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Research Article

The Efficacy of *Haematococcus pluvialis* Culture as a Bioremediator for Tofu Effluent

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Abstract

Wastewater management is one of the issues at the heart of the Sustainable Development Goals. This is urgent because waste that is not properly managed can cause damage to water bodies, reducing clean water sources that can be used for household, office and industrial needs. One of the low-cost yet effective wastewater management strategies which also produce valuable products is the use of microalgae as remediators to improve the quality of polluted waters. The aim of this study was to evaluate the efficiency of *H. pluvialis* culture as a bioremediator for tofu effluent. The research stages consisted of activation of *H. pluvialis* microalgal starter in Walne medium, followed by cultivation of microalgae in tofu effluent medium, observation of microalgal growth in the effluent, and measurement of water quality parameters such as pH, colour, BOD and COD, as well as the aroma of tofu effluent before and after use as a microalgal growth medium. According to the growth curve of *H. pluvialis* in the tofu wastewater medium, it is known that this microalgae can grow well in the effluent and can improve wastewater quality. Where the pungent odour typical of soya waste began to disappear from the second day of cultivation, the pH increased from acidic to neutral, and BOD and COD levels were drastically reduced. In conclusion, *H. pluvialis* microalgae can be used as a bioremidiator of tofu ffluent as it can effectively improve the quality of the wastewater.

Keywords: microalgae; wastewater treatment; water quality

1. Introduction

The United Nations (UN) defines waste as any material that is not a primary product and for which the producer has no further use in production, transformation, or consumption. This material is discarded or intended for discard by the producer. With regard to the physical form of waste, three classifications can be distinguished: solid, liquid, and gaseous. Solid waste encompasses a wide range of materials, including municipal solid waste, biomedical waste, industrial waste, agricultural waste, hazardous waste, and electronic waste. Liquid waste encompasses various types of effluent, including sewage, refinery waste, and liquid by-products. Concurrently, the term "gas waste" encompasses a variety of elements, including stack gas, emitted gas products, and foam (Aziz et al., 2021).

A total of three Sustainable Development Goals (SDGs) and six indicators have been identified as relevant to the correlation of waste management: municipal solid waste management (11.6.1), food loss and waste (12.3.1), information transmitted under chemicals and waste conventions (12.4.1), hazardous waste generated and treated (12.4.2), national recycling rate (12.5.1), and coastal eutrophication and plastic debris density (14.1.1). Consequently, the issue of waste has emerged as a pervasive problem with global implications. In Indonesia alone, the annual waste generation reached 68 million tons in 2020 (Sintawardani et al., 2022). A salient issue concerning liquid waste in Indonesia pertains to tofu effluent. Given its status as a major producer of tofu, it is unsurprising that Indonesia has a considerable amount of tofu liquid waste. Furthermore, the majority of tofu production in Indonesia is characterized by small-scale industrial enterprises. Consequently, the management of tofu wastewater is suboptimal.

A plethora of strategies have been devised for the treatment of wastewater, including physical, chemical, biological, and combinations of these methodologies (Abdelfattah et al., 2023). The primary objective of wastewater treatment is to achieve a substantial reduction in the content of organic matters, thereby enhancing the quality of the effluent (Mohsenpour et al., 2021). The primary parameters employed to ascertain the quality of wastewater include the concentration of nitrogen (N) and phosphorus (P), the levels of biological oxygen demand (BOD) and chemical oxygen demand (COD), and the acidity or pH of the water (Abdelfattah et al., 2023; Mohsenpour et al., 2021). A plethora of studies have demonstrated the efficacy of microalgae in the treatment of wastewater, thus indicating a potentially viable solution for environmental remediation. A number of species of microalgae have been demonstrated to be effective bioremediators for heavy metals, pollutants, and pathogens present in wastewater. These include Scedosporium, Chlorella, Botryococcus, Phormidium, Limnospira (formerly Arthrospira, Spirulina), and Chlamydomonas (Abdelfattah et al., 2023). However, there is a paucity of literature addressing the potential of *Haematococcus pluvialis* in this context. The utilization of wastewater treatment-based microalgae confers several advantages, including: The system under consideration is characterized by its low cost, high tolerance to wastewater toxins, and capacity to produce valuable products from the harvested biomass. Additionally, it has been demonstrated to reduce CO2 concentration in the air (Abdelfattah et al., 2023). Consequently, microalgae cultivation in wastewater has the potential to offer significant environmental and economic advantages.

