



## Effect of Tofu Wastewater Addition on the Growth and Carbohydrate-Protein-Lipid Content of *Spirulina platensis*

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### ABSTRACT

This study was conducted to investigate the effect of tofu wastewater (TW) addition to the growth medium on the growth of *Spirulina platensis*. The TW addition was varied in range of 0 – 8 v/v%. The results showed that the growth rate ( $\mu$ ) of *S. platensis* at TW addition of 0, 2, 4, 6, 8 v/v% was 0.007, 0.084, 0.074, 0.088, 0.086 mg/L.d, respectively. The medium with TW content of 6 v/v% (carbon:nitrogen:phosphorous=161:17:1; carbon/nitrogen=9.55) was the best medium for biomass production. The growth rate of *S. platensis* was successfully modeled using modified Gompertz equation ( $R^2 = 0.93-0.98$ ). In prediction through modified Gompertz, the maximum biomass production (2.17 mg/L) was resulted from medium IV (TW of 6 v/v%). Medium IV (TW of 6 v/v%), medium II (TW of 2 v/v%) and medium III (TW of 4 v/v%) resulted in biomass containing the highest amount of protein (66.62%), carbohydrate (61.23%) and lipid (19.66%), respectively.

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## 1. INTRODUCTION

Tofu is a traditional oriental food that is popular in Indonesia. It is produced from soybean as raw material through some processes. Production of tofu begins from washing out the soybeans and then soaking the beans in clean water for 6 hours. After that, the soybeans are grinded and mixed with water to obtain a slurry. Furthermore, the produced slurry is cooked and filtered to separate the liquid starch from its dregs. Moreover, acetic acid solution is added into the liquid starch where it forms clumps of tofu. Finally, the clump of tofu is pressed using press equipment giving out liquid as a wastewater and tofu as a product [1]. To product 80 kg tofu, a tofu industry generates 2610 kg wastewater. Currently, tofu wastewater (TW) from many tofu industries is not completely treated, so that it directly enters the environment and produces odor, greenhouse gasses (GHG) emission and pollution in water and soil [2, 3].

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Wastewater from tofu industry, which is as an organic waste, can be treated using biological method, such as microalgal cultivation. Chemical oxygen demand (COD), total nitrogen (TN) and phosphorus (P) that present in wastewater are utilized by microalgae as source nutrients to grow. Many authors investigated the potential of TW as nutrient source of microalgae. Rizkytata et al. [4] reported that *Chlorella vulgaris* grew well in 20-30% tofu waste medium. The most lipid content of the microalgae was resulted from 30% TW medium. Furthermore, Nugroho et al. [5] also cultivated *Chlorella vulgaris* to remove BOD, phosphate, ammonium and nitrate contained in TW. However, they did not investigate the carbohydrate-protein-lipid content in microalgae. Putnarubun et al. [6] stated that tofu waste could be used as cultivation medium at concentration of 1 mL/L saltwater for microalgae *Tetraselmis sp.*

Based on information above, utilization of TW as nutrient source for *Spirulina platensis* has not been conducted and reported by others yet. The microalgae *S. platensis* has superiorities. The biomass size of *S. platensis* is larger than others, so it is easy in harvesting.

It can live in extreme condition, which is in pH range of 8-11. Also, it can live in mixotrophic condition optimally. Cheunbarn and Peerapornpisal [7] found that *S. platensis* had the best growth rate in medium containing 10% digested swine wastewater and synthetic nutrient. Budiyo et al. [8] reported that *S. platensis* thrived in medium containing 0.8% digested vinasse and synthetic nutrient. Hadiyanto et al. [9] stated that *S. platensis* was cultivated successfully in medium containing remediated palm oil mill effluent (POME) and synthetic nutrient. Hence, in this study the authors focused on *S. platensis* cultivation under mixotrophic condition with combination of TW and synthetic nutrient. The objectives of this study were investigation of the growth rate and carbohydrate-protein-lipid content of *S. platensis*. Some authors stated that the microbial growth rate was strongly dependent to culture medium composition [10-13]. Hence, in this study, the microalgal growth rate was monitored until 20 days. Furthermore, the authors modeled the growth rate data obtained using modified Gompertz equation to predict the maximum biomass production.

