The kinetics of tempeh wastewater treatment using *Arthrospira platensis*

Lieke Riadi, Theresia Desy Askitosari, Ragil Puspita Dutaning Widhi, Melvina Laurensia, Yuana Elly Agustin and Yalun Arifin

ABSTRACT

The microalga *Arthrospira platensis* (*Spirulina*) was used for tempeh wastewater treatment. Microalga growth and the kinetics of chemical oxygen demand (COD) degradation under different light intensities (2,100 and 4,300 lux), tempeh wastewater concentrations (0, 0.5, 1, 1.5% v/v), and sodium nitrate concentrations (0, 0.75, 1, 2, 2.5 g/L) were studied. Improved cell growth in wastewater indicated that mixotrophic growth was preferred. The addition of sodium nitrate up to 2 g/L increased COD removal. The highest COD removal was 92.2%, which was obtained from cultivation with 1% v/v tempeh wastewater, 2 g/L sodium nitrate, 2,100 lux, and the specific growth rate of 0.33 ± 0.01 day^{-1}. The COD removal followed a pseudo-first-order kinetic model with the kinetic constant of 0.3748 day^{-1} and the nitrate uptake rate of 0.122 g/L-day. The results can be used to design a pilot-scale tempeh wastewater treatment facility using *A. platensis* for tertiary treatment. Based on the kinetic model, a 20 m^3 reactor can treat tempeh wastewater to reduce the COD from 400 to 100 ppm in 4 days and produces approximately 32.8 kg of dried microalgae.

Key words | *Arthrospira platensis*, COD degradation, kinetic model, microalgae, tempeh wastewater

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HIGHLIGHTS

- The alga *Arthrospira platensis* can reduce COD value in diluted tempeh wastewater up to 90% COD removal.
- The addition of sodium nitrate up to 2 g/L improves the COD reduction.
- The COD degradation follows a pseudo-first-order kinetic model.
- The results can be used to design a pilot-scale tempeh wastewater treatment facility using *A. platensis* for tertiary treatment.

INTRODUCTION

Tempeh is a high-protein traditional food from Indonesia made by fermenting soybean. The soybean processing can generate 2 m^3 of wastewater per 100 kg of soybean (Puspawati & Soesilo 2018). Tempeh wastewater contains high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) up to 27,000 mg/L and 14,000 mg/L, respectively (Puspawati *et al.* 2019). *Arthrospira platensis* (previously known as *Spirulina platensis*) is a microalga that belongs to the phylum of Cyanobacteria. It is a non-nitrogen-fixing photoautotroph (Fujisawa *et al.* 2010) that has a high content of protein, vitamins, and minerals compared to many other foods (Jung *et al.* 2019). This alga is also capable of mixotrophic metabolism, in which CO_2 and organic carbon are simultaneously assimilated (Chojnacka & Zielińska 2012; Subashchandrabose *et al.* 2013; Pereira *et al.* 2019). The

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applications of this alga include food additives, animal feed, cosmetics, and pharmaceuticals (Vo *et al.* 2015). This edible alga has a protein content of around 60–65% on a dry basis, which is higher than other edible algae such as *Chlorella vulgaris* and *Isochrisis galbana* (Tokuşoglu & Ünal 2003).

Microalgae are important biomass for food, feed, pharmaceuticals, cosmetics, biofuels, and bioplastics (Morais Junior *et al.* 2020). Previous studies have demonstrated the use of microalgae *A. platensis* for the treatment of diluted wastewater from soybean processing (Syaichurrozi & Jayanudin 2017; Sahaq & Hadiyanto 2019). This microalga has also been utilized for the treatment of wastewater from swine (Cheunbarn & Peeraporpisal 2010; Mezzomo *et al.* 2010), fish farming (Wuang *et al.* 2016; Nogueira *et al.* 2018), sago starch (Phang *et al.* 2000), agricultural by-product (Taufiqurrahmi *et al.* 2017) and human urine (Chang *et al.* 2013).

