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Effect of synthetic nutrient concentrations in medium containing tofu wastewater on *Spirulina platensis* biomass production

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Abstract

Microalgae cultivation employing a combination of organic nutrients from tofu wastewater (TW) and synthetic nutrients is an intriguing and potentially lucrative approach for concurrent biomass production and wastewater treatment. The optimization of the synthetic nutrient concentrations in mediums containing TW for *Spirulina platensis* cultivation was studied to reduce future operating costs on a commercial scale. The microalgae were cultivated in mediums with TW of 6%v/v and various synthetic nutrient concentrations which were 0, 25, 50, 75, and 100% of complete synthetic nutrients (CSN) consisting of $NaHCO_3$ of 1 g/L and urea of 0.05 g/L. The results showed that the optimal biomass production with a high protein content was obtained from a medium with TW of 6%v/v and 75% of CSN, having C/N and N/P ratios of 10.83 and 11.82, respectively. The biomass productivity and specific growth rate of microalgae in the medium were 8.55 mg/L/day and 0.089 day^{-1} with a doubling time of 7.80 days. The protein content as the main valuable biochemical in the microalgae was 67.38% dry cell weight. In the medium, the shape of *S. platensis* looked helix without any defects. In conclusion, about 25% of the synthetic nutrient need can be reduced to get the optimal results in biomass and protein production of *S. platensis*. This study contributes to informing the optimal synthetic nutrient concentration (which is 75% of CSN) in an *S. platensis* cultivation medium containing TW of 6%v/v, hence it can reduce the operating costs for commercial purposes in the future.

Keywords: Biomass production, Cultivation, Microalgae, Protein production, *Spirulina platensis*, Synthetic nutrients, Tofu wastewater

1. Introduction

Microalgae have the potential as an important source of biomass since they contain valuable biochemicals such as lipids, carbohydrates, protein, pigments, and antioxidants [1,2]. Some authors have conducted substantial studies on the microalgae field for a variety of objectives, including microalgae cultivation for functional foods or animal feed and wastewater remediation [3,4]. *Spirulina platensis* has many superiorities over the other microalgae, including the following: (i) its biomass size is bigger (more than 20 μm), so it is easy in harvesting just using a simple filter with a pore size of 20 μm (Desmorieux and Decaen 2005) (ii) it can live in extreme conditions with a pH range of 8-11 [6], a salinity up to 70 g/L [7], a temperature of 20-40°C [8], and (iii) it contains a valuable protein that is useful for the human body [6]. *S. platensis* is cultivated on a commercial scale around the world as a food or feed source. *S. platensis* is often utilized as a functional food or single-cell protein for humans because of its high protein content, which ranges between 60 - 70% of cell dry weight (CDW) [6,7].

The *S. platensis* specific growth rate, biomass productivity, doubling time, and chemical composition (carbohydrate, protein, and lipid) depend on the nutrients in the cultivation medium [9]. The combination of natural (organic) and synthetic (inorganic) nutrients for enhancing the microalgae growth has gained considerable interest, because (i) it can produce the biomass yield nearly identical to those obtained when cultivating exclusively with synthetic nutrients, (ii) it can reduce operating costs, and (iii) it can treat wastewater before it is discharged directly into the water bodies [9,10].

Some authors have studied the potency of some wastewaters as a growing medium for microalgae, which were palm oil mill effluent (POME) [11-13], digested POME [14], digested vinasse [6], coconut milk skim effluent [15], tofu wastewater [9,16]. The summary of these studies is shown in the (Table 1).

Table 1 Utilization of the wastewaters as organic nutrients for microalgae.

Wastewater	Microalgae	Results	References
POME	*Chlamydomonas incerta*	*Chlamydomonas incerta* can grow well in a medium with POME concentration of 250 mg/L and can consume about 67.35% of COD	[11]
POME	*Chlorella sorokiniana*	*Chlorella sorokiniana* can grow well in a medium with POME concentration of 250 mg/L resulting in the specific growth rate and biomass productivity of 0.099 day^{-1} and 8.0 mg/L/day, respectively	[12]
POME	*Chlorella pyrenoidosa*	The optimum growth rate is obtained when the POME is used as a medium with an initial COD concentration about 700 mg/L	[13]
Digested POME	*S. platensis*	The utilization of digested POME of 20%v/v can reduce the synthetic nutrient need up to 50% (from Modified Bangladesh No.3)	[14]
Digested vinasse	*S. platensis*	The optimum condition is a combination of the digested vinasse of 0.8%v/v and synthetic nutrients of 50% (from nutrients of 1 g/L $NaHCO_3$, 0.05 g/L urea, 10 mg/L TSP)	[6]
Coconut milk skim effluent	*S. platensis*	The utilization of coconut milk skim effluent of 20% can reduce the synthetic nutrient need up to 80% (from Modified Bangladesh No.3)	[15]
Tofu wastewater	*Chlorella vulgaris*	The optimum growth rate is obtained when a cultivation medium consists of POME volume of 20-30%.	[16]
Tofu wastewater	*S. platensis*	*S. platensis* can grow well at tofu wastewater (TW) concentration of 6% v/v with synthetic nutrients of 100% (from 1 g/L $NaHCO_3$ (purity 98%) and 0.05 g/L urea (46% N content))	[9]

For a purpose of food or feed source, the current study focused on the *S. platensis* cultivation. Based on the (Table 1), *S. platensis* has been cultivated in mediums containing wastewaters of digested POME [14], digested vinasse [6], coconut milk skim effluent [15], and tofu wastewater [9]. Of all the wastewaters, the most potential waste used as an organic nutrient source for enhancing the biomass production and protein content of the *S. platensis* is tofu wastewater due to its high nitrogen content. Based on our literature studies, the utilization of tofu wastewater for *S. platensis* cultivation medium is still limited. Based on the (Table 1), in our previous study [9], we found that *S. platensis* could grow at TW concentrations ranging from 2 to 8%v/v, with the optimal TW concentration of 6%v/v resulting in the highest biomass production. However, the previous study did not investigate the possible drop in the synthetic nutrient need when the TW was used as an organic nutrient source. Thus, the possible reduction of the synthetic nutrients by combining with the optimal TW concentration of 6%v/v should be explored to find the best medium resulting in the high biomass production and protein content of *S. platensis* and then to lower the future production costs.

