Utilization of tofu wastewater as a cultivation medium for
*Chlorella vulgaris* and *Arthrospira platensis*

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**Abstract.** Tofu is one of favourite daily meal in Indonesia. It is produced freshly in some small to large-scale industries, and generates a large volume of wastewater. Generally, untreated wastewater from tofu industries in Indonesia is still directly disposed to the environment that can cause severe pollution in water resources. Tofu wastewater contains very high organic compounds and nutrients, but it has an acidic pH. The possibility of utilizing tofu wastewater as a cultivation medium for microalgae is intriguing. The objective of this research was to utilize tofu wastewater as a cultivation medium for *Chlorella vulgaris* and *Arthrospira platensis*. Cultivation of microalgae was conducted for ten days using 3, 5, and 10 % of diluted tofu wastewater. For comparison, the tofu wastewater anaerobic digestion effluent (TW-ADE) was used as the medium. Walne medium was used as a positive control, and distilled water was used as a negative control. Both microalgae showed the highest biomass and protein production was in Walne medium (positive control), followed in 5% tofu wastewater medium for *C. vulgaris*, and 3 % tofu wastewater medium for *A. platensis*. The highest chlorophylls content of *C. vulgaris* and *A. platensis* was in 5 % tofu wastewater and Walne medium, respectively. The most top carotenoid content of *C. vulgaris* and *A. platensis* was in 5 % and 10 % tofu wastewater medium, respectively. On the other hand, biomass, chlorophylls, carotenoids, and protein content of both microalgae in TW-ADE medium had the lowest result. Cultivation of *C. vulgaris* and *A. platensis* in tofu wastewater offer a wide range of environmental and economic benefits, as a sustainable strategy to treat wastewater and produce high-value products from microalgae.

**Keywords:** *Arthrospira platensis*; carotenoids; *Chlorella vulgaris*; tofu wastewater; tofu wastewater anaerobic digestion effluent (TW-ADE)

**1. Introduction**

Tofu industries in Indonesia achieved more than 84,000 units from household to large scale with production capacity about 2.56 million tons/year generated 20 million m³/year of tofu wastewater and about 1,024 million tons of solid waste [1,2]. The solid waste can be used as a feed, but the liquid waste is usually directly disposed to the environment that can cause severe pollution in water resources [1]. Tofu wastewater contains very high organic compounds, mostly proteins and lipids, about 40 to 60 % (226.06 mg/L to 434.78 mg/L), carbohydrate (25 % to 50 %), and fat (10 %) [3].
According to Widyarani et al. [4], the protein content constituted more than half of organic material in the tofu wastewater. The wastewater has Chemical Oxygen Demand (COD) 7,500 to 14,000 mg/L, Biological Oxygen Demand (BOD) 6,000 to 8,000 mg/L, Total Suspended Solid (TSS) of more than 1,000 mg/L, total nitrogen, and phosphate with low acidity at pH 5 to 6 [1,5]. Anaerobic decomposition of tofu wastewater is very promising, considering this high organic content.

Microalgae are microscopic photosynthetic organisms that can be found in both marine and freshwater environments. Naturally, two primary farm systems for the production of microalgae and cyanobacteria are photoautotrophic and heterotrophic. The photoautotrophic refers to the process algae need light to grow and generate new biomass. The photoautotrophic production system can be integrated with the industrial’s wastewater treatment system and CO2 mitigation to reduce the industrial CO2 emission footprint. Heterotrophic refers to cultivate algae without light and feed on carbon sources, for example, sugars, acetate. Some microalgae are mixotrophic, which can be heterotrophic and autotrophic [6].

Microalgae can be utilized as food supplements, pharmaceuticals, biofuels, animal feed, aquaculture, and cosmetics [3] because they contain lipids, carbohydrates, proteins, pigments, vitamins, and other compounds. The advantages of microalgae cultivation are shorter reproduction cycle, less area required compared to land-based crops, ability to recycle nutrients from unconventional water resources (e.g., wastewater and brackish water including oceans) [7,8,9,10] and also as strategy to reduce emission of CO2 [11,12].