The cultivation of microalgae in tofu effluent is a viable option due to the residual presence of essential nutrients, including nitrogen and phosphorus, which are found in the wastewater (Musa et al., 2021). The discharge of tofu effluent into watercourses is prohibited due to its detrimental impact on the physicochemical parameters of these ecosystems. Specifically, the effluent contains elevated concentrations of chemical oxygen demand (COD) and biological oxygen demand (BOD), as well as an acidic pH level, which poses a significant threat to the biodiversity of the aquatic environment. The chemical oxygen demand (COD) levels in the water sample range from 7,500 to 14,000 milligrams per liter (mg/L) (ppm), while the biological oxygen demand (BOD) levels are within the range of 6,000 to 8,000 mg/L. The pH level of the water sample is approximately 5 to 6 (Ajijah et al., 2020).

The tofu production process is comprised of seven distinct stages, which are as follows: initial washing, soaking, grinding, cooking, filtration, clumping, and pressing. During the process of washing, water is introduced at the milling stage. Conversely, during the washing, clumping, and pressing stages, water is expelled or retained, subsequently becoming tofu effluent. The tofu wastewater utilized in this study is the residual water of the tofu clumping process (whey), which still contains 75% water from the total water added during the production process (Sjafruddin et al., 2022). The objective of this research is to assess the efficacy of Haematococcus pluvialis culture as a bioremediator for tofu effluent. The parameters that are being evaluated include cell growth, physical characteristics (i.e., aroma, color, and pH), and biochemical characteristics (i.e., BOD and COD) of the effluent before and after its use as a medium for microalgae growth.

2. Material and Method

Microalgae Activation

Prior to the initiation of the cultivation process, the *H. pluvialis* microalgae starter, which had been stored in a refrigerated environment, was subjected to a three-day activation period. This procedure was undertaken to acclimate the microalgae to a novel environment, employing Walne's medium for the purpose of facilitating growth. The compositions of Walne's medium are enumerated in Table 1. The total volume of the culture was determined to be 900 milliliters, with an algal inoculum (starter) volume of 90 milliliters, constituting 10% of the total culture volume. The nutrient solution was added in a volume of 900 microliters, corresponding to 0.1% by volume, while the vitamin solution was added in a volume of 90 microliters, representing 0.01% by volume (Andersen, 2005). Following the initiation of the process, the microalgae culture was subsequently transferred to a novel medium for cultivation. This new medium was utilized in conjunction with tofu effluent and Walne's medium to facilitate a comparative analysis.

Table 1. Walne's medium compositions

A solution (nutrient)	Quantity
$NaH_2PO_4.2H_2O$	20 gr/L
$NaNO_3$	100 gr/L
Na_2EDTA	Five gr/L
Na_2SiO3	40 gr/L
$MnCl_2.H_2O$	0.36 gr/L
$FeCl_3$	1.3 gr/L
H_3BO_3	10 gr/L
Aquadest	1000 ml

B solution (trace metal)	Quantity
$ZnCl_2$	21 gr/L
$CoCl_2.6H_2O$	Two gr/L
$(NH_4)_8.Mo_7O_{24}.4H_2O$	0.9 gr/L
$CuSO_4.7H_2O$	20 gr/L
$FeCl_3.6H_2O$	3.15 gr/L
Aquadest	100 ml

C solution (vitamin)	Quantity
B12	0.1 gr/L
Thiamine	20 gr/L
Biotin	0.1 gr/L

Microalgae Cultivation

The growth conditions of *H. pluvialis* during activation and cultivation were identical: light intensity of ±2500 lux; photoperiod of 24 hours light, temperature range between 29-33°C, and aeration for 24 hours (Putri & Alaa, 2019). In the cultivation of microalgae in Walne's medium (designated W), the nutrient and vitamin solutions were added in equivalent quantities to those employed during the activation phase. However, no nutrients or vitamins were added to the algae cultivated in the tofu wastewater, as the algae obtain their nutrients from the effluent. The cultivation process was executed in Walne's medium for a duration of 16 days, whereas the tofu effluent was cultivated for 8 days.

Table 2. Microalgae culturing conditions

Photoperiod (light: dark) hour(s)	Light (lux)	Temp (^oC)	Aeration
24:0	2500	29-33	24 hours

The tofu effluent employed as a growth medium underwent filtration with filter paper to separate the residual tofu curds (solid waste) contained in the effluent. The tofu wastewater growth medium is composed of two distinct treatments: the first treatment involves aeration of the medium the day prior to its use (designated as "code A"), and the second treatment consists of a lack of aeration or its direct use as a growth medium (designated as "code NA").