In producing 80 kg of tofu, the tofu factories result in 2610 kg of TW [17]. Nowadays, in developing countries like Indonesia, TW from many tofu factories has not been thoroughly treated since the companies have not yet discovered effective and efficient treatment procedures, so the waste has a negative influence on the environment when it reaches the river body directly [17]. Despite the fact that, according to [16], the TW contain a variety of nutrients that can be used to replace the synthetic nutrient needed in microalgae cultivation

The modified Gompertz model is a growth model that is used seldom to determine the growth pattern of microalgae. This model evolved from the Gompertz model, which was initially used to predict the growth rate of microorganisms or microbes. At first, the Gompertz model depicted a sigmoidal curve and included three kinetic parameters that had no biological meaning. As a solution, Zwietering et al. modified the model by substituting the kinetic parameters with three new kinetic parameters which were had biological meaning [18].

Based on the information above, this study aimed to examine the potency in reducing the synthetic nutrients for *S. platensis* cultivation medium containing TW of 6%v/v and the effect of those conditions on the growth profile, pH profile, specific growth rate, biomass productivity, doubling time, biochemical components such as protein and lipid of *S. platensis* biomass, and shape of *S. platensis*. A modified Gompertz model was used to simulate and forecast the maximum biomass concentration and biomass productivity of *S. platensis*. For 18 days, the microalgae culture and medium pH were examined. The final biomass was evaluated to determine the chemical compositions with the greatest protein content as a potential target for optimization since *S. platensis* has the potential to be used as a single-cell protein in the future.

This study was conducted to reduce the use of synthetic nutrients in microalgae cultivation so that if microalga cultivation is conducted on a commercial scale, it will not require a substantial amount of synthetic nutrients because they have been replaced with natural nutrients derived from the tofu wastewater. This study has never been conducted previously, hence its inclusion in this paper is an academic novelty.

2. Materials and methods

Materials and methods which were used in this study will be explained in detail in the sub-sections below.

2.1 Materials

S. platensis culture was taken from the collection of Balai Besar Pengembangan Budidaya Air Payau (Center for Brackish Water Aquaculture Development) in Jepara Regency, Indonesia. Tofu wastewater was collected from a tofu production plant in Serang, Banten Province, Indonesia. To keep the wastewater fresh, the TW was stored in a 25 L container without the addition of continual aeration or filtration. The TW contained chemical oxygen demand (COD) of 7666.21 mg/L, Total Carbon of 2874.83 mg/L, Total Nitrogen of 179.68 mg/L, P-PO_4 of 33.43 mg/L, pH level of 4.2 [9]. The chemicals of $NaHCO_3$ (purity of 98%), urea (46% N content), and NaOH (technical grade) were commercial-grade chemicals purchased from local chemical markets in Jakarta and Cilegon City, Indonesia.

2.2 Microalgae cultivation set-up

The initial concentration of *S. platensis* culture was equal to the absorbance value at a wavelength of 680 nm (OD_{680}) of 0.1. The microalgae culture was maintained in 1 L conical flasks with 1 L working volume which consisted of inoculum of 100 mL, TW of 60 mL (6%v/v of total volume) as the optimum concentration of organic nutrients based on a previous study [9], and tap water of 840 mL. Into the 1 L total volume, 0, 25, 50, 75, and 100% of complete synthetic nutrients (CSN) was added, in which the CSN consisted of $NaHCO_3$ of 1 g/L and urea of 0.05 g/L. Cultivation was conducted under a temperature of 28-32°C with a photosynthetic photon flux density of 40.5 μmol/m^2/s from a tube light lamp was placed at a distance of 15 cm from the conical flasks under dark/light cycle of 0:24. The pH of the microalgae culture was first adjusted to 9.0 by adding the NaOH (technical grade). Each culture was grown for 18 days while homogenized using an aerator. In this study, the experiments were conducted with no replication.

2.3 Microalgae biomass analysis

The microalgal growth was observed daily using a ultra violet -visible (UV-Vis) spectrophotometer (Spectroquant® Prove 600; New York, USA) at 680 nm. Biomass was estimated using an equation used by a study of [19] which converted the OD_{680} to the biomass of microalgae *S. platensis* (Equation (1)), following:

$$W = (0.5273 \times OD_{680} - 0.0138) \times 1000 \qquad (R^2{=}0.9982) \tag{1}$$

where W is the biomass concentration (mg/L) and optical density $(OD)_{680}$ is the absorbance of microalgae culture at 680 nm. The absolute growth rate (AGR) or biomass productivity of *S. platensis* was calculated using the Equation (2), following:

$$AGR = \frac{W_2 - W_1}{t_2 - t_1} \tag{2}$$

where AGR is the absolute growth rate (mg/L/day), and W and t are the biomass concentration (mg/L) and time (days) during the cultivation, respectively. This was also important to measure the specific growth rate or growth rate constant [20], following the Equation (3):

$$\mu = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \tag{3}$$

where μ is the specific growth rate (day^{-1}), and W and t are the biomass concentration (mg/L) and time (days) during the cultivation as explained before. Doubling time is also one important parameter which should be known in the microalgae cultivation. The doubling time was calculated using the Equation (4):

$$T_d = \frac{\ln 2}{\mu} \tag{4}$$

where T_d is the doubling time (days) and μ is the specific growth rate (day^{-1}). Another important parameter to study was maximum biomass productivity [21]. The maximum biomass productivity was equal to the value of AGP_{max} obtained from the modified Gompertz model (section 2.4).