## 2. METHODS

**2.1. *S. platensis* and Tofu Wastewater** *Spirulina platensis* was obtained from the collection of Balai Besar Pengembangan Budidaya Air Payau (BBPBAP) Jepara, Indonesia. It was a common species of *S. platensis*, so that there was no specific code number for the species. Culture of *S. platensis* that had OD<sub>680</sub> value ~ 0.6 was used as inoculum. Inoculum preparation was conducted in BBPBAP Jepara. The *Spirulina platensis* was cultivated in Walne medium with addition of B-12 vitamin. The light intensity of cultivation was 3000 Lux and the medium pH was adjusted to 9-11. TW was obtained from a tofu industry located in Serang city, Banten Province, Indonesia. The TW contained 7666.21 mg/L COD, 2874.83 mg/L total carbon, 179.68 mg/L total nitrogen and 33.43 mg/L P-PO<sub>4</sub> with pH level of 4.2.

**2.2. Experimental Set Up** The nutrient source consisted of organic nutrient (TW) and inorganic

nutrient (synthetic nutrient). For synthetic nutrient, the authors modified the nutrient proposed by Budiyo et al. [8]. In this study, the synthetic nutrient contained 1 g/L NaHCO<sub>3</sub> (purity 98%) and 0.05 g/L urea (46% N content). These values were adopted from study of Budiyo et al. [8]. In previous study, authors varied the TW concentration at 0, 10, 20, 30, 40, 50 v/v% in medium to determine the maximum concentration of TW that was tolerable for *S. platensis*. The results showed that, *S. platensis* just could live in 0 and 10 v/v% (the data was not published). Hence, in this study, TW was added at concentrations below 10 v/v% which were 0, 2, 4, 6 and 8 v/v%. The cultivation was conducted at room temperature (28–32 °C) and pressure of 1 atm. Artificial light as light source was obtained from tube light (TL) lamp 18 Watt with distance of ±15 cm. Initial pH of medium was adjusted to 9.0 using HCl 1 M or NaOH 1 M. *S. platensis* could grow well in pH of 8-11 [8].

**2.3. Experimental Design** Medium cultivation with total volume of 1 L was carried out in batch system in Erlenmeyer flask. TW was added into medium with variations of 0, 2, 4, 6, 8 v/v% medium. Inoculum of *S. platensis* as much as 10 v/v% was added into medium. All media contained synthetic nutrients: 1 g/L NaHCO<sub>3</sub> (purity 98%) and 0.05 g/L urea (46% N content). Initial pH of all media was adjusted to 9.0. Table 1 shows the amount of TW, tap water and inoculum in all media.

**2.4. Experimental Procedures** Cultivation was done in Erlenmeyer flask within 20 days. Optical density of the culture was measured using UV-vis spectrophotometer at λ 680 nm once in two day. This method was also applied by Budiyo et al. [8] and Hadiyanto et al. [9]. Value of medium pH was measured using pH meter once in two day. The obtained data were used to calculate the growth rate, growth curve and pH profile. Chen and Lee [14] reported that the data obtained for *S. platensis* cultivation determined by measuring OD at wavelength of 680 nm could be converted into biomass dry weight (W) using Equation (1). The growth rate of *S. platensis* was calculated using logarithmic phase (Equation (2)).

**TABLE 1.** Experimental design

Medium	Concentration of TW (v/v%)	Volume of TW (mL)	Tap water (mL)	Inoculum <i>S. platensis</i> (mL)	Total volume (mL)
I	0	0	900	100	1000
II	2	20	880	100	1000
III	4	40	860	100	1000
IV	6	60	840	100	1000
V	8	80	820	100	1000

Remarks: TW; Total volume = TW volume + tap water volume + inoculum volume

The doubling time was calculated using Equation (3).

$$W = 0.479 \times OD_{680 \text{ nm}} + 0.032 \quad (R^2 = 0.987) \quad (1)$$

$$\mu = \ln(W_i - W_0) / (t_i - t_0) \quad (2)$$

$$t_d = \ln(2) / \mu \quad (3)$$

Remarks :  $\mu$ , growth rate (mg/L.d);  $W_i$ , biomass concentration at  $t_i$  (mg/L);  $W_0$ , biomass concentration at  $t_0$  (mg/L);  $t_i$ , cultivation time  $i$  (day);  $t_0$ , cultivation time 0 (day);  $OD_{680 \text{ nm}}$ , optical density at 680 nm;  $t_d$ , doubling time (day).