Cell density observation

The growth of *H. pluvialis* in two media was observed on a daily basis under the microscope. The algal cell density was enumerated using a hemocytometer and calculated with the following formula. Subsequently, the cell density data were utilized to generate a growth curve for *H. pluvialis*.

$$Cell\ density = \frac{(numbers\ of\ cells\ counted) \times (dilution\ factor)}{number\ of\ squares\ counted} \times 10^4\ cells/ml$$

Measurement of the physical and biochemical properties of tofu effluent before and after use

In order to ascertain the function of microalgae in enhancing wastewater quality, a meticulous examination was conducted. This examination entailed the measurement of pH, color, COD, and BOD, in accordance with the stipulated Indonesian national standard (SNI). The quantity of tofu liquid waste was measured in a controlled experiment before and after its use as a microalgae growth medium. This measurement was used to calculate the percentage reduction. The methods employed for this measurement are delineated in Table 3.

Table 3. The methods use for physico-biochemical measurement

Measurement	**Method**
pH	SNI 6989.11:2019
Color	Colorimetry (IKM.BLL-3.3)
COD	Colorimetry (IKM.BLL-3.4)
BOD	SNI 6989.72:2009

3. Results and Discussion

The objective of this study was to assess the efficacy of Haematococcus pluvialis culture as a bioremediator for tofu wastewater. In order to achieve this objective, the microalgae *H. pluvialis* was cultivated in a medium consisting of tofu wastewater. The cultivation was performed under two distinct conditions: under conditions of aeration the day prior (designated as "code A") and under conditions of no aeration or utilization as a growth medium directly (designated as "code NA"). These conditions were then compared with those of *H. pluvialis* cultivated on Walne medium (designated as "code W"). The Walne medium contains complete nutrients for microalgae growth. The tofu effluent utilized in this study was obtained from a local tofu producer located in Abian Tubuh Village, Mataram City, who engages in continuous tofu production on a household scale. The physical properties of the fresh tofu effluent utilized are enumerated in Table 4.

Table 4. Physical characteristics of fresh tofu wastewater

Appearances	Aroma	Temperatur	pH
The liquid is light brown in colour and contains residual tofu curds	Intense soya odour (beany off-flavor)	±62 °C	5,9 (weak acid)

The tofu effluent utilized was subjected to pre-treatment, specifically aeration, prior to its application, as outlined in the method established by (Liu et al., 2021). A comparative analysis was conducted with cultures employing tofu effluent that had not undergone prior aeration. When subjected to aeration, the wastewater produced from tofu resulted in substantial foam formation, with the rate of aeration directly correlating to the rate of foam production (Figure 1). The process of foam formation entails a reduction in the liquid's volume. It has been demonstrated that an increase in the production of foam is concomitant with an increase in the consumption of liquid. The phenomenon of tofu effluent foaming during aeration is attributed to the presence of surfactant compounds within the liquid matrix (Collivignarelli et al., 2020). Aeration was conducted for a duration of one day, resulting in alterations to the color, aroma, and pH of the liquid (refer to Table 5 for detailed metrics). The effluent from the aerated tofu process is characterized by a browner color, a more pronounced aroma, and an increased acidity level, as indicated by a lower pH value. Despite exhibiting lower physical properties compared to the unaerated effluent, the aerated tofu effluent facilitated more efficient microalgae cultivation, as evidenced by well-mixed microalgae on medium A and a substantial attachment of microalgae to the bottle or L-pipe on NA medium. This phenomenon can be attributed to the process of overnight aeration of the tofu effluent, which facilitates the growth and decomposition of organic matter by bacteria. This decomposition releases nutrients that are essential for the growth of microalgae. Additionally, the production of CO_2 by these bacteria is crucial for the photosynthesis process in algae. Consequently, microalgae cells in A medium exhibit greater density compared to those in NA medium.