Chlorella is a unicellular algal that has a circular shape [13], while Arthrospira platensis is a filamentous green-blue algal from the cyanobacteria family [14]. Both Chlorella and A. platensis have a high protein, chlorophyll, carotene, vitamin, antioxidant, antimicrobial, and other nutrients [15]. They also have the ability to grow in the poor medium and degrade ammonia, nitrate, nitrate, and phosphate in the medium [5,16]. Research conducted by Dianursanti et al. [5] showed that 20 to 30% of tofu wastewater was the optimum medium for Chlorella growth. On the other hand, research conducted by Widayat et al. [3] showed the fastest growth rate of C. vulgaris was in 10% tofu wastewater. C. vulgaris also can degrade BOD, COD, phosphates, and ammonia in tofu wastewater. A. platensis in 0-8% tofu wastewater medium showed the fastest growth rate and highest protein content in 6% tofu wastewater [17].

Cultivation of microalgae in wastewater offers a wide range of economic and environmental benefits, as a sustainable strategy to produce high-value products from microalgae, wastewater treatment by assimilation of organic and inorganic pollutants, eliminating of CO2 emissions [9,18,19]. If the wastewater is treated by an anaerobic process, 90% of COD could be degraded, and the rest should be treated aerobically. Both tofu wastewater and the effluent from the anaerobic treatment plant of tofu wastewater still contain nutrients that could be utilized as a cultivation medium for microalgae. The objectives of this research were to use tofu wastewater and the tofu wastewater anaerobic digestion effluent (TW-ADE) from the anaerobic plant in Giriharja-Sumedang, Indonesia as a cultivation medium for Chlorella vulgaris and Arthrospira platensis. As well as the effect of medium variation on biomass, protein, chlorophylls content, and carotenoids content of both microalgae.

2. Materials and Methods

2.1. Tofu wastewater sampling

The samples of tofu wastewater were collected from a tofu home industry, whereas the tofu wastewater anaerobic digestion effluent (TW-ADE) was obtained from Biogas Plant, both located in Giriharja Village, Sumedang, Indonesia. Tofu wastewater contained 9,225 mg/L COD, and TW-ADE included 613.5 mg/L COD.
2.2. Cultivation media
Tofu wastewater media were prepared in 3 variations of concentration (3%, 5%, and 10%) by diluting the tofu wastewater using distilled water. The TW-ADE medium was used without dilution. Walne medium was used as a positive control, and a distilled water medium was used as a negative control. The composition of Walne medium [20] was presented in Table 1. All media were added with 1 g/L NaHCO₃ as an inorganic carbon source. pH medium was adjusted to 8.5. The medium was sterilized in an autoclave at 121 °C, 1 atm, 15 min. COD, nitrate, and phosphate concentration of media were presented in Table 2.

Table 1. Walne medium composition.

| Nutrients Stock Solution | Trace Elements Stock Solution | Vitamins Stock Solution |
|--------------------------|------------------------------|-------------------------|
| Materials                | Materials per liter          | Materials per liter     |
| NaNO₃                    | 100 g                        | ZnCl₂                   | 21 g                     |
| H₃BO₃                    | 33.6 g                       | CoCl₂.6H₂O              | 20 g                     |
| Na₂EDTA                  | 45 g                         | (NH₄)₆Mo₇O₂₄.4H₂O       | 9 g                      |
| NaH₂PO₄.2H₂O             | 20 g                         | CuSO₄.5H₂O              | 20 g                     |
| FeCl₃.6H₂O               | 1.3 g                        |                         |                          |
| MnCl₂.4H₂O               | 0.36 g                       |                         |                          |

Quantity of used stock solution per liter
Nutrients solution: 1 mL
Trace elements solution: 1 mL
Vitamins solution: 100 µL

Table 2. The concentration of COD, nitrate, and phosphate.

| Medium                      | COD | Phosphate | Nitrate |
|-----------------------------|-----|-----------|---------|
| 3 % tofu wastewater         | 374 | 0.2       | 13.7    |
| 5 % tofu wastewater         | 461 | 0.7       | 15.4    |
| 10 % tofu wastewater        | 716 | 1.9       | 19.2    |
| TW-ADE                      | 613.5 | 1.3      | 12.8    |
| Walne medium (positive control) | 441.5 | 17.7 | 83.9 |
| Distilled water (negative control) | 0 | 0 | 0 |

2.3. Microalgae cultivation
Chlorella vulgaris and Arthrospira platensis stock cultures (7,200 mg/L and 5,000 mg/L biomass in Walne broth medium) were obtained from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara, Indonesia. Each medium was inoculated with either C. vulgaris or A. platensis stock culture at 10% v/v. PET cylinders (1,000 L total volume, 750 L working volume) were used as reactors. The cultures were maintained at room temperature, under artificial illumination using Philip Tube Light 18W (photoperiod 12:12 h L/D cycle), and aerated using aquarium pump for 10 days. Optical Density (OD) was quantified every day using UV-VIS spectrophotometer Agilent Cary 60 at a wavelength of 680 nm then converted to microalgae biomass. Microalgae biomass was quantified with the gravimetric method. Protein was quantified according to the Lowry method [21]. Chlorophyll a, chlorophyll b, and total carotenoids content were quantified, according to Dere et al. [22].
2.4. Data analysis

Data were compared using ANOVA with Duncan post-hoc test. The p-value < 0.05 was regarded as significant. IBM SPSS 22 was used in the study.