After cultivation for 20 days, *S. platensis* biomass was filtered using 10 mm filter cloth, and broth was dried at 60 °C until the constant weight of biomass. Then, the composition of carbohydrate-protein-lipid was analyzed using proximate method.

## 2. 5. Kinetic Modeling of *S. platensis* Growth

The microbial growth rate can be modeled using Gompertz equation [15]. However, many authors did not study the microalgal growth rate modeling. Previously, Zwietering et al. [16] reported that predictive modeling of microorganism growth allowed the prediction of shelf life of products, detection of critical parts of the production and optimization of production. Zwietering et al. [16] used the modified Gompertz equation to model the growth of microorganism, which is written as Equation (4). The authors also used Equation (3) to model the growth rate of *S. platensis* in mixotrophic condition.

$$y = A \cdot \exp\{-\exp[(U \cdot e/A) \cdot (\lambda - t) + 1]\} \quad (4)$$

Remarks:  $y$ , biomass concentration at any time (mg/L);  $A$ , maximum biomass production (mg/L);  $U$ , maximum specific growth rate (mg/L.d);  $\lambda$ , lag time (days);  $e$ , mathematical constant (2.718) and  $t$ , time (days)

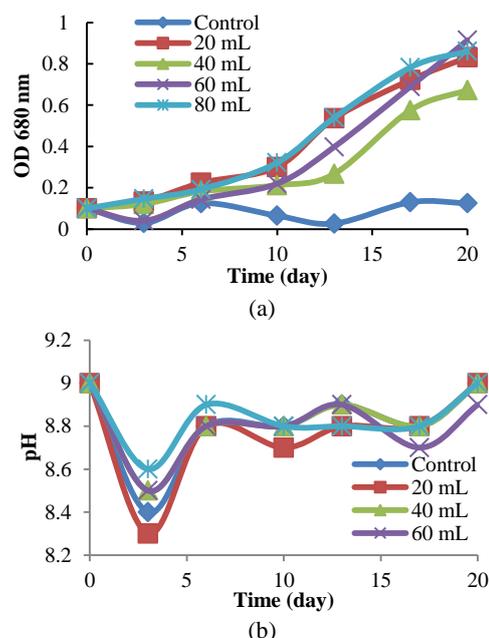
## 3. RESULTS AND DISCUSSION

### 3. 1. Growth and pH Profile

In previous study, *S. platensis* was cultivated in medium with variation of TW in the range of 0 – 50 v/v%. After cultivation, the results showed *S. platensis* could not grow in medium containing more than 10 v/v%. Use of high COD concentration in the medium caused dark color and high turbidity, so that the penetration of light into the medium was low. Thus, the photosynthetic rate of *S. platensis* was slow [7, 17]. The COD value in the media containing 0, 10, 20, 30, 40, 50 v/v% of TW was 0, 766.62, 1533.24, 2299.86, 3066.48, 3833.11 mg/L. *S. platensis* was tolerant to COD concentration below 766.62 mg/L. Whereas, Travieso et al. [18] stated that the growth of microalgae was hampered at COD

concentration (settled piggery wastewater) above 242 mg/L. Thus, the difference of tolerance level to COD for *S. platensis* was caused by the kind of waste added into the medium. The settled piggery wastewater might be darker than TW, so that the light penetrated easier into the medium of TW than into medium of settled piggery wastewater.