The common method for treating wastewater with high COD value consists of primary, secondary, and tertiary treatments (Metcalf & Eddy Inc. *et al.* 2013). The primary and secondary treatment processes such as coagulation, flocculation, and aerobic and/or anaerobic digestion eliminate most of the COD content. We propose a tertiary treatment process that applies the alga *A. platensis* cultivation. Unlike the cost – centered processes in the primary and secondary treatments, the use of the alga will create a valuable product. Although algae grow slower than bacteria, the tertiary treatment using algae can be coupled with the valuable biomass production (Abdel-Raouf *et al.* 2012). In our case, the *A. platensis* biomass can be used as animal feed.

In this research, we studied several factors that affect the growth of *A. platensis* such as light intensity, organic compound concentration in wastewater, and the availability of nitrogen sources. The conditions for the cultivation of these microalgae have been described (Borowitzka 2005; Habib *et al.* 2008; Soni *et al.* 2019). The alga grows in an alkaline medium at pH 9–10 and a temperature of 30–38 °C with a light intensity of 1,500–4,500 lux (Sukenik *et al.* 1991). Nitrate can be used as a nitrogen source for *A. platensis.* The combination of nitrate and high light intensity gives good results for its biomass and pigment biosynthesis improvement (Ajayan *et al.* 2012). One study reported that nitrogen limitation in a fed-batch cultivation can lead to mixotrophic growth that increases biomass productivity (Li *et al.* 2018).

The mixotrophic cultivation of *Arthrospira platensis* was conducted in a batch system to study the growth of *A. platensis* and organic compounds chemical oxygen demand (COD) degradation under different light intensity, organic compound, and nitrate concentration. The purpose to add nitrate in the system was to substitute the N-contents in the wastewater due to the dilution of wastewater or depletion in the primary and/or secondary treatment. The study aims to determine the kinetic parameters both for growth and COD degradation in tempeh wastewater which has not been studied before. The kinetic parameter values are important for the design of the algae-based wastewater treatment facilities in soybean-based processing. The results obtained would also serve as a platform for a future pilot-scale tempeh wastewater treatment and to produce algae biomass.

METHODS

Microalgae, media, and tempeh wastewater

The *Arthrospira platensis* was obtained from PT Maris Indonesia. The medium used for the control cultivation is based on the standard MSI medium from previous research (Taufiqurrahmi *et al.* 2017). It consists of 0.5 g/L $NaHCO_3$, 0.5 g/L NaCl, 0.05 g/L urea, 0.02 g/L triple superphosphate (TSP), and 0.01 g/L cobalamin (vitamin B12). Tempeh wastewater was obtained from a tempeh-making home industry in Surabaya, Indonesia with 5 m^3/day wastewater produced. This organic wastewater was generated from the soaking of the dehulled soybeans and the subsequent cooking in tempeh production (Chaerun 2009). It was analyzed for COD, BOD, pH, heavy metals, total nitrogen, and total suspended solids (TSS). It was then sterilized in a 2 L container at 121 °C, 15 psi for 15 min, prior to use. For cultivation with tempeh wastewater, urea and TSP were replaced with wastewater at 0.5%, 0.75%, 1%, and 1.5% (v/v). The pH was adjusted to 9 with the addition of NaOH.

Algae cultivation and tempeh wastewater degradation

The alga was cultured for 7 days in a 2 L medium at a room temperature of 30 °C and with aeration at 4 L/min. Dissolved oxygen concentration was measured by the AppliSens DO probe. Here, 0.5 g/L $NaHCO_3$, 0.5 g/L NaCl and 0.01 g/L cobalamin were added every 2 days to the culture (Taufiqurrahmi *et al.* 2017). The constant light was provided using fluorescence lamps, with an intensity of 2,100 or 4,300 lux (16.6 or 34.0 W/m^2) to study the light effect on growth and COD degradation under different concentrations of wastewater. A no-alga study was carried out at 1.0% (v/v) tempeh wastewater without *A. platensis*. The medium with 1.0% (v/v) tempeh wastewater was later used to study the effect of nitrate. In this experiment, sodium nitrate ($NaNO_3$) was added at various concentrations of 0 (control), 0.75, 1, 2, and 2.5 g/L. The culture was run for 7 days. The sample was taken daily and was measured for optical density at 680 nm, pH, COD, and nitrate contents.