3. Results and Discussions

The growth curve of both *C. vulgaris* and *A. platensis* was evaluated for 10 days (Fig.1). In all media except the TW-ADE, *C. vulgaris* growth was better than *A. platensis*. The log phase of *C. vulgaris* grown in the Walne medium (positive control) occurred between the 2nd and the 8th day. On the other hand, the log phase of *C. vulgaris* grown in the tofu wastewater media showed had started from the 1st day, but the increase of biomass was not significant until the 10th day. Biomass of *C. vulgaris* increased from 720 mg/L on the 1st day to 1,595 to 3,282 mg/L on the 10th day in all media except in the TW-ADE medium, which decreased from 720 mg/L to 671 mg/L (Fig. 1a). In the positive control, biomass increased significantly from the 1st to 10th day with a growth rate of 0.17 mg/L per day. The highest biomass concentration (2,015 mg/L) and growth rate (0.114 mg/L per day) were observed in 5 % tofu wastewater medium; however, the difference with the other tofu wastewater media and the negative control was not significant.

Biomass of *A. platensis* also increased from 500 mg/L on the 1st day to 818 – 1,715 mg/L on the 10th day in all media except TW-ADE, which decreased from 500 mg/L to 0 mg/L (Fig. 1b). In the positive control, the highest growth rate was 0.14 mg/L per day, and the highest biomass concentration of 1,924 mg/L was observed on the 8th day. Similar to *C. vulgaris*, the log phase for *A. platensis* grown in the three tofu wastewater media also started from the 1st day, but the increase of biomass was not significant until the 10th day. The highest biomass concentration (1,404 mg/L) and growth rate (0.10 mg/L per day) were observed in 3% tofu wastewater medium. The difference between the three tofu wastewater media, as well as with the negative control, was significant.

A research conducted by Widayat *et al.* [3] showed that *Chlorella* sp. had the highest growth rate in 10 % tofu wastewater compared with 30 %, 50 %, 70 %, 90 % tofu wastewater media. Those media were enriched with 30 mg/L nutrition consisted of NPK fertilizer, NaHCO₃, B1, and B12 vitamins [3]. Syaichurrozi and Jayanudin [17] cultivated *A. platensis* in 2, 4, 6, and 8 % tofu wastewater enriched with 1 g/L NaHCO₃ and 0.05 g/L urea. The highest growth rate was observed in 6% tofu wastewater. In this study, after ten days of incubation, the growth in the Walne medium (positive control) was significantly higher than all tofu wastewater media for both *C. vulgaris* and *A. platensis*. Phosphate and nitrate concentrations in tofu wastewater media were lower than in Walne medium (Table 2), which might contribute to the lack of growth in the tofu wastewater media.

This research showed that microalgae could not grow in the TW-ADE medium. It might be affected by turbidity, COD value, or nutrients content such as phosphate and nitrate content. Phosphate and nitrate concentrations of TW-ADE were lower than Walne medium, but in the same order of magnitude as tofu wastewater media (Table 1). Syaichurrozi and Jayanud [17] suggested that high COD concentration might inhibit *A. platensis* growth. The highest COD concentration that could support *A. platensis* growth was 767 mg/L. In this study, the COD concentration of TW-ADE was 613.5 mg/L. For further, chemical analysis for the characterization of TW-ADE is needed to optimize TW-ADE as a cultivation medium for microalgae.