In control medium (medium I), *S. platensis* could grow very slowly until end of cultivation with the final  $OD_{680}$  of 0.12. While in media II to V, *S. platensis* biomass reached the highest  $OD_{680}$  value of 0.83, 0.67, 0.91, 0.86, respectively (Figure 1(a)). The higher the  $OD_{680}$ , the more biomass was obtained [9]. *S. platensis* cultured in mixotrophic condition (natrium bicarbonate and TW) produced higher biomass than it in photoautotrophic condition (natrium bicarbonate). Chainapong et al. [19] stated that *S. platensis* grew well with combination of bicarbonate and acetate compared with bicarbonate only. On the other hand, too high concentration of acetate could inhibit the microalgal growth [20]. Generally, microalgae growth by assimilating acetate has to possess a glyoxylate cycle pathway to efficiently incorporate acetyl groups of acetyl-CoA to carbon skeletons. The operation of the glyoxylate cycle requires synthesis of enzymes such as isocitrate lyase (EC 4.1.3.1) and malate synthetase (EC 2.3.3.9). The two enzymes were induced when cells were transferred to medium containing acetate [20]. In tofu production process, acetic acid solution is added into the liquid starch to form clumps of tofu [1].



**Figure 1.** The effect of TW addition on (a) growth profile and (b) pH profile of *S. platensis*

Hence, TW contained acetic acid in large amount. That could be proved with the low pH value of TW, which was 4.2. Addition of TW in amount of 2 to 6 v/v% supplied the acetic acid in appropriate amount. However, at TW addition of 8 v/v%, the acetic acid presented in the medium with high concentration. Thus, biomass production from medium V was less than that from medium IV.

The least of OD<sub>680</sub> value in medium I (control) was also caused by phosphorus nutrient. *S. platensis* needed nutrient to grow, but control medium did not supply the nutrient. Phosphorus (P) was a macro-nutrient required in cellular metabolic processes to form various structures and functions of components (cell, nucleotides, nucleic acids), which were required for the growth and development of microalgae. On the other hand, in media II-V, the microalgae could grow well because the phosphorus was supplied by TW added in the medium (Table 2).

In media II-V, pH decreased at the beginning of cultivation from 9 to 8.3-8.5 and then went up (Figure 1(b)). This phenomenon was caused by activity of oxidative bacteria contained in the wastewater [8]. When higher concentration of TW was added, more amount of these bacteria were contained in the medium. At the beginning of cultivation, the oxidative bacteria converted organic matter of TW into CO<sub>2</sub> via respiration process. The formed CO<sub>2</sub> reacted with water to form carbonate, so that the medium to be acidic. The carbonate was used by *S. platensis* for photosynthetic process and released OH<sup>-</sup>, so medium pH gradually increased. Besides, activity of bacterial oxidation might be participated in the system, *S. platensis* also utilized organic carbon as source of carbon to produce CO<sub>2</sub> through respiration (heterotrophic condition). Carbon dioxide formed caused acidic condition in medium. Furthermore, *S. platensis* used CO<sub>2</sub> for photosynthesis (photoautotrophic condition). Because of photoautotrophic growth, medium pH gradually increased. Andrade and Costa [21] reported that the higher the rate of photosynthetic activity, the higher was the medium pH. Photoautotrophic and heterotrophic processes taken place simultaneously in cell is called mixotrophic growth [22].

In control medium, medium pH also decreased at first (from 9 to 8.6). This was caused by the presence of synthetic nutrient in medium. Sodium bicarbonate (NaHCO<sub>3</sub>) was dissolved into Na<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> (ion bicarbonate). The HCO<sub>3</sub><sup>-</sup> was carbon source for *S. platensis*, but control medium did not contain phosphorus nutrient. *S. platensis* needed phosphorus for photosynthesis activity. Thus, the HCO<sub>3</sub><sup>-</sup> was not used directly by *S. platensis* and was accumulated in the system, so that the medium pH decreased. Furthermore, Na<sup>+</sup> was used as micro nutrient by *S. platensis*, while HCO<sub>3</sub><sup>-</sup> was converted to CO<sub>2</sub> and OH<sup>-</sup> with the help of enzyme carbonic anhydrase [8]. The formed CO<sub>2</sub> was utilized as carbon source in photosynthesis process. Ion OH<sup>-</sup> accumulated in the medium caused an increase in medium pH [23].

