conducted following the method described by Kumar et al. [28] using the reactor illustrated in Fig. 1. At this stage, analysis was conducted by comparing the day-to-day biogas production and accumulated biogas between control (only cow dung) and treatment (50:50 v/v cow dung and *P. stratiotes*' biomass). A volume of 2 L bottle was used as a reactor at this stage. The reactor was filled with 1 L tofu effluent +500 mL cow dung as substrate (for control) and 1 L tofu effluent +250 mL cow dung +250 mL (previously blended) *P. stratiotes*' biomass as substrate. The biogas collection pipe was connected to the sealed bottle and gas collection unit. Biogas production was observed daily by measuring the overflow water volume until the 45-day of observation period.

2.5. Statistical analysis

For each stage in this research, three replications were made, and data was presented in terms of mean $\pm$ SD. One-way analysis of variance (ANOVA) was used to analyze the correlations between the tested treatment (PC, EC, and T) and the tested period. ANOVA was run under a 95 % confidence interval and $\alpha = 5$ %, and a significant difference was

noted by p -value ≤ 0.05 using SPSS version 16.0 software [29,30].

3. Results and discussion

3.1. RFT results

Based on Table 1, it can be seen that several parameters of the initial characteristics of the tofu effluent were below, exceeding, and/or outside the permissible effluent standard range. It can be seen that pH was acidic and below the permissible range, TSS was higher than the standard, DO was below the minimum of 3, while COD, BOD, ammonia, total nitrogen, and phosphate were far higher than the permissible limit. The obtained results were in line with previous findings, which stated that tofu wastewater was acidic [31], contained a high amount of organic compounds [5], and nutrients [1]. Previous studies mentioned that *P. stratiotes* can withstand COD concentrations up to 500 mg/L [20,24,25], which became the basis for the determination of choosing the maximum 20 % concentration during the RFT test.

Based on the visual analysis (Table 2), all 5 tested plants in 0 % tofu effluent concentration (100 % tap water) showed good growth. This concentration acts as the control to define the overall growth of other tested plants exposed to tofu effluent in the media. After 14 days of observation, a small yellowing was obtained in 0 % tofu effluent. This was subjected to the lack of nutrients in tap water, which then resulted in low chlorophyll production and photosynthetic activity. Tested plants in 5 % and 10 % concentrations showed fresh green petals after 14 days of observations. In contrast to 5 % and 10 %, the plants living in 15 % and 20 % concentrations of tofu effluent seem to be severely affected by the exposure.

Based on these observations, a 10 % concentration of tofu effluent was selected to be the main pollutant treated during the phytotreatment test. In phytotreatment, used wastewater concentrations should not affect the plant's growth significantly [15,17]. Plants should be able to grow in the used wastewater, not only to survive but also to ensure that the treatment process will be carried out well until the end of the test period [26].

3.2. Phytotreatment of tofu effluent

In the phytotreatment stage, 10 g of plants' wet weight was used as an initial plant biomass to treat the tofu effluent. Based on the weight observations (Fig. 2), both the treatment (T) and plant control (PC) systems showed an increment of growth, including their wet and dry weights. At

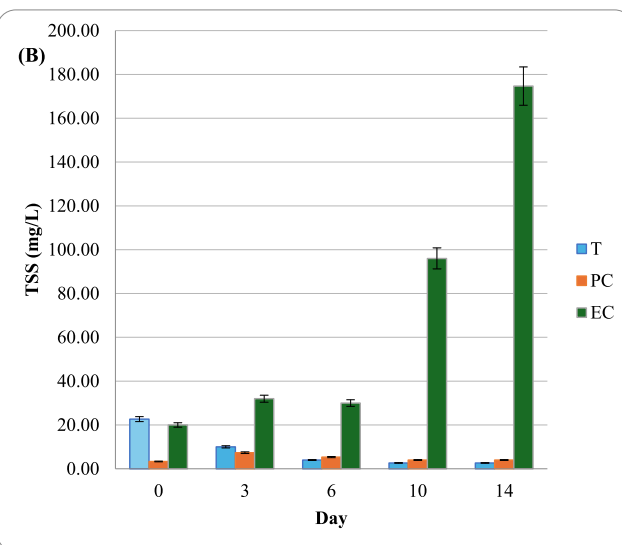
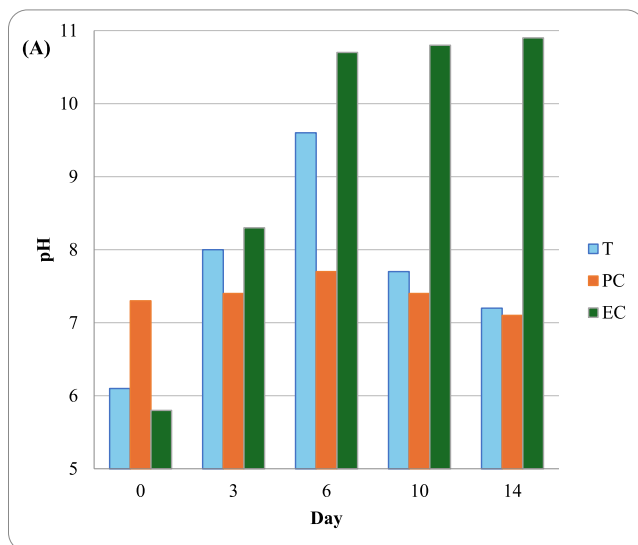