2.4 Kinetic Model of S. platensis growth

It is critical to do simple modelling to determine the robustness of data in microalgae research. One of the simple models is the modified Gompertz model published by [18], who found that predictive modelling of microbe growth enabled the prediction of product shelf life, the identification of important manufacturing components, and production optimization. By adapting the modified Gompertz model to describe a microbe development, the equation (5) presented the modified Gompertz model used to simulate the growth of *S. platensis* under mixotrophic conditions in this study.

$$W_t = W_{max} . \exp\left\{-\exp\left[\frac{AGR_{max}.e}{W_{max}}(\lambda - t) + 1\right]\right\} \quad (5)$$

where W_t is the biomass concentration after t days (mg/L), W_{max} is the maximum biomass concentration achieved (mg/L), AGR_{max} is the maximum absolute growth rate or maximum biomass productivity (mg/L/day), λ is lag time (days), and e is the mathematical constant (value = 2.718282)

2.5 Biochemical

Total protein (as a percentage of cell dry weight) was determined using the Kjeldahl total nitrogen technique and a conversion factor of 6.25 for total nitrogen to protein [22]. The total lipid concentration (as a percentage of cell dry weight) was determined using the Soxhlet extraction technique [23].

2.6 Morphology characterization of S. platensis

A microscopy approach using a high-quality digital microscope Leica ICC50 (Wetzlar, Germany) was employed to explore the morphology of *S. platensis*. Through the utilization of reflecting light, a lens was employed to produce high-resolution pictures at 400x magnification.

3. Results and discussion

3.1 Medium characterization

The mediums were examined for the presence of several nutrients necessary for microalgae development, including carbon, nitrogen, and phosphorus. Total carbon, nitrogen, and phosphorus were acquired from a variety of synthetic nutrient sources such as $NaHCO_3$ and urea, as well as from an organic nutrient source which was TW of 6% v/v. As indicated in the (Table 2), the difference in the combination of organic and synthetic nutrients in the mediums resulted in different C:N:P ratios, which affected the microalgae development. Microalgae cannot grow optimally if the nutritional composition is unbalanced, which is produced by disruption of metabolic processes inside the microalgae cells as a result of a nutrient deficit or excess. From the (Table 2), mediums containing TW of 6% v/v with 0, 25, 50, 75, and 100% of CSN had C:N:P weight ratios of 86:5.37:1, 100.02:7.52:1, 114.04:9.67:1, 128.06:11.82:1, and 142.08:13.97:1, respectively. With an increase in the synthetic nutrient addition, the carbon and nitrogen contents increased, but the phosphorus content was always the same because phosphorus was only obtained from the TW in which the TW concentration was fixed, 6% v/v. The C/N, C/P and N/P weight ratios in all mediums are also presented in the Table 2. Meanwhile, some other authors have recommended microalgae cultivation mediums with C:N:P weight ratio, C/N weight ratio and N/P weight ratio which are presented in the Table 3.

Table 2 Nutrients composition in the medium of microalgae *S. platensis*.

Percentage of the synthetic nutrient addition (%)	Synthetic Nutrients		Organic Nutrients from TW			Total Nutrients			Ratios			
	C^1 (mg)	N^1 (mg)	C^2 (mg)	N^2 (mg)	P^1 (mg)	C^3 (mg)	N^3 (mg)	P^2 (mg)	C/N	C/P	N/P	C:N:P
0	0	0	229.99	14.37	2.67	229.99	14.37	2.67	16	86	5.37	86:5.37:1
25	37.50	5.75	229.99	14.37	2.67	267.49	20.12	2.67	13.29	100.02	7.52	100.02:7.52:1
50	75	11.50	229.99	14.37	2.67	304.99	25.87	2.67	11.79	114.04	9.67	114.04:9.67:1
75	112.50	17.25	229.99	14.37	2.67	342.49	31.62	2.67	10.83	128.06	11.82	128.06:11.82:1
100	150	23	229.99	14.37	2.67	379.99	37.37	2.67	10.17	142.08	13.97	142.08:13.97:1

Remarks: TW = tofu wastewater; C = carbon; N = nitrogen; P = phosphorous

C^1 = [(BM C/BM $NaHCO_3$) × g $NaHCO_3$ × 98% (purity)] + [(BM C/BM urea) ×g urea]; N^1 = (BM N/BM urea) ×g urea

C^2 = (BM C/BM O_2) × COD contained in TW; N^2 = total nitrogen contained in TW; P^1 = P-PO_4 contained in TW

$C^3 = C^1 + C^2$; $N^3 = N^1 + N^2$; $P^2 = P^1$

$C/N = C^3/N^3$; $C/P = C^3/P^2$; $N/P = N^3/P^2$; $C:N:P = C^3:N^3:P^2$.

Table 3 The recommended C:N:P, C/N and N/P weight ratios.