Between the 1st and 5th day, chlorophylls content of *C. vulgaris* increased from 0.2 – 0.6 mg/L to 0.4 – 1.6 mg/L (Figure 2a). Likewise, carotenoids content increased from 9 – 19.5 mg/L to 28.3 – 71.7 mg/L. Between the 5th and 10th day, chlorophylls and carotenoids content increased for culture in Walne (positive control) and 5 % and 10 % tofu wastewater media. The highest chlorophylls and carotenoids content of *C. vulgaris* for 5th days incubation were in 10 % tofu wastewater medium (1.38 and 6.34 mg/L) while for 10th days incubation in 5 % tofu wastewater medium (3.19 and 201.69 mg/L). It showed that the higher biomass not always followed by the higher chlorophylls and carotenoids content due to the highest biomass of *C. vulgaris* found in Walne medium but chlorophylls and carotenoids content lower than in 5 % and 10 % tofu wastewater medium. There was a significant difference (p<0.05) in chlorophylls and carotenoids content of *C. vulgaris* in Walne, 5 %, and 10 %
tofu wastewater medium. Research conducted by Sari et al. [23] showed that *C. vulgaris* had the highest chlorophylls content in 35 % tofu wastewater than in 5 %, 15 %, and 25 % tofu wastewater.

![Growth Curve of *C. vulgaris*](image1.png)

![Growth Curve of *A. platensis*](image2.png)

**Figure 1.** Growth Curve of (a) *A. platensis* and (b) *C. vulgaris* in different media

Between the 1st and 5th day, chlorophylls content of *A. platensis* increased from 0.1 – 0.3 mg/L to 0.3 – 0.7 mg/L (Figure 2c). Likewise, carotenoids content increased from 4.8 – 13.0 mg/L to 16.0 – 35.2 mg/L (Figure 2d). Between the 5th and 10th day, chlorophylls and carotenoids content increased for culture in Walne (positive control) and tofu wastewater (3 %, 5 %, 10 %) media. *A. platensis* had the highest chlorophylls content in Walne medium for 5th and 10th days incubation (0.7 and 1.3 mg/L) while the highest carotenoid content was in 5 % tofu wastewater medium (1.16 mg/L) for 5th days incubation and in 10 % tofu wastewater medium (72.20 mg/L) for 10th days incubation. There was a significant difference (p<0.05) in chlorophylls content of *A. platensis* in Walne, 3 %, 5 %, and 10 % tofu wastewater medium. Carotenoid content of *A. platensis* in Walne, 3 % and 5 % tofu wastewater medium showed no significant difference (p>0.05), but in 10 % tofu wastewater medium showed significant difference (p<0.05).

Decreasing nitrogen to 50 % in the medium resulted in a reduction of biomass, lipid, protein, carotenoids, and phyocyanin productivities but decreasing 100 % phosphorus enhanced lipids and carotenoids productivities [24]. Various cultivation conditions and microalgal strain affect the pigments, lipid, protein, and carbohydrate composition. Changes in culture conditions, nutrients deficiency, physical modifies, and regimen growth are developed as strategies in microalgae cultures to increase compounds of interest [25].
Figure 2. Chlorophylls and carotenoids content of *C. vulgaris* and *A. platensis* in different media.
After ten days of incubation, the Walne medium as a positive control had the highest protein content of \textit{C. vulgaris} (272.83 mg/L) and \textit{A. platensis} (219.17 mg/L) (Fig. 3). There was a significant difference in protein content of both microalgae cultivated in Walne medium for 10th-day incubation to other media. The variation of tofu wastewater as cultivation medium of microalgae showed that the highest protein content of \textit{C. vulgaris} (135.83 mg/L) in 5 \% tofu wastewater medium and \textit{A. platensis} (42.50 mg/L) in 3 \% tofu wastewater medium. It was shown that higher biomass also followed by the higher protein content due to the highest biomass of \textit{C. vulgaris}, and \textit{A. platensis} was in those concentrations. The protein content of \textit{A. platensis} in 10 \% tofu wastewater and TW-ADE medium was not detected. It might be caused by the biomass of \textit{A.platensis} very low, so the protein content also very low.

![Figure 3. Protein Content of \textit{C. vulgaris} and \textit{A. platensis} in different media](image)

4. Conclusion
Microalgae can utilize nutrients content in the tofu wastewater medium for their growth, even though the growth was less than the positive control using Walne medium. The highest biomass and protein in the tofu wastewater medium was observed in 5 \% tofu wastewater for \textit{C. vulgaris} and 3 \% tofu wastewater for \textit{A. platensis}. Despite the lower biomass content compared with the Walne medium, the highest of chlorophylls and carotenoids content for both isolates was in the tofu wastewater medium. On the other hand, microalgae grown in TW-ADE had the lowest growth rate, chlorophylls, carotenoids, and protein content. Our observation on chlorophylls, carotenoids, and protein contents confirmed that cultivation conditions such as medium influence the biochemical composition of the biomass.

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