Fig. 3. (a) pH and (b) TSS concentration during phytotreatment (T: treatment, PC: plant control, and EC: effluent control).

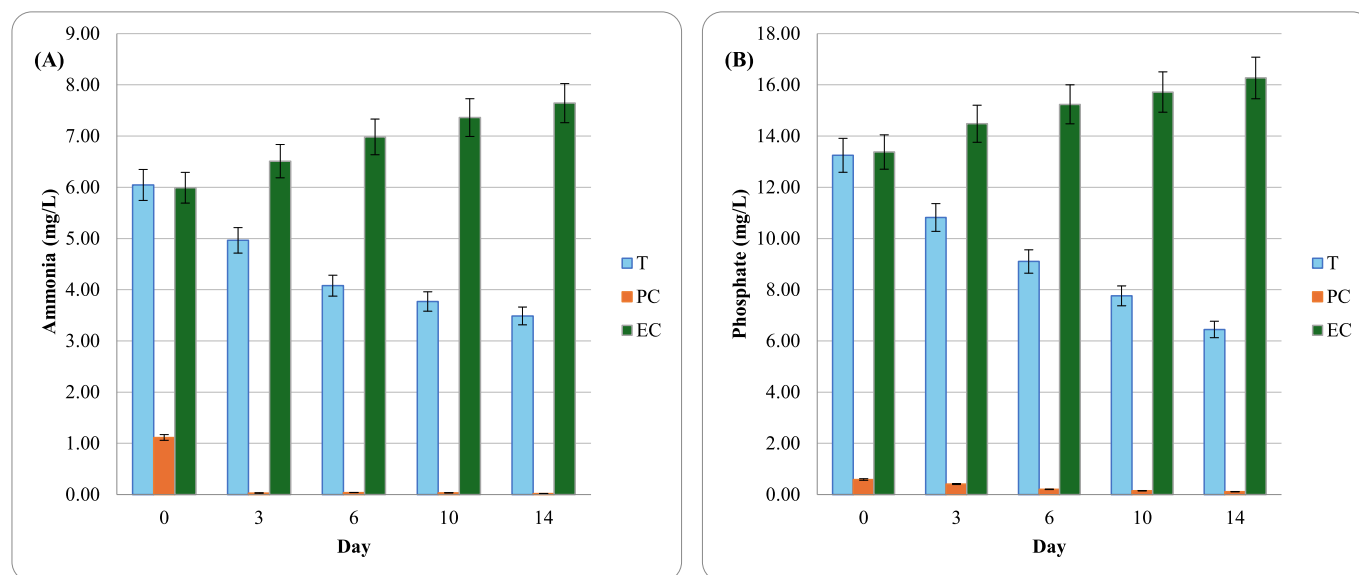


Fig. 4. (a) Ammonia and (b) phosphate concentrations during phytotreatment (T: treatment, PC: plant control, and EC: effluent control).

the end of the phytotreatment period (14 days), PC showed a higher wet and dry weight, reaching up to 18.77 g and 1.33 g, respectively. The wet and dry weights of plants in T were 18.42 g and 1.20 g, respectively. Even though there was a difference in terms of the weight, overall data showed that there is no significant difference between T and PC. In addition to that, there was also no significant difference in weight between two consecutive testing periods. Similar results were obtained by previous researchers, which showed that the use of 20 % coffee wastewater did not significantly affect the growth of *E. crassipes*, while 10 % of it even increased its final weight as compared to the control [17]. In this case, it showed that the use of tofu effluent did not significantly affect the growth of *P. stratiotes*, which is a good indication for phytotreatment [15,17,20].