Microalgae	C:N:P	C/N	N/P	W_{max} (mg/L)	AGR_{max} (mg/L/d)	μ_{max} (day^{-1})	References
S. platensis	62:11.4:1	5.4	11	540	18.1	0.412	[24]
S. platensis	60.5:6.2:1	9.75	6.2	-	-	0.220	[6]
S. platensis	-	10.7	-	206	63	0.371	[19]
S. platensis	55.7:7.3:1	7.6	7.3	-	-	0.142	[25]

Based on the (Table 3), the recommended C/N ratio is in the range of 5.4-10.7 and N/P is in the range of 6.2-11. In this study, a medium with the most suitable C/N and N/P ratios based on the recommended C/N and N/P ratios was a medium with TW of 6%v/v and 75% of CSN, containing C/N of 10.83, N/P of 11.82 and C:N:P of 128.06:11.82:1 (Table 2).

3.2 Growth Rate and pH Profile

The growth curve of *S. platensis* in mediums at various C/N and N/P ratios of mixed nutrients is shown in the (Figure 1A). From the figure, *S. platensis* which was cultivated in mediums containing TW of 6% v/v and 25-75% of CSN had a bigger biomass concentration than that in mediums containing TW of 6% v/v and 0 and 100% of CSN. A medium containing TW of 6% v/v and 0% of CSN had a C/N of 16 which was much higher than the recommended C/N ratio (which was 5.4-10.7) so *S. platensis* could not grow well. Meanwhile, a medium containing TW of 6% v/v and 100% of CSN had a C/N of 10.17 which was almost the same as the recommended C/N (which was 5.4-10.7), but the medium had an N/P ratio of 13.97 which was much higher than the recommended N/P (which was 6.2-11), so the *S. platensis* also could not grow well. The abundance of carbon and nitrogen content in a medium increased toxicity in the medium during cultivation. Unbalanced nutrients changed the environmental conditions of cultivation and inhibited the microalgae growth by disturbing the microalgae metabolism. According to [26], excessive nutrient enrichment in ecosystems, particularly freshwater ecosystems (including freshwater algae), resulted in more significant problems, one of which was the development of compounds inside cyanobacteria that were more deadly than cobra venom [27,28]. In detail, a medium with TW of 6% v/v and 75% of CSN resulted in the highest final biomass concentration of all mediums (Table 4). It was caused by that the medium contained the most suitable C/N and N/P ratios which were almost the same as the recommended ratios as explained in the sub-section of 3.1.

The pH profile during cultivation is shown in the (Figure 1B). As shown in the figure, the pH of all variables decreased from day 0 to day 2 because the *S. platensis* consumed the organic carbon through the respiration process and then resulted in CO_2. That condition was called the heterotrophic condition. Furthermore, the CO_2 reacted with water to result in carbonate and then decreased the medium pH. After two days, the pH began to rise steadily until the cultivation process was complete (day 18). The increase in medium pH was caused by that *S. platensis* utilised the carbonate for the photosynthesis proses and released OH^-. That condition was called the photoautotrophic condition. Heterotrophic and photoautotrophic processes occurred simultaneously in *S. platensis* cell. That condition was called the mixotrophic condition [29]. The increase in medium pH was also caused by the synthetic nutrient presence, particularly $NaHCO_3$. It was dissolved to become Na^+ and HCO_3^-. Furthermore, HCO_3^- was converted to become CO_2 and OH^- with the assistance of the carbonic anhydrase enzyme [6]. The CO_2 was utilized by the microalgae for the photosynthesis process, the Na^+ ions raised the salinity of the medium and can be utilized by the microalgae as a micronutrient, and the OH^- ions caused the medium pH to rise during cultivation [9,30,31].

The heterotrophic and photoautotrophic processes caused the decrease and increase in pH respectively. The final medium pH at synthetic nutrient addition of 0, 25, 50, 75, and 100% CSN was 8.8, 9, 9.1, 9.3, and 9.3 (Figure 1B). It showed that the more the synthetic nutrients were added, the higher the final medium pH would be because the photoautotrophic process was more dominant occurred in the system than the heterotrophic process.

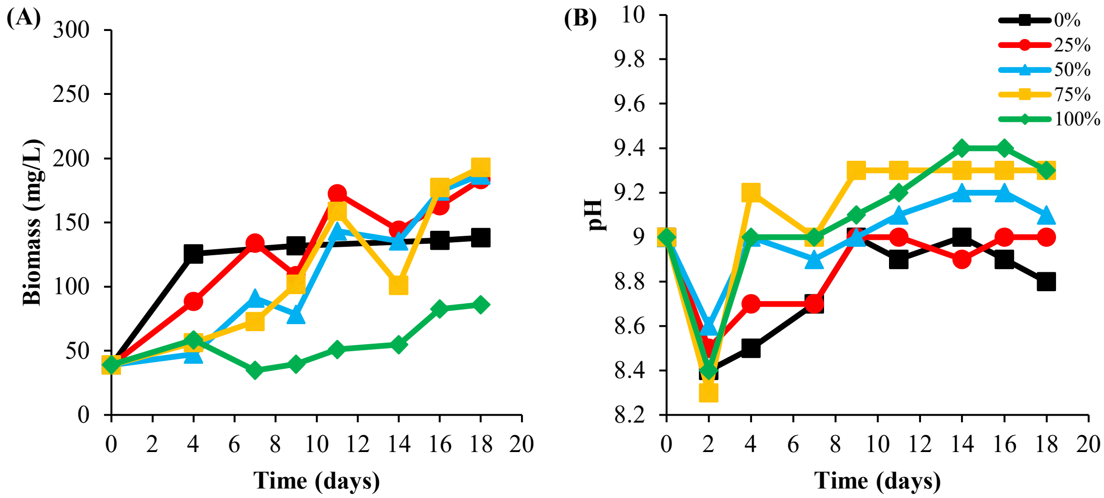

Figure 1 The effect of various synthetic nutrient additions on (A) the growth profile, and (B) the pH profile during the *S. platensis* cultivation.