**3. 2. Growth Rate of *S. platensis*** The final *S. platensis* biomass density at TW concentrations of 0–8 v/v% was 0.09, 0.43, 0.35, 0.47, 0.45 mg/L, respectively (Table 2). The microalgae in control medium did not receive phosphorus nutrient, so that *S. platensis* could not grow well. Besides that, organic compounds such as soluble sugars, amino acids and various mineral elements contained in wastewater could enhance biomass productivities [24]. Therefore, *S. platensis* thrived in media II-V and produced higher biomass compared to control medium (medium I). The growth rate ( $\mu$ ) of *S. platensis* on each medium can be seen in Table 2. The higher the biomass value, the higher was the growth rate of microalgae. Media I–V had  $\mu$  values of 0.007, 0.084, 0.074, 0.088, 0.086 mg/L.d, respectively. These results showed that the best growth rate of *S. platensis* was 0.088 mg/L.d, which was in medium IV (TW addition of 6 v/v%). The C/N ratio of medium IV was 9.55 (Table 3). Budiyo et al. [8] reported a C:N:P= 60.5:6.2:2.1 (C/N = 9.75) for mixotrophic medium.

The doubling time ( $t_d$ ) in control medium was very long, which was 100.634 days. It means medium I (control medium) was not suitable to cultivate *S. platensis*. Meanwhile, media II-V were good for the microalgae, because they just needed 7-9 days to generate biomass in double amount (Table 2).

**TABLE 2.** The effect of various TW additions on maximum OD, biomass, maximum growth rate and doubling time

Medium	TW addition (v/v%)	TW volume (mL)	COD (mg/L)	Final OD <sub>680</sub>	Biomass (mg/L)	$\mu$ (mg/L.d)	$t_d$ (days)
I	0	0	0	0.12	0.09	0.007	100.634
II	2	20	153.32	0.83	0.43	0.084	8.25
III	4	40	306.65	0.67	0.35	0.074	9.33
IV	6	60	459.97	0.91	0.47	0.088	7.84
V	8	80	613.30	0.86	0.45	0.086	8.09

Remarks: TW, Tofu wastewater; COD, Chemical Oxygen Demand; OD, Optical Density;  $\mu$ , growth rate;  $t_d$ , doubling time

**TABLE 3.** The composition of C: N: P in 1 L growth medium

Medium code	TW addition (v/v%)	Nutrient in TW			Synthetic nutrient			Total nutrient			Ratio			
		C <sup>1</sup> (mg)	N <sup>1</sup> (mg)	P <sup>1</sup> (mg)	C <sup>2</sup> (mg)	N <sup>2</sup> (mg)	C <sup>3</sup> (mg)	N <sup>3</sup> (mg)	P <sup>3</sup> (mg)	C/N	C/P	N/P	C:N:P	
I	0	0	0	0	150	23	150	23	0	6.52	-	-	-	
II	2	57.50	3.59	0.67	150	23	207.50	26.59	0.67	7.80	310.34	39.77	310:40:1	
III	4	114.99	7.189	1.34	150	23	264.99	30.19	1.34	8.78	198.17	22.57]	198:23:1	
IV	6	172.49	10.78	2.00	150	23	322.49	33.78	2.00	9.55	160.78	16.84	161:17:1	
V	8	229.99	14.37	2.67	150	23	379.99	37.37	2.67	10.17	142.08	13.97	142:14:1	

Remarks: TW, tofu waste; C, carbon; N, nitrogen; P, phosphorous

C<sup>1</sup> = (BM C/BM O<sub>2</sub>) × COD contained in TW; N<sup>1</sup> = total nitrogen contained in TW; P<sup>1</sup> = P-PO<sub>4</sub> contained in TW

C<sup>2</sup> = [(BM C/BM NaHCO<sub>3</sub>) × g NaHCO<sub>3</sub> × 98% (purity)] + [(BM C/BM urea) × g urea]; N<sup>2</sup> = (BM N/BM urea) × g urea

C<sup>3</sup> = C<sup>1</sup> + C<sup>2</sup>; N<sup>3</sup> = N<sup>1</sup> + N<sup>2</sup>; P<sup>3</sup> = P<sup>1</sup>

C/N = C<sup>3</sup>/N<sup>3</sup>; C/P = C<sup>3</sup>/P<sup>3</sup>; N/P = N<sup>3</sup>/P<sup>3</sup>; C:N:P = C<sup>3</sup>:N<sup>3</sup>:P<sup>3</sup>

This proved that the presence of phosphorus nutrient was important for *S. platensis* growth. Chainapong et al. [19] found that *S. platensis* had the best t<sub>4</sub> value when the microalgae cultivated in mixotrophic medium (NaHCO<sub>3</sub> and sodium acetate), which was 9.24-11.62 days. Hence, mixotrophic medium (NaHCO<sub>3</sub> and TW) in this study was better for *S. platensis* activity than mexorophic medium proposed by Chainapong et al. [19].