In the treatment system (T), the initial pH is 6.1, which then gradually increases up to pH 9.6 after 6 days of exposure (Fig. 3a). This increment happens due to active photosynthesis by the plants that remove carbon dioxide in the solution, increasing the pH of wastewater [32]. But towards the end of the treatment, the pH is back to neutral with a final pH of 7.2. The reduction may be contributed to by a lack of available nutrients in the system that slows down the photosynthesis process. It also indicates the shift of microbial activity towards acidic metabolic byproducts as the nutrient load decreases [33]. Comparing T and PC, a significant difference in pH values was observed on days 0, 3, and 6, as the plants might be adapting to the different living medium. After day 10, there was no significant difference in the reading of pH observed for the PC and T systems. It maintains a neutral pH in the range of 7.1 to 7.7, as there is no organic influx supplied to the system, causing minimal photosynthesis to occur. This might correlate with the physical observation of the plants that show withering characteristics after 7 days of exposure to tap water, as low nutrient supply was available for the growth. As for the EC system, the pH continues to rise from 5.8 on day 0 to a final pH of 10.9 after 14 days. It started significantly lower as compared to T and PC, while becoming significantly higher starting on day 6. This is due to microbial activity originally existing in the effluent, where decomposition occurs, releasing byproducts such as ammonia that contribute to pH increments [34]. These findings highlight the importance of the existence of *P. stratiotes* plants in the treatment system to moderate the pH, providing a balanced environment by absorbing nutrients and facilitating oxygenation.

Phytotreatment had also been widely known as an approach to treating suspended solids in wastewater. As mentioned by Mirzaee et al. [35], phytotreatment was able to remove up to 99 % of TSS after some

treatment duration. A similar observation was found in this research where TSS concentration is successfully decreased during the treatment period. The initial TSS of the wastewater was 23 mg/L, but the treatment system (T) was able to further polish the quality of the wastewater by decreasing the TSS as low as 3 mg/L (88 % removal) after 10 days (Fig. 3b). This is contributed to by the natural sedimentation process that occurred along the exposure period, as well as nutrient uptake by the plant, phytotreatment processes, and microbial activities [36]. While for PC systems, the TSS increases by 55 % from initial concentration from day 0 to day 3. As plants in respective systems withered due to a lack of nutrients for their growth, the remaining plants might suspend into wastewater, contributing to an increment in suspended solids concentration. After 6 days, the concentration of TSS decreases slightly and maintains at the range of 5.3 to 4.0 mg/L TSS till the end of exposure. These insignificant changes in TSS removal were due to the originally low concentration of pollutant load without effluent, contributed to by slight microbial activity by decomposing existing organic content in the TSS. While for the EC system, the TSS concentration is overall showing an increment pattern of TSS over time and significantly higher as compared to T and PC after day 3. Naturally existing microbes in the wastewater undergo a decomposition process, breaking down the organic matter into smaller particles that contribute to the TSS concentration [37]. With the absence of plants to absorb the nutrients, the particulate matter was accumulated in the system. The growth of the suspended microbial population also contributes to the increase of TSS in the EC system, while some of the microbial population in T and PC systems were attached to the plant's root [38–40].

Fig. 4 shows the concentration of ammonia and phosphate in tofu wastewater effluents after 14 days of treatment. In the experiment aimed at reducing ammonia and phosphate levels with *P. stratiotes*, the concentration of ammonia in the systems containing the aquatic plant (T and PC) significantly decreased over time. A significant ammonia reduction was observed between day 0 and 3 for PC, while for T, it was observed between day 0 & 3 and day 3 & 6. As *P. stratiotes* have assimilated this chemical, its concentrations have gradually diminished from initial elevated levels. This demonstrates the plant's utility in efficient phytotreatment. The EC systems without *P. stratiotes* exhibited an insignificant ($p > 0.05$) rise in ammonia content over the same duration. In Fig. 4a, ammonia concentration reduced from 6 mg/L to 3.5 mg/L (42.3 %) after 14 days of treatment using *P. stratiotes*. PC showed the lowest ammonia concentration throughout the entire research period, while significantly lower ammonia concentration in T as