3.3 Kinetic analysis

Apart from the growth profile, it was critical to analyze *S. platensis* growth kinetics, including the absolute growth rate of biomass productivity, specific growth rate, and doubling time. As shown in the (Table 4), the final *S. platensis* biomass concentration which cultivated in mediums with TW of 6% v/v and 0, 25, 50, 75, 100 % of CSN was 138.06, 183.41, 187.10, 192.90, 85.86 mg/L respectively. These results were compatible with the growth profile of *S. platensis* in the Figure 1A, where *S. platensis* which was cultivated in mediums with TW of 6% v/v and 25-75 % of CSN had more or less similar biomass production. The absolute growth rate and specific growth rate were also more or less similar in that three conditions with the range of absolute growth rate of 8.03-8.55 mg/L/day and the range of specific growth rate of 0.086 – 0.089 per day. In detail, the highest final biomass concentration (192.90 mg/L) was obtained in a medium with TW of 6% v/v and 75% of CSN because the medium contained the most suitable C/N and N/P ratios for *S. platensis* cultivation (see explanation in sub-section 3.1). This value was almost the same as a result of a study of [19] with a biomass concentration of 206 mg/L.

The fastest doubling time can be found in *S. platensis* which was cultivated in a medium with TW of 6% v/v and 75 % of CSN in which the value was 7.80 days, 21% faster than *S. platensis* which was cultivated with nutrients of TW of 6% v/v without any addition of synthetic nutrients in which the value was 9.86 days. The doubling time of *S. platensis* which was cultivated in mediums with TW of 6% v/v and 25 and 50 % of CSN was 8.05 and 7.95 days. Surprisingly, the *S. platensis* which was cultivated in a medium with TW of 6% v/v and 100 % of CSN had the slowest doubling time which was 15.77 days. The delayed doubling time might be a result of microalgae metabolism being disrupted by the abundance of nutrients. As previously stated, an abundance of nutrients that are not consumed by microalgae will be harmful to the medium. This toxic medium will affect the growth rate of microalgae or will accumulate toxic compounds inside the microalgae. All of the doubling time results were consistent with the growth profile of *S. platensis* shown in the Figure 1A. This showed that the C/N and N/P ratios in a medium with TW of 6% v/v and 75% of CSN were the most suitable of all ratios in all mediums in this study so the microalgae doubling time occurred fast.

Table 4 The effect of various synthetic nutrients additions on the final OD_{680}, final biomass concentration, absolute growth rate, specific growth rate, and doubling time.

Concentration of synthetic nutrients (% w/v)	Final OD_{680}	Final biomass concentration (mg/L)	AGR (mg/L/d)	μ (day^{-1})	t_d (days)
0	0.288	138.06	5.51	0.070	9.86
25	0.374	183.41	8.03	0.086	8.05
50	0.381	187.10	8.23	0.087	7.95
75	0.392	192.90	8.55	0.089	7.80
100	0.189	85.86	2.61	0.044	15.77

Remarks: TW = Tofu wastewater; OD = Optical Density; AGR = absolute growth rate; μ = specific growth rate; t_d = doubling time.

A pseudo-first-order kinetic model was built to corroborate the microalgal growth profile. The pseudo-first-order kinetic model equation is presented in the Equation (6).

$$\ln\left(\frac{W_t}{W_0}\right) = kt \tag{6}$$

where W_t is the biomass concentration after t days (mg/L), W_0 is the biomass concentration at t=0 (mg/L), k is the kinetic constant of the pseudo-first-order kinetic model (day^{-1}), t is the cultivation time (days).

The modelling results through the pseudo-first-order kinetic are shown in the (Figure 2). From that figure, mediums with TW of 6% v/v and 25 - 75 % of CSN resulted in a higher slope (k) than those with TW of 6% v/v and 0 and 100 % of CSN with a comparison value between 0.0931 - 0.1022 day^{-1} for the former and 0.0335 - 0.0863 day^{-1} for the latter. Those results were well-matched with the results shown in the (Figure 1A).

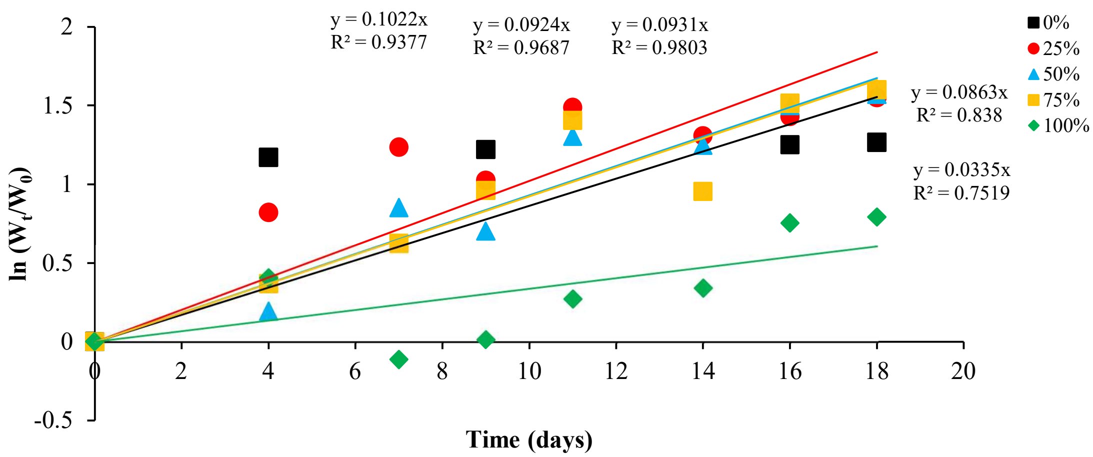

Figure 2 Pseudo first-order kinetic model results.