Results in Table 4 showed that the difference of nutrient source in medium resulted in different final ODs and growth rates of *S. platensis*. Other authors reported that *S. platensis* could be cultivated in mixotrophic condition with combination of synthetic and organic nutrient. According to Table 4, the results of this study were better than study of Andrade and Costa [21]. In that study, the growth rate value was just 0.064 g/L. Whereas, the best growth rate in this study was 0.088 g/L. That might be caused by the characteristic of molasses that was dark and had too

high COD content. However, the other studies showed that the growth rate (0.134-220 g/L) was better than this study. That might be caused by the difference of C:N:P ratio in medium. The C/N ratio in this study (9.47) was similar to other studies (7.6-9.76), but the C/P and N/P ratios in this study were too high. That means the medium still contained too little amount of phosphorous nutrient, although the medium was the best in this study.

**3. 3. Kinetic Analysis** In this section, the growth rate of *S. platensis* modeled by using modified Gompertz equation successfully (R<sup>2</sup>=0.93–0.98). Kinetic parameters (A, U, λ) obtained are shown in Table 5. The measured and predicted biomass production are plotted in Figure 2. According to Table 5, medium contained TW of 2–8 v/v% which resulted in maximum biomass (A) of 1.97, 2.04, 2.17 and 1.85 mg/L in prediction.

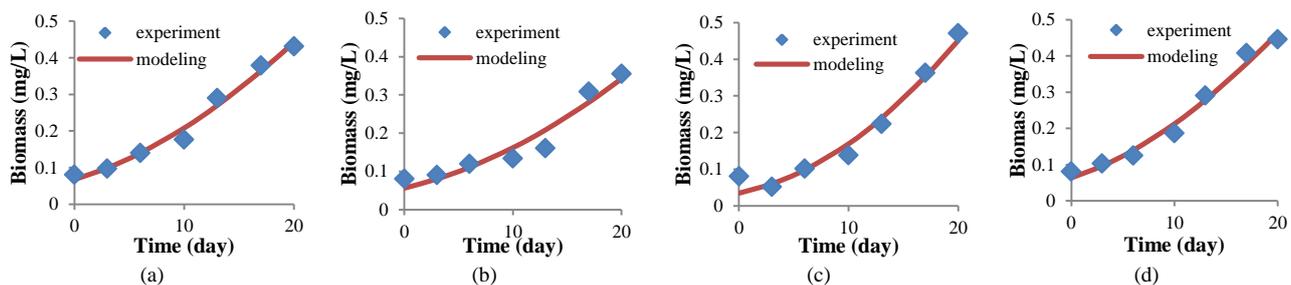
**TABLE 4.** Comparison of study for *S. platensis* cultivation at mixotrophic condition

Microalgae	Synthetic nutrients	Wastewater	Final OD	μ (g/L)	C:N:P	C/N	C/P	N/P	Ref
<i>S. platensis</i>	100% (1 g/L NaHCO <sub>3</sub> , 0.05 g/L urea, 10 ppm TSP)	Digested vinasse 0.8 %	1.070	0.143	64.6:7.7:1	8.39	64.6	7.7	[8]
<i>S. platensis</i>	50% (1 g/L NaHCO <sub>3</sub> , 0.05 g/L urea, 10 ppm TSP)	Digested vinasse 0.8 %	0.780	0.220	60.5:6.2:1	9.76	60.5	6.2	[8]
<i>S. platensis</i>	-	POME 10 %	-	0.134	-	-	-	-	[25]
<i>S. platensis</i>	50% (Modified Bangladesh No.3)	POME 20 %	-	0.142	55.7:7.3:1	7.6	55.7	7.3	[17]
<i>S. platensis</i>	8 g/L NaHCO <sub>3</sub> , 1.5 g/L NaNO <sub>3</sub>	Digested swine wastewater 10%	1.09	-	-	-	-	-	[7]
<i>S. platensis</i>	Zarrouk medium	Molasses 0.75 g/L	-	0.064	-	-	-	-	[21]
<i>S. platensis</i>	20% (Modified Bangladesh No.3)	Coconut milk skim effluent 20 %	-	0.179	-	-	-	-	[26]
<i>S. platensis</i>	1 g/L NaHCO <sub>3</sub> and 0.05 g/L urea	TW 6%	0.91	0.088	161:17:1	9.47	161	17	This study