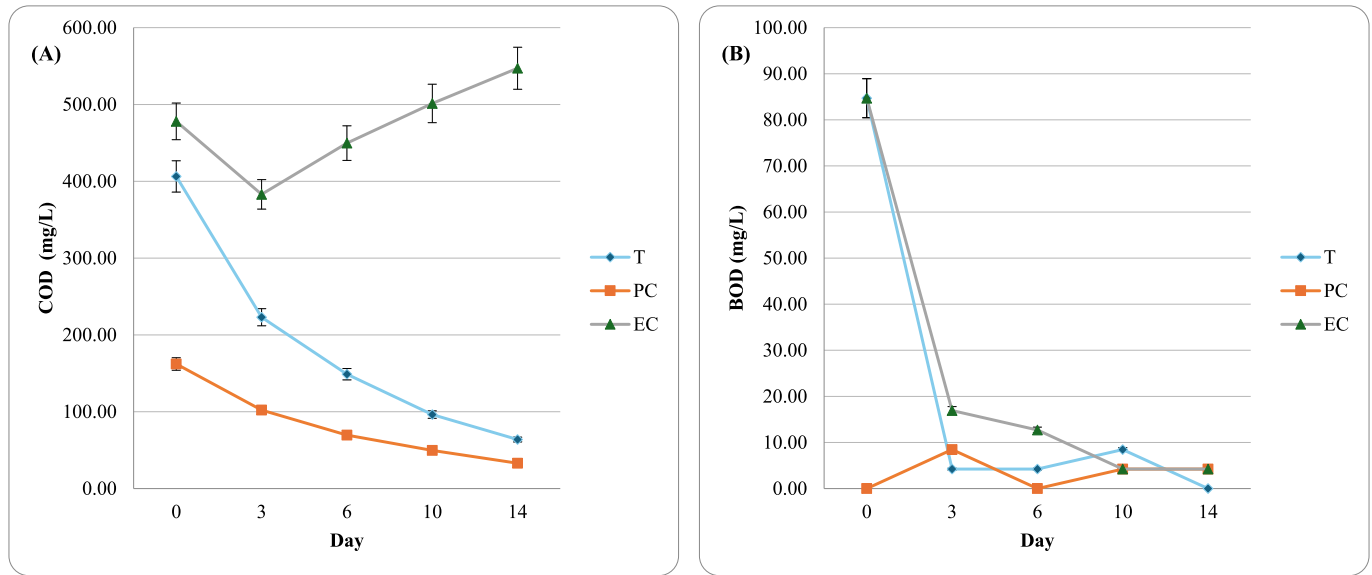


Fig. 5. (a) COD and (b) BOD concentrations during phytotreatment (T: treatment, PC: plant control, and EC: effluent control).

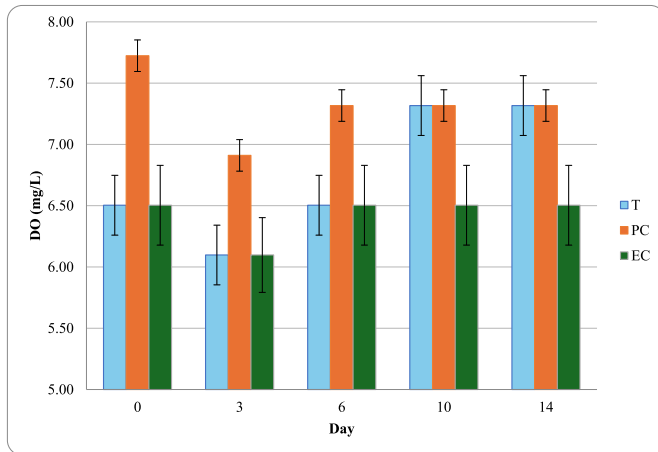


Fig. 6. DO concentration during phytotreatment (T: treatment, PC: plant control, and EC: effluent control).

compared to EC systems can be observed clearly. The absence of a physiologically mediated ammonia uptake pathway suggests that environmental factors influence the buildup. These findings ultimately position *P. stratiotes* as a viable alternative for the cleanup of ammonia in aquatic environments.

P. stratiotes exhibited the best total nitrogen removal from treated swine wastewater at 63.15 % [41]. Ali et al. [42] and Lu et al. [43] showed that *P. stratiotes* can lower ammonium levels by 50 % or more when compared to controls that do not include the plant. In certain studies, removal rates have been observed to reach as high as 69 % in a short timeframe, depending on the specific conditions of the wastewater being treated. Meanwhile, almost 100 % removal of ammonia from sewage wastewater was achieved with an initial concentration of 6.62 mg/L [44]. *P. stratiotes* are known to reduce the concentration of ammonium ions in water by initially utilizing $\text{NH}_4\text{-N}$ as its nitrogen source. It only begins to use $\text{NO}_3\text{-N}$ once all the $\text{NH}_4\text{-N}$ has been depleted [42].