It was essential to predict the growth profile of *S. platensis* to assess the robustness of growth data obtained from *S. platensis* culture employing a combination of natural and synthetic nutrients. As seen in the Figure 3 and Table 5, the modified Gompertz model was used to predict the microalgae growth profiles. From the Figure 3 and Table 5, we were able to get kinetic parameters such as W_{max}, AGP_{max}, and λ by modelling the experimental growth profile with good MAPE values which were below 20%. According to the (Table 3), the maximum biomass concentration (W_{max}) which can be achieved by cultivating the *S. platensis* in mediums with TW of 6% v/v and 0, 25, 50, 75, and 100 % of CSN were 135.95, 602.08, 602.29, 635.85, and 450.03 mg/L, while the values of the maximum absolute growth rate (AGP_{max}) were 43.97, 10.27, 10.48, 11.17 and 3.35 mg/L/d, respectively. Hence, the maximum biomass productivity value was the maximum absolute growth rate (AGP_{max}). The λ or lag time of *S. platensis* which was cultivated in all mediums was almost the same which was around 0, which means the microalgae did not need more time to adaption to those new conditions. Microalgae biomass immediately increased after the microalgae were cultivated in the mediums with mixed nutrients.

Table 5 Kinetic parameter values from the modelling using the modified Gompertz model.

Kinetic Parameters	Synthetic Nutrient Addition (%)				
	0	25	50	75	100
W_{max}	135.95	602.08	602.29	635.85	450.03
AGP_{max}	43.97	10.27	10.48	11.17	3.35
λ	0	0.16	0.16	0.75	0
MAPE (%)	16.32	13.69	12.23	13.11	19.04

Remarks: W_{max} = biomass production potential (mg/L); AGP_{max} = the maximum absolute growth rate (mg/L/d); λ = lag phase period or minimum time to produce biomass (days); MAPE = Mean Absolute Percentage Error.