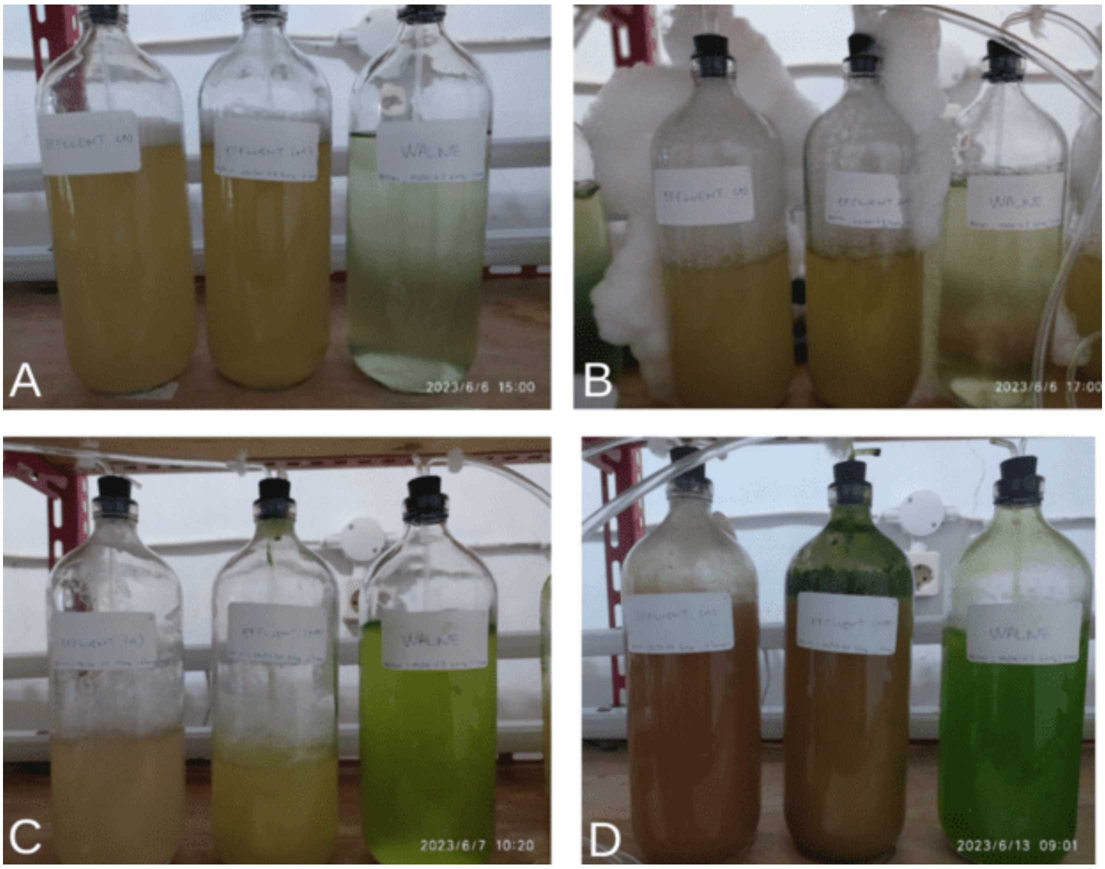

Figure 1. Cultivation process of H. pluvialis in 2 media {left to right: aerated tofu waste (A), non-aerated tofu waste (NA), Walne medium (W)}. Description: A (freshly cultivated microalgae culture), B (after 2 hours of cultivation, the volume of the tofu wastewater medium decreased by more than a third), C (after a day, the volume of the tofu wastewater medium culture decreased by more than half of the initial volume and microalgae sediment appeared under the NA culture bottle), D (after 7 days of cultivation, the colour of the medium cultures A and NA becomes getting darker brown and more microalgae sediment appears on the neck of the NA culture bottle, while the colour of culture W is becomes greener).

During the cultivation process, both media types produced foam, as all cultures were subjected to aeration for a duration of 24 hours. Consequently, the culture volume of both medium A and NA decreased, with the NA medium experiencing a greater reduction in volume and a significant number of microalgae settling to the bottle's base. Following a two-day cultivation period, a marked dissipation of the potent and pungent odor of the tofu wastewater medium became evident, accompanied by a discernible shift in the culture's coloration. The culture exhibited a transition from a greenish yellow hue (D-0) to a whitish beige shade (D-1), subsequently transitioning to a beige color (D-2), and then to a brownish beige (D-3), followed by a light brown phase (D-4). Notably, a progressive intensification in the pigmentation was observed, culminating in a dark brown state by the fifth day. In summary, odors in wastewater can be attributed to two activities: the anaerobic decomposition of biodegradable materials or the direct emission of specific chemicals with wastewater discharges (Ren et al., 2019).