In Fig. 4b, the concentration of phosphates significantly decreased over time during the 14-day treatment period with *P. stratiotes* (T), indicating the plant's effectiveness in wastewater treatment. The

phosphate concentrations decreased markedly from 13.3 mg/L at the beginning of the experiment to around 6.4 mg/L at the end of the treatment period, indicating a reduction efficiency of roughly 51.3 %. Similar to the ammonia analysis result, the control system (EC) showed an insignificant ($p > 0.05$) increase in phosphate concentration over the same time period. A significantly lower phosphate concentration was observed after day 3 between T and EC systems. This shows that the plant is effective at absorbing and getting rid of nutrients. Imron et al. [20] studied that up to 80.4 % total phosphate eradication from domestic wastewater was achieved in 14 days of treatment. This underscores the potential of *P. stratiotes* for the treatment of phosphate contamination in aquatic environments. According to Buta et al. [45], *P. stratiotes* exhibit the greatest phosphorus accumulation efficiency at 95 % with an initial phosphorus concentration of 3.14 ± 3.15 mg/L, followed by *Lemna minor* after a 7-day contact period. This highlights the possibility of using *P. stratiotes* for the treatment of water bodies from phosphorus pollution.

COD and BOD are important parameters in detecting organic matter loading in wastewater, where higher values indicate high organic pollutant content and directly contribute to higher dissolved oxygen depletion [36]. As observed, the COD concentration in the treatment system (T) showed a significant declination pattern over time, similar to the PC system with no tofu effluent involved (Fig. 5a). The treatment system (T) shows a faster removal rate of 45 % after only 3 days of exposure and achieved higher COD removal at 84 % after 14 days, compared to the PC system achieving 37 % removal after 3 days and

Table 3

Final characteristics of tofu effluent.

Parameter	Unit	Initial	Final	Effluent standard ^a	Analysis method
pH	–	5.8	7.2	6–9	pH meter
TSS	mg/L	22.67	2.67	Max 200	Gravimetric
DO	mg/L O ₂	6.5	7.32	Min 3	Winkler titration
COD	mg/L	406.33	63.83	Max 300	Close reflux
BOD	mg/L	84.69	0	Max 150	5-day BOD test
Ammonia	mg/L	6.05	3.49	Max 1	UV Spectro
Phosphate	mg/L PO ₄ -P	13.25	6.45	Max 5	UV Spectro

^a Indonesian Government Effluent Standard for Industrial Activities No. 52014.

Table 4Comparative performance of *P. stratiotes* with previous studies.

Plant species	Type of wastewater	Period (days)	Pollutant removals	Country	References
<i>P. stratiotes</i>	Tofu effluent (10 %)	14	TSS: 88.22 % BOD: 100 % COD: 84.29 % Ammonia: 42.31 % Phosphate: 51.32 %	Indonesia	This study
<i>P. stratiotes</i> L.	Sugar mill effluent (75 %)	60	TDS: 57.26 % BOD: 75.72 % COD: 69.4 % TKN: 72.86 % P: 71.49 %	India	[21]
<i>P. stratiotes</i>	Domestic (25 %)	14	BOD: 99.72 % COD: 99.8 % Ammonia: 46.4 % Nitrate: 100 % Nitrite: 100 %	Indonesia	[20]
<i>P. stratiotes</i>	Domestic	30	TP: 80.4 % TDS: 83 % TSS: 82 % BOD: 82 % COD: 81 % Ammonia: 84 %	Pakistan	[42]
<i>P. stratiotes</i>	Domestic (50 %)	22	COD: 97.4 % Ammonia: 68.5 % Phosphate: 54 %	Malaysia	[44]
<i>P. stratiotes</i>	Husbandry effluent	30	TSS: 97.66 % BOD: 82.56 % COD: 82.89 % TKN: 63.75 % TP: 89.75 %	Vietnam	[57]
<i>P. stratiotes</i>	Domestic	20	TDS: 39.7 % TSS: 95.2 % BOD: 86 % COD: 89 % Ammonium: 39.2 % Nitrate: 90.1 % Phosphate: 73.9 %	Cameroon	[58]
<i>P. stratiotes</i>	Lake	7	BOD: 41.3 % Ammonia: 51.4 % TN: 25.8 % TP: 37.7 %	Egypt	[59]
<i>P. stratiotes</i>	Domestic	42	COD: 79.81 % TN: 100 % TP: 100 %	Brazil	[60]
<i>Eichhornia crassipes</i>	Tofu effluent	10	TSS: 98.13 % BOD: 97.70 % COD: 97.74 % Ammonia: 80.19 %	Indonesia	[61]
<i>Lemna minor</i>	Tofu effluent (10 %)	15	BOD: 45 % TN: 62.24 % TP: 54.23 %	Indonesia	[56]
<i>Vetiveria zizanioides</i>	Tofu effluent (38 %)	15	TSS: 75.28 % BOD: 71.78 % COD: 76 %	Indonesia	[62]