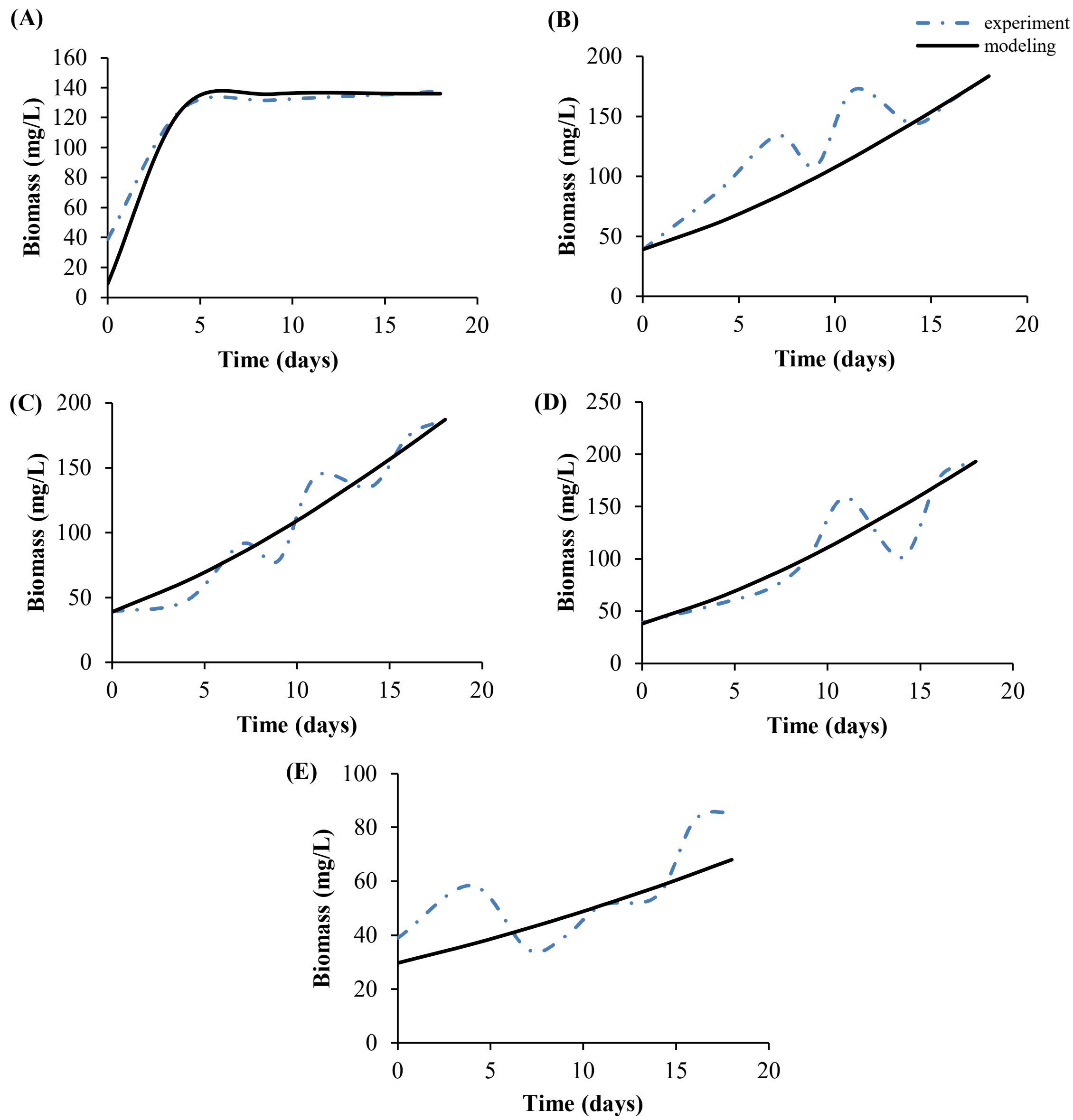

Figure 3 Correlation between experimental and predicted biomass production value obtained from the modified Gompertz equation at various synthetic nutrient additions of (A) 0%, (B) 25%, (C) 50%, (D) 75%, (E) 100% into the microalgae medium.

Based on the (Table 5), the best medium was with TW of 6%v/v and 75% of CSN because it resulted in higher W_{max} and AGP_{max} values than the other mediums, which were 635.85 mg/L and 11.17 mg/L/d, respectively. The suitable nutrient composition (C/N and N/P ratios) in that medium supported the microalgae to grow well. The W_{max} (635.85 mg/L) of this study was higher than that in a previous study [24] having a value of 540 mg/L. The previous study used POME as an organic nutrient. The POME contained more complex compounds than the TW. Thus, the difference in those values might be due to the different complexity of the POME and TW used in the previous study and this study.

3.4 Biochemical content

Biochemicals inside the cultivated *S. platensis* such as lipid and protein were analyzed to determine the relationship between the nutrient composition in the mediums and the resulting biochemical content, as shown in the Figure 4. Protein content in *S. platensis* which was cultivated in mediums with TW of 6% v/v and 0, 25, 50, 75, and 100% of CSN was 33.52, 39.46, 37.67, 67.38, and 39.59% dry cell weight (DCW), respectively. An increase in synthetic nutrient addition from 0% to 75% of CSN decreased the C/N ratio in mediums from 16 to

10.83. In other words, the nitrogen content in mediums increased with increasing the synthetic nutrient addition. According to [32], an increase in the protein content in *S. platensis* biomass was due to an increase in the nitrogen content in the mediums. During cultivation, microalgae took nitrogen from the mediums and converted it to protein, in which the primary component of protein was nitrogen. It can be shown in the Figure 4 that *S. platensis* cultivated in a medium with TW of 6% v/v and 75 % of CSN had the highest protein content (67.38% DCW) compared to the others. However, microalgae at the addition of 100% of CSN had a lower protein than that at the addition of 75% of CSN due to the disruption of microalgae metabolism by the abundant nutrients in the medium. It made protein production low.

Lipid content in *S. platensis* cultivated in mediums with TW of 6% v/v and 0, 25, 50, 75, and 100% of CSN was 2.14, 3.17, 2.86, 1.76, and 0.98% DCW, respectively, relatively low compared to the protein content. The low lipid content can be caused due to the amount of phosphorus in mediums, and stoichiometrically, it is not related to protein produced by the microalgae [33]. Microalgae tend to accumulate more lipid under stress and mixotrophic condition [34]. The microalgae cultivated in mediums with TW of 6% and 75 - 100% of CSN resulted in a lower lipid content than that in the other mediums. In the case of *S. platensis* cultivated in a medium with TW of 6% v/v and 75 % of CSN, the lower lipid was due to unstressed microalgae. When nutrients were in balance, *S. platensis* metabolism occurred normally and did not suffer from stress, and the majority of nutrients might be converted to protein, which was the primary biochemical product of *S. platensis*. On the other hand, the low lipid content in microalgae grown with nutrients of TW of 6% v/v and 100% of CSN was a result of microalgae metabolism being disrupted by an abundance of nutrients. Protein and lipid content in the microalgae *S. platensis* depended on the nutritional composition of the medium used for cultivation.

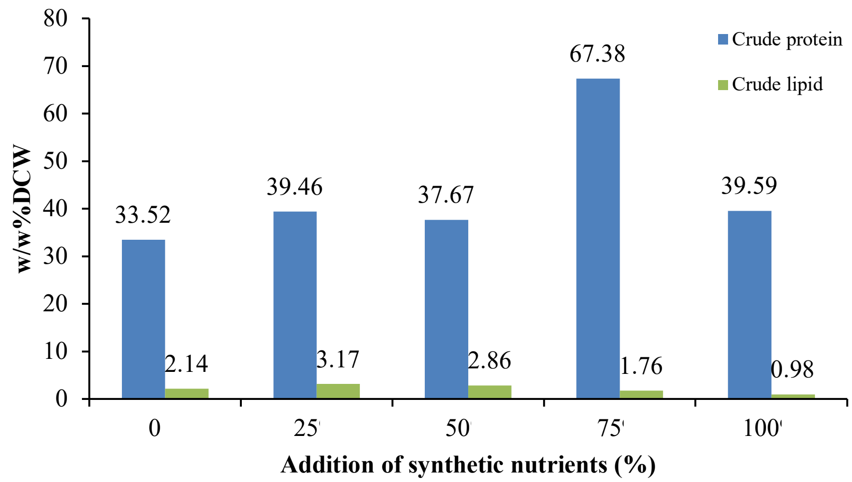

Figure 4 The effect of various synthetic nutrients addition on protein and lipid content of *S. platensis*.