**TABLE 5.** Estimated parameters of modified Gompertz equation

Kinetics parameters	Media			
	II TW 2 v/v%	III TW 4 v/v%	IV TW 6 v/v%	V TW 8 v/v%
A (mg/L)	1.97	2.04	2.17	1.85
U (mg/L.d)	0.03	0.03	0.04	0.03
$\lambda$ (day)	5.36	7.99	8.71	4.86
R <sup>2</sup>	0.98	0.93	0.97	0.98
RMSD	0.01	0.01	0.01	0.01

Remarks: A, biomass production potential; U, the maximum biomass production rate;  $\lambda$ , lag phase period or minimum time to produce biomass; R<sup>2</sup>, correlation coefficient; RMSD, Root Mean Square Deviation



**Figure 2.** Plotting between measured and predicted biomass production value obtained from modified Gompertz equation. (a) TW 2 v/v%, (b) TW 4 v/v%, (c) TW 6 v/v%, (d) TW 8 v/v%

The A value increased with the increasing of TW addition from 2 to 6 v/v%. Furthermore, on TW addition of more than 8 v/v%, the A value decreased. In medium IV (TW 6 v/v%), the C:N:P ratio of 161:17:1 was good for *S. platensis* activities (Table 3).

The more A value was obtained, the more U value was resulted (Table 5). The *S. platensis* had the best growth rate per day (U) in medium IV. Photosynthesis activity was done well with nutrient composition of C:N:P = 161:17:1 by *S. platensis*. The  $\lambda$  value indicated how long the *S. platensis* need to adapt to the medium condition. The more the kinetic parameter of  $\lambda$  value, the more the lag time (adaptation time) was needed by the microalgae. The increasing of TW addition from 2 to 6 v/v% the lag time was increased from 5.36 to 8.71 days. Meanwhile at TW addition of 8 v/v%, *S. platensis* had lag time that was less than TW concentration of 6 v/v%.

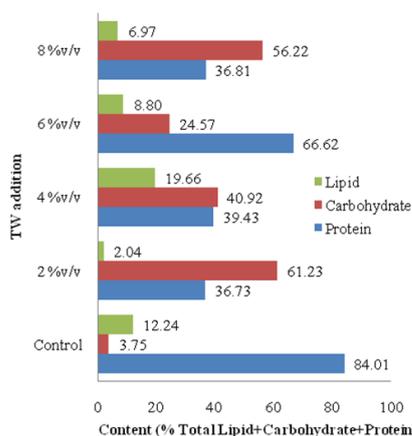
### 3. 4. Carbohydrate, Protein, Lipid Content of *S. platensis*

The presence of TW in medium could change the carbohydrate, protein and lipid content in *S. platensis* biomass. The protein content of *S. platensis* biomass increased from 36.73 to 66.62% at TW addition from 2 to 6 v/v%. This phenomenon was caused by increasing of nitrogen concentration from 26.59 to 33.78 mg/L (Table 3). Uslu et al. [27] stated that the more the presence of nitrogen source, the more the *S. platensis* biomass contained protein. On the other hand, at TW addition more than 6 v/v% (TW addition of 8 v/v%), protein content decreased (36.81%). At TW

addition of 8 v/v%, medium V contained carbon content of 379.99 mg/L and nitrogen of 37.37 mg/L. The nitrogen concentration in medium V might be too high, so that the *S. platensis* growth was hampered (Figure 1 (a)) and the protein content was low (Figure 3). On the other hand, the high carbon concentration in medium V led to high carbohydrate content. Markou [28] stated that the high carbon concentration increased carbohydrate and decreased protein content of *S. platensis*. Thus, the best medium to get high protein content in *S. platensis* biomass was medium with TW addition of 6 v/v%.