final removal at 80 % at the end of exposure. This proves the ability of plants to reduce the organic load in wastewater by their natural mechanism of nutrient uptake and enhancing surrounding microbial activity [33]. While the minimal decrease of COD in PC systems might be due to the minimal removal of non-biodegradable compounds in the absence of tofu effluent. The final concentration of COD in the treatment system is 64 mg/L. Meanwhile, in the EC system, removal of 20 % COD occurred during the first three days but then continued to increase to as high as 547 mg/L [33]. Throughout the entire testing period, the treatment system (T) showed a significantly lower COD concentration as compared to the EC system, indicating the good performance of *P. stratiotes* in removing COD from the media.

Biological oxygen demand measured biologically oxidizable pollutants in wastewater [46]. A significant BOD removal was observed for the

first 3 days of exposure in T and EC systems (Fig. 5b). A total of 95 % of BOD concentration was able to be removed by the T system on day 3, followed by a slight increment to 8.47 mg/L BOD at day 10 before being completely removed (100 % removal) at day 14, consistent with the COD concentration trends that decrease per time. This indicates that organic matter has been consumed almost entirely or stabilized by plants and microorganisms [47]. Meanwhile, the EC system shows almost similar removal patterns, with slower removal of 80 % after 3 days and gradually decreasing to 4.34 mg/L after 10 days and maintained until the end of the exposure period. Overall BOD analysis showed that the T system achieved significantly lower values as compared to the EC system. These results also indicated that microbial degradation contributed highly during the treatment process, where the organic matter has been broken down through natural processes but not

as efficiently as with the existence of the plant to expedite the removal process and achieve better final removal [8]. Plants help in absorbing the nutrients and supporting the microbial communities around the rhizosphere. The PC system otherwise has zero BOD concentration initially with no effluent present, which then increases to 4.23 mg/L as the final reading after 14 days. This observation reflects the typical plant metabolism with limited organic matter, which also caused the small variations in BOD reading.

Phytotreatment using *P. stratiotes* has emerged as an alternative in treating numerous types of pollutants, including heavy metals, antibiotics, and nutrients in various wastewaters [24,48,49]. Imron et al. [20] studied the potential of *P. stratiotes* treating domestic wastewater that has high organic and inorganic substances, which successfully removed 99.8 % COD, 97.2 % BOD, 46 % ammonia, and 80 % total phosphate in a 25 % wastewater concentration. This study shows similar capabilities with the removal of COD at 84 %, 100 % BOD, and 88 % TSS for wastewater with 10 % concentration. This treatment also has a comparable performance to the highly technical treatment of Fenton oxidation in treating tofu wastewater with a final removal of 81 % TSS and 95 % COD, with lower cost and environmental benefit [5].

Looking at the DO observations (Fig. 6), it clearly showed that the use of *P. stratiotes* was able to increase the dissolved oxygen

concentration inside the effluent. As compared to the DO in EC (which was mostly stable at 6.5 mg/L), the DO in T showed a significant increment over time (starting at 6.5 mg/L with a final concentration of 7.32 mg/L). In the PC reactor, the DO concentration even showed a significantly higher value of 7.72 mg/L (as compared to T and EC), which was then reduced to 7.32 mg/L at the end of the treatment period (subjected to the oxygen consumption by plants and microbial communities) [50]. This showed that *P. stratiotes* have the capability of oxygen transfer from the air into the rhizosphere area [50–52].