Sample assays and data analysis

The data obtained were in triplicate. The microalgal biomass concentrations were determined daily at the same hour by measuring the optical density at 680 nm using UV-Vis Spectrophotometer HP 8453 (Hewlett-Packard, Palo Alto, USA). The biomass dry weights were measured using the dry weight method at 105 °C for 2 hours after the cells were separated from the growth medium by filtration with a 100-mesh filter cloth. COD measurements were carried out using the closed reflux method (Lapara *et al.* 2000). The samples were digested in a COD reactor at 148 °C for 2 hours and the COD values were measured using a spectrophotometer at 620 nm. Nitrate was analyzed using Anion Exchange Chromatography Shimadzu SIL-10A with Conductivity Detector (Shimadzu, Kyoto, Japan). The sample was diluted with 9 mM sodium carbonate buffer with a ratio 1:1 prior to injection. The specific growth rates were obtained using the exponential growth curve with the minimum correlation coefficient at 0.98.

The undiluted tempeh wastewater was analyzed using the following techniques. The COD was analyzed with the same method mentioned above. The TSS was measured using the gravimetry method (Kamarudin *et al.* 2018). The BOD was analyzed using a BOD meter (VELP, Usmate Velate, Italy) at 20 °C and incubated for 5 days. Total nitrogen was analyzed using the Kjeldahl method. Soluble P was analyzed using spectrometry (EPA 1978). Heavy metals Mg, Mn, Zn, Hg, and Ni were measured using ICP-MS (Inductively Coupled Plasma Mass Spectrometry) Nex Ion 300X (Perkin Elmer, Massachusetts, USA).

RESULTS AND DISCUSSION

COD removal at various concentration of wastewater and different light intensity

Table 1 shows the characteristic of tempeh wastewater. The main problem in the wastewater was the high COD and BOD levels. This COD level does not allow algae growth. Our unpublished data show that the growth is inhibited when the algae was cultured at the medium containing 2% (v/v) tempeh wastewater. Therefore, any economic analysis of the tempeh wastewater treatment should consider the primary and secondary treatments as the main factors. The tertiary treatment using the alga *Spirulina* will serve as the additional step that allows the production of high-value products from the wastewater.

Table 1 | Tempeh wastewater characteristic

Parameter	Concentration [mg/L]	The standard for wastewater discharge
pH	4.3	6–9
COD	31,243	100
BOD	7,650	50
TSS	1,946	100
Total N	350	100
Soluble P	17,000	n/a
Zinc	1.31	10
Nickel	0.44	0.5
Mg	242	n/a
Mn	1.27	5
Hg	0	0.005

The standard is based on The East Java regulation for wastewater discharge nr.72/2013.

COD removed during the cultivation of *A. platensis* at different tempeh wastewater concentrations is given in Table 2. The final COD values were below the limit required for discharge to the environment. The percentage of COD reduction is higher compared to the result from another study using *A. platensis* for the treatment of 8.5% and 17.5% swine wastewater that gives 43.9% and 53.7% COD reduction, respectively (Mezzomo *et al.* 2010). There was a slight change in the COD value in the no-alga experiment (1% v/v, without *A. platensis*). This demonstrates that in the presence of *A. platensis* most of the organic substances in tempeh wastewater are metabolized by the alga.

The degradation of the organic compounds (R-Ox) in the tempeh wastewater via cell respiration by *A. platensis* can be explained by Equation (1). The organic compound is identified as the COD. The oxygen in the Equation (1) is the dissolved oxygen:

$$R-Ox+O_2 \xrightarrow[light]{A.\ platensis} CO_2 + H_2O + biomass \tag{1}$$

The reaction rate is derived as follows:

$$-r_{COD}=k\,[COD][O_2] \tag{2}$$

Table 2 | The removal of organic carbon as COD at various concentration of wastewater