S. platensis has the potential to be employed as a nitrogen source in the future owing to its high protein content, which reached 60-70% when *S. platensis* is found in nature. This research showed that when *S. platensis* was grown in a mixotrophic condition with a combination of TW of 6% v/v and 75% of CSN, the protein content of *S. platensis* could reach 67.38 %. This result was better than the results of the other authors, for instance, Nur et al. [15] reported cultivation of *S. platensis* in a mixotrophic condition by using a mixture of coconut milk skim effluent (CMSE) and synthetic nutrients resulted in biomass with a protein content of 39%. Nur et al [35] also found that mixed nutrients from Zarrouk medium and POME resulted in *S. platensis* biomass containing protein of 49.05%. Moreover, Volkmann et al. [36] cultivated *S. platensis* with mixed nutrients of desalinated wastewater and Paoletti medium resulting in biomass with a protein content was 56.17%. According to the comparisons above, the combination of TW of 6%v/v and 75% of CSN has the potential to increase the protein content of *S. platensis* biomass by more than 60%.

3.5 Shape of S. platensis

It was critical to investigate the influence of a mixture of organic and synthetic nutrients on the shape of *S. platensis*. In theory, *S. platensis* can have a morphology of linear filament fragmentation, devoid of any helix (spiral) structure. This disease is induced by microalgal oxidative stress [37]. Unbalanced nutrients in the medium ingested by microalgae may contribute to the algae's unstable metabolism and may influence the cell's resistance to abiotic variables such as temperature or light [38,39]. Under such conditions, *S. platensis* will experience the oxidative stress, altering the structure of the cell. On the other hand, *S. platensis* cultivated in a medium with balanced nutrients will have a normal morphology which is a form of a helix. Based on the microscope analysis, *S. platensis* grown in a combination of TW of 6% v/v and 75% of CSN maintained its typical morphology (Figure 5). Microalgae's regular metabolism might protect them from the oxidative damage. It gave *S. platensis* a normal, helix form.

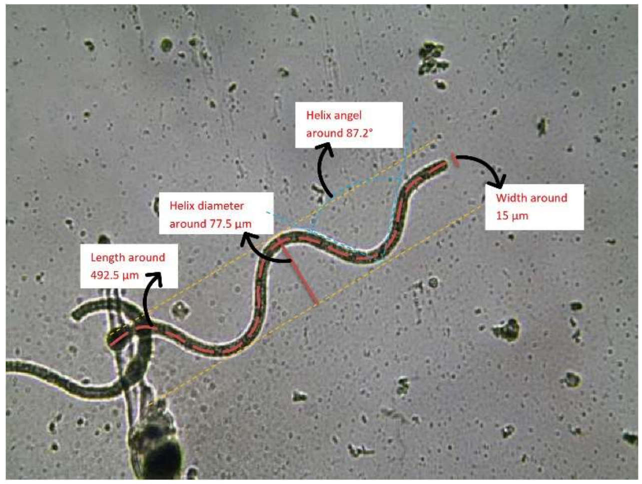

Figure 5 The *S. platensis* shape cultivated in TW of 6%v/v and 75% of CSN.

In detail, the *S. platensis* cell Comparison between *S. platensis* shape in this study and that in the other studies is shown in the Table 6. Based on the Table 6, the morphology of *S. platensis* in this study was normal because the values of length, width, helix diameter, and helix angle were in the range of values obtained from the other studies.

Table 6 Comparison between *S. platensis* shape in this study and that in the other studies.

Parameters	This study	References				Summary value from the other studies
		[40]	[41]	[42]	[43]	
Length (µm)	492.5	-	100-500	-	150-400	100-500
Width (µm)	15	6-16	5.25-7.5	-	-	5.25-16
Helix diameter (µm)	77.5	30-70	20-60	-	35-40	20-70
Helix angle (°)	87.2	-	-	50-121	-	50-121

4. Conclusion

The expansion of microalgae's application in all fields promotes the commercialization of microalgae cultivation. An increase in the production costs will result from the necessity for a large quantity of synthetic nutrients. Utilizing organic nutrients obtained from organic waste, in this case, tofu wastewater, has a favourable influence on the cost-effectiveness of future large-scale microalgae cultivation. Different ratios of synthetic to organic nutrients were used to culture microalgae, while growth and biochemical levels such as lipids and proteins were measured. The results of this research demonstrated unequivocally the suitability of tofu wastewater as a nutrient source for *S. platensis* biomass development. The best result was achieved by adding a 75% of CSN to TW of 6% v/v as organic nutrients. From the medium, the absolute growth rate or biomass productivity of *S. platensis* was 8.55 mg/L/day, the specific growth rate was 0.089 day^{-1}, the doubling time was 7.80 days and maximum biomass productivity was 11.17 mg/L/day (value of AGP_{max}). *S. platensis* biomass from the medium contained the highest protein content which was 67.38% DCW. *S. platensis* cultivated in the medium had a regular form, helix, when it was observed using a microscope. *S. platensis* growth data were well predicted using the modified Gompertz equation, which indicated that all data in this experiment were robust. According to the experiment's purpose, 25% of CSN may be eliminated to achieve the optimum microalgae biomass production. The findings showed that tofu wastewater is an organic nutrient that has the potential to promote the development of the microalgae *S. platensis* due to its high protein content for a variety of applications, such as natural fertilizer or animal feed concentrate. *S. platensis* biomass may be employed in the pharmaceutical industry, although microalgae grown in wastewater should not be ingested by people due to ethical considerations. Therefore, the utilization of organic nutrients may raise the productivity of microalgae farming for the global betterment of life.

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