3.3. Overall performance

The overall performance of *P. stratiotes* in phytotreating tofu effluent is summarized in Table 3. Based on the results, it can be seen that after treatment, effluent has a pH in the range of acceptable standards, as well as TSS, DO, COD, and BOD. However, the content of ammonia and phosphate in the after-treatment effluent is still higher than the permissible standard. Although plants uptake a high amount of N and P, there might be insufficient time to produce lower than the permissible standard effluent. With these results, it is suggested to prolong (or optimize) the treatment period so that lower ammonia and phosphate concentrations can be achieved because the trend of ammonia and

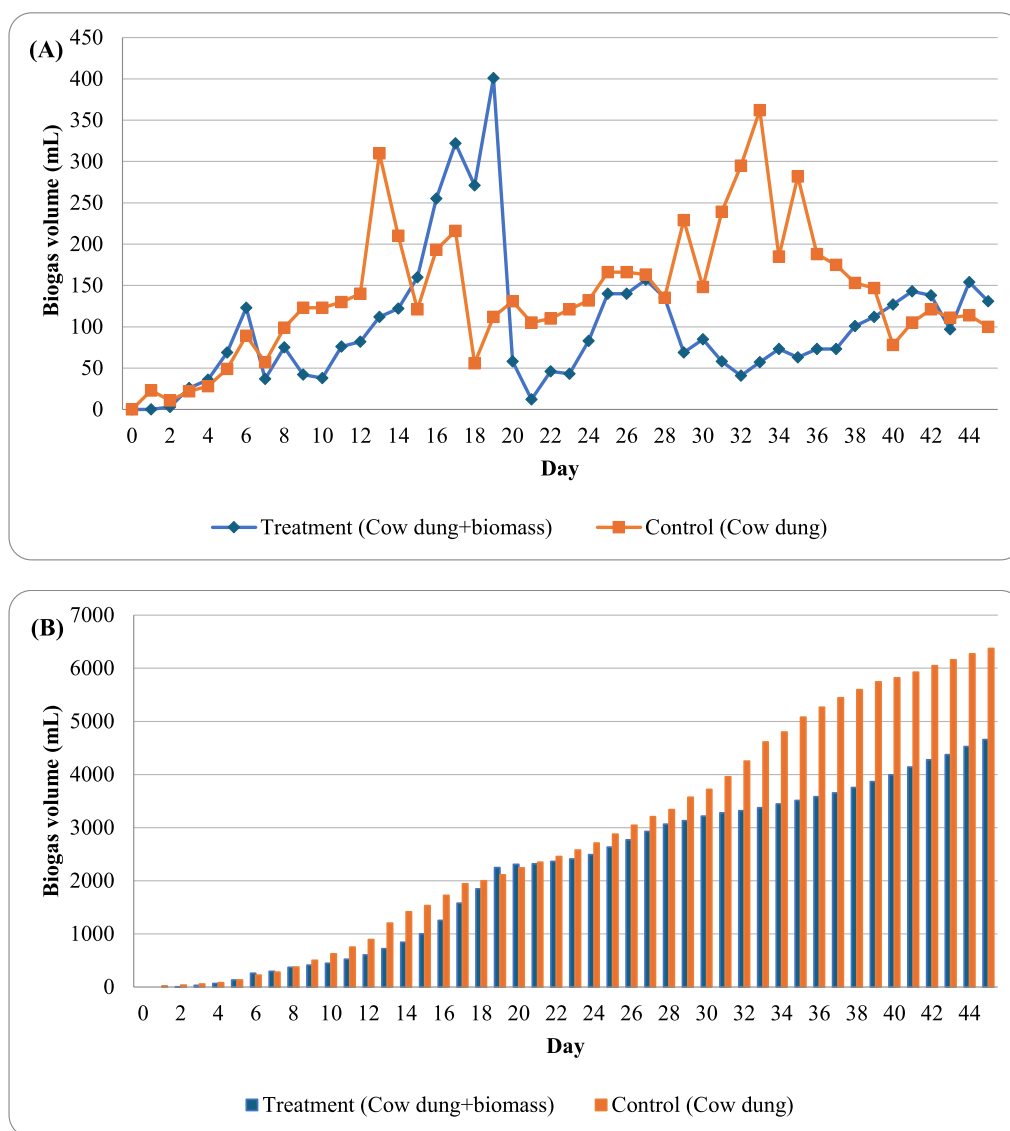


Fig. 7. (a) Fluctuation and (b) accumulation of biogas production.

phosphate removals still continues to decrease even at the end of the treatment period (day 14).

A comparison of *P. stratiotes*' performance in treating various types of wastewaters and also the treatment of tofu effluent using various plants is summarized in Table 4. The performance of *P. stratiotes* in treating various types of wastewaters is quite stable and promising. Most of the research mentioned that *P. stratiotes* achieved BOD and COD removals around 80 % and more, while for ammonia and phosphate, the ranges were between 30 % and 70 %. Based on the collected data, some factors affecting *P. stratiotes*' performance were initial wastewater characterization and also treatment period [53–55]. Comparing the performance of *P. stratiotes* with other plants in treating tofu effluent, this research achieved similar results in terms of pollutant removals. *P. stratiotes* showed higher removal of BOD when compared to *Eichhornia crassipes* in treating 10 % tofu effluent [56].