Figure 2 illustrates the cell morphology of *H. pluvialis* following a seven-day cultivation period. *H. pluvialis* cells are characterized by a round morphology and a green pigmentation, which is attributable to the prevalence of lutein, a pigment that constitutes 75-80% of the total pigmentation (Shah et al., 2016). In both medium types, the cell morphology of tofu wastewater and Walne remained round and green in color, respectively. The distinguishing factor in this case was the presence of a lower cell density in the wastewater medium. This observation was accompanied by a predominance of white *H. pluvialis* cells, indicating a significant decrease in pigment production. Furthermore, the culture water in the NA medium exhibited a higher degree of turbidity compared to medium A, necessitating multiple dilutions of the culture for observation. This observation stood in stark contrast to the W medium culture, which was characterized by clarity and the absence of any discernible contamination. Medium A cultures exhibited a lower level of turbidity compared to NA cultures. This phenomenon can be attributed to the process of overnight aeration, which facilitates the breakdown of complex organic compounds in the effluent into smaller, more straightforward molecules by other microorganisms, such as bacteria (Gerardi, 2006). This process contributes to a reduction in turbidity.

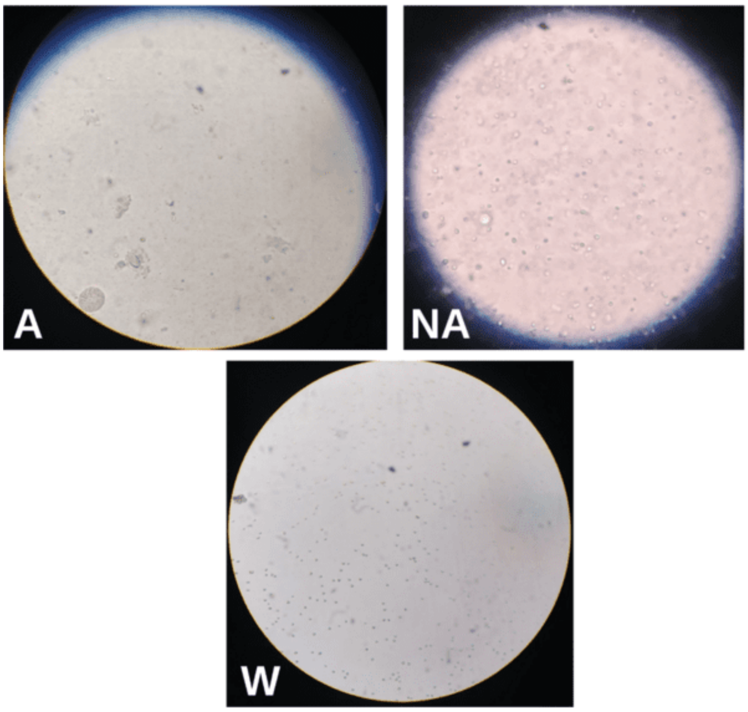

Figure 2. Cell appearance of *H. pluvialis* under the microscope on day 7: A (effluent medium+aeration treatment), NA (effluent medium +non-aeration treatment), W (walne medium)

Preliminary findings indicate that *H. pluvialis* exhibits robust growth potential in tofu wastewater, with a cell density that is double that observed in the Walne medium. However, the growth phase remains unaltered, with the exception of the tofu wastewater medium, which necessitates a prolonged adaptation period of two days. This requirement arises due to the exclusive utilization of Walne medium during the initial starter activation phase, as depicted in Figure 3. In summary, the microalgae *H. pluvialis* will experience the death phase on day 14 (see Figure 4). Therefore, it can be concluded that tofu wastewater can be utilized as a growth medium for *H. pluvialis* microalgae. Furthermore, it is recommended that the tofu wastewater be aerated prior to use.

The utilization of tofu liquid effluent as a microalgae growth medium has been demonstrated to enhance wastewater quality. In this instance, the beany off-flavor aroma has been neutralized, and the pH and biological oxygen demand (BOD) levels have improved and met the Indonesian National Standard (SNI). Despite the fact that the COD levels remain significantly above the standards established by the Indonesian government, a substantial reduction in concentration has been observed (refer to Table 5). Consequently, further research is necessary to reduce COD concentrations in tofu effluent, with the objective of meeting the SNI before the effluent is discharged into water bodies.