With increase of TW concentration from 2 to 6 v/v%, carbohydrate content decreased, then it increased at concentrations above 6 v/v%. The profile of carbohydrate content had contradict with protein content in *S. platensis* biomass (Figure 3). This profile was similar to the report of Markou [28], where the variation of C/N could increase carbohydrate content but decreased protein content, and vice versa. Thus, the best medium to increase carbohydrate content in the *S. platensis* biomass was medium with TW addition of 2 v/v%.

At TW addition of 2 until 4 v/v%, the lipid content increased from 2.04 to 19.66%. Furthermore, with TW addition of 6 and 8 v/v%, the lipid content decreased to 6.97%. In this study, the profile of protein content was similar with lipid content (Figure 3). However, Markou [28] reported that increase of lipid and carbohydrate of *S. platensis* biomass was followed by decreasing of protein.



**Figure 3.** The effect of various media on carbohydrate-protein-lipid content of *S. platensis*

Moreover, Markou et al. [29] found that the lipid content could not be related to carbohydrate or protein content, but it had correlation with phosphorus concentration in medium. In this study, the best phosphorous concentration was 1.34 mg/L to get high lipid content in biomass. Therefore, the best medium to obtain high lipid content of *S. platensis* was medium with TW addition of 4 v/v% .

#### 4. CONCLUSION

The final biomass and growth rate of *S. platensis* at TW addition of 0 to 8 v/v% was 0.09, 0.43, 0.35, 0.47, 0.45 mg/L and 0.007, 0.084, 0.074, 0.088, 0.086 mg/L.d, respectively. The growth rate of *S. platensis* modeled by using modified Gompertz equation ( $R^2=0.93$  to 0.98). Medium IV (C:N:P =161:17:1) was good for biomass production. The highest carbohydrate content of biomass (61.23%) was obtained from medium II (C:N:P = 310:40:1). Meanwhile, the highest protein content of biomass (66.62%) was resulted in medium IV (C:N:P=161:17:1). Furthermore, the best medium to get high lipid content (19.66%) was medium III (C:N:P=198:23:1).

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## Effect of Tofu Wastewater Addition on the Growth and Carbohydrate-Protein-Lipid Content of *Spirulina platensis* RESEARCH NOTE

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این مطالعه به منظور بررسی تأثیر پساب توفو (TW) بر محیط رشد اسپیرولینا پلتنزیس صورت گرفت. افزودن TW در محدوده ۰ تا ۸ درصد وزنی/وزنی متغیر بود. نتایج نشان داد که سرعت رشد (μ) پلتنزیس در ۰، ۲، ۴، ۶، ۸٪ به ترتیب ۰،۰۰۷، ۰،۰۸۴، ۰،۰۷۴، ۰،۰۸۸، ۰،۰۸۶ میلی گرم بر لیتر در روز بود. محیط با محتوای پساب ۶٪ (کربن: نیتروژن: فسفر = ۱:۱۶:۱۷؛ کربن/نیتروژن = ۹،۵۵) بهترین محیط تولید بیومس بود. نرخ رشد پلتنزیس با استفاده از معادله گمپرتز اصلاح شده (R<sup>2</sup> = ۰/۹۳-۰/۹۸) با موفقیت مدل سازی شد. در پیش بینی از طریق گمپرتز اصلاح شده، حداکثر تولید بیومس (۲،۱۷ میلی گرم بر لیتر) از محیط حاوی ۶٪ پساب به دست آمد. محیط کشت شماره ۴ (حاوی پساب ۶٪)، محیط کشت شماره ۲ (حاوی پساب ۲٪) و محیط کشت شماره ۳ (حاوی پساب ۴٪) به ترتیب منجر به تولید بیومس حاوی بیشترین مقدار پروتئین (۶۶،۶۲٪)، کربوهیدرات (۶۱،۲۳٪) و لیپید (۱۹،۶۶٪) شدند.

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