3.4. Biogas production

The potential of biogas production by *P. stratiotes* biomass was compared to the commonly used substrate of cow dung. In Fig. 7a, it can be seen that the phase of methanogenesis occurred faster in the control reactor, which was recorded at days 12 to 16, while the second cycle seems to be happening on days 28 to 36. In the treatment reactor, methanogenesis phases occurred on days 16 to 20, and it seems to only have 1 cycle throughout the tested period. Although the production of biogas tends to be slower in the treatment reactor, the amount of biogas produced in the treatment reactor was recorded to be higher. The slower phases of biogas production in the treatment reactor might be attributed to the content of plant biomass, which is cellulose, hemicellulose, and lignin [63]. These compounds are harder to be degraded by anaerobic bacteria, especially for lignin, but give higher caloric value and biogas conversion [64]. The highest biogas produced by the control reactor was 310 mL on day 13, while 401 mL was produced by the treatment reactor on day 19. It can also be seen in Fig. 7b that biogas accumulation by the treatment reactor on day 19 significantly surpassed the control reactor (2250 mL vs. 2112 mL). However, the control reactor overcame the treatment reactor, starting on day 21, which then continued until the end of the treatment period. At the end of the treatment period (day 45), biogas accumulation in the control reactor reached 6373 mL, while only 4659 mL in the treatment reactor.

3.5. Authors perspective and future research directions

It was clear that phytotreatment of tofu effluent by *P. stratiotes* showed a very promising potential and offered a new possibility to be used as a polishing unit before final discharge into the environment. The main limitation of this method is that effluent needs to be diluted before treatment; in the real case, it needs to be pretreated until achieving the desired concentration. This is one of the limitations of biological treatment using plants. However, the performance of *P. stratiotes* in phytotreating tofu effluent was excellent. Dilution to only 10 % concentration gave pH, COD, ammonia, and phosphate that were still outside the permissible limit, and phytotreatment was able to increase the pH and reduce the COD to the permissible range/limit. Considering the ammonia and phosphate, *P. stratiotes* showed a considerably good removal, and the trend still continues at the end of the tested period. Prolonging the tested period may be beneficial to reduce ammonia and phosphate concentrations. Future research is suggested to optimize the tested period to obtain the best performance of phytotreatment while also producing effluent lower than the permissible standard. In addition to that, optimizing the initial plant biomass can be conducted to obtain higher removal in proportion to the treatment agent (plants) [15,17]. Speaking about biogas production, the use of *P. stratiotes*' biomass clearly showed a good potential; however, pretreatment is needed to break down the hardly degraded component, such as lignin. Some available biomass pretreatments suitable before anaerobic digestion are

acid or enzyme hydrolysis [65].

4. Conclusion

Phytotreatment of tofu effluent (10 % concentration) using *Pistia stratiotes* was able to remove TSS by 88 %, ammonia by 42.3 %, phosphate by 50 %, COD by 84 %, and BOD by 95 % from an initial concentration of 22.67 mg/L TSS, 6.05 mg/L ammonia, 13.25 mg/L phosphate, 406.33 mg/L COD, and 84.69 mg/L BOD. *P. stratiotes* were also able to stabilize pH from 6.1 to 7.2 while also being capable of transferring oxygen from air into the rhizosphere area. Prolonging and/or optimizing the phytotreatment periods were suggested to obtain higher removal performance, especially for ammonia and phosphate, before discharging into the water bodies. Further treatment of the plant's biomass using anaerobic digestion suggested a good potential for biogas production, as shown by higher maximum daily biogas production. However, the overall biogas accumulation is still lower than the control. Further biomass pretreatment using acid or enzyme hydrolysis is suggested to be conducted to unravel the real potential of the plant's biomass by breaking down the hardly degraded compounds such as cellulose, hemicellulose, and lignin.

CRediT authorship contribution statement

Setyo Budi Kurniawan: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Funding acquisition, Data curation. **Muhammad Fauzul Imron:** Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Rikky Ramadhan Mustofa:** Investigation, Formal analysis. **Dhuroton Najiya:** Investigation, Formal analysis. **Nor Sakinah Mohd Said:** Writing – original draft. **Junaidah Buhari:** Writing – original draft. **Hajjar Hartini Wan Jusoh:** Resources. **Azimah Ismail:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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