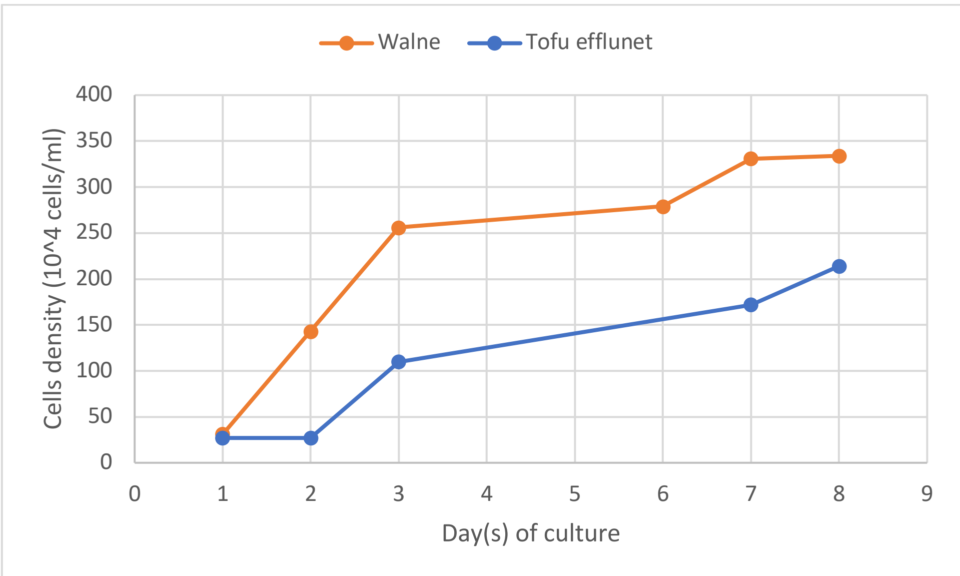

Figure 3. Comparison of H. pluvialis growth in 2 mediums (Walne & tofu effluent)

Fresh tofu wastewater is characterized by a temperature of approximately 62°C, a pH level of around 5.9 (indicative of a weak acid), and a distinct beany aroma. Following its utilization as a growth medium for microalgae, the pungent odor that is characteristic of soybean waste began to dissipate on the second day. Concurrently, the pH level increased to 7 (neutral), and the COD and BOD levels decreased, exhibiting a reduction efficiency of 27-30% and 90%, respectively. According to Delgadillo-Mirquez et al. (2016), an increase in the pH was observed as a consequence of the absorption of carbon dioxide by microalgae in the medium. Concurrently, the decline in COD and BOD levels is concomitant with the rise in dissolved oxygen (Wei et al., 2018) yielded by microalgae cells through photosynthesis as they proliferate. In summary, *H. pluvialis* microalgae have been demonstrated to possess the capacity to effectively remediate wastewater associated with tofu production.

Table 1. Results of effluent quality analysis before and after use as a microalgae growth medium

Treatment(s)	Parameters	Before (D-0)	After (D-8)	Effluent water quality standard*	Description
Effluent with no-aeration (NA)	Aroma	Beany off-flavor	Neutral	–	Improving the aroma
	pH	5.7 (acid)	7.09 (neutral)	6-9	has met the standard
	Color	1,850 PCU	10,350 PCU	–	–
	BOD	96 mg/L	9.74 mg/L	150 mg/L	has met the standard
	COD	69,200 mg/L	50,500 mg/L	300 mg/L	has not met the standard
Effluent with aeration (A)	Aroma	Unpleasant odour	Neutral	–	Improving the aroma
	pH	4 (acid)	6.91	6-9	has met the standard
	Color	2,990 PCU	5350 PCU	–	–
	BOD	14 mg/L	20.82 mg/L	150 mg/L	has met the standard
	COD	70,900 mg/L	49,800 mg/L	300 mg/L	has not met the standard

Description:

*SNI for soybean processing business, according to Regulation of the Minister of Environment of the Republic of Indonesia Number 5 of 2014 concerning Wastewater Quality Standards

Conclusion

H. pluvialis microalgae demonstrates robust growth in tofu wastewater medium. The utilization of tofu wastewater as a medium for microalgae cultivation has been demonstrated to enhance the quality of wastewater, thereby ensuring its safe discharge into water bodies. The tofu effluent quality parameters that met the SNI after treatment were pH and BOD. While the COD level post-treatment did not reach the SNI, it demonstrated the capacity to curtail the COD concentration by up to 30%. Furthermore, the pungent odor characteristic of industrial soybean waste was effectively mitigated from the second day of cultivation. Therefore, *H. pluvialis* can be utilized as a bioremediator for tofu liquid waste.

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