	COD_0 (ppm)		**COD_7 (ppm)**		**Reduction (%)**	
Concentration [%(v/v)]	**4,300 lux**	**2,100 lux**	**4,300 lux**	**2,100 lux**	**4,300 lux**	**2,100 lux**
1 (control)	271.12 ± 5.85	303.16 ± 6.54	240.52 ± 8.90	264.23 ± 5.70	11.27 ± 3.13	12.83 ± 1.24
0.5	120.62 ± 12.50	123.66 ± 4.37	54.14 ± 5.05	40.77 ± 3.30	55.12 ± 4.82	67.03 ± 2.00
1	277.15 ± 6.81	297.06 ± 5.72	62.56 ± 15.15	49.63 ± 6.80	77.43 ± 5.44	83.29 ± 2.20
1.5	392.16 ± 18.25	333.26 ± 14.38	77.71 ± 16.20	59.82 ± 2.86	80.36 ± 3.22	82.05 ± 1.39

COD_0 is the initial COD, while COD_7 is the final COD after 7 days.

where $-r_{COD}$ is the COD reaction rate; k, (COD), and (O_2) are the kinetic constant, COD, and oxygen concentrations, respectively. The dissolved oxygen concentration is constant at 5.8 ppm during the experiment; therefore Equation (2) becomes:

$$-r_{COD} = k'[COD] \quad (3)$$

$$k' = k\,[O_2] \quad (4)$$

The experiment was carried out in a batch system. Thus, the kinetics constant can be obtained by using Equation (5) (Levenspiel 1999)

$$[COD] = [COD]_o \exp(-k't) \quad (5)$$

where (COD) is the COD concentration at a certain time, $(COD)_0$ is initial COD concentration, k' is the rate of reaction constant, and t is the time of reaction. The COD removal is influenced by the COD reduction rate, which is a function of COD concentration. Therefore, the rate of COD degradation is proportional to the COD concentration as described in (Equation (3)). This kinetic model is suitable under following conditions: (1) all available COD is due to the biodegradable organic compounds, (2) low COD concentration (<400 ppm), and (3) constant dissolved oxygen level.

Based on Table 2, we used 1% v/v to investigate the kinetic of COD degradation at different nitrate concentrations and light intensity. The cultivation batch reactor volume can be determined after degradation time is calculated by Equation (5), as presented in Equation (6):

$$V = t \cdot Q \quad (6)$$

where t = batch time and Q is flow rate of wastewater.

Effect of light intensity and tempeh wastewater to *Arthrospira platensis* growth

The cell-specific growth rates and final cell concentrations are presented in Table 3. The relatively low growth rate and cell concentration at the control culture (0% tempeh wastewater) at 2,100 lux indicates the suboptimal growth condition due to the limited amount of light. The intensity falls into the light-limited region that is less than 30 W/m^2 or 3,800 lux (Chojnacka & Noworyta 2004). The growth rate is similar to the previous result obtained using mineral medium (Prates *et al.* 2018). Both specific growth rate and cell concentration in the culture with 2,100 lux light intensity increased with the addition of the tempeh wastewater.

Table 3 | Growth of *Arthrospira platensis* in different tempeh waste concentrations and light intensities

	4,300 lux			**2,100 lux**		
Media %(v/v)	**μ [day^{-1}]**	**X [g/L]**	**Yield [mg X/mg COD]**	**μ [day^{-1}]**	**X [g/L]**	**Yield [mg X/mg COD]**
control	0.22 ± 0.01	1.19 ± 0.02	n/a	0.12 ± 0.01	0.49 ± 0.02	n/a
0.5%	0.27 ± 0.01	2.29 ± 0.08	26.33 ± 6.60	0.22 ± 0.01	0.69 ± 0.03	6.24 ± 0.28
1%	0.24 ± 0.01	2.38 ± 0.04	8.72 ± 0.81	0.26 ± 0.01	0.78 ± 0.06	2.61 ± 0.24
1.5%	0.23 ± 0.01	2.41 ± 0.05	5.55 ± 1.44	0.29 ± 0.02	0.93 ± 0.02	2.89 ± 0.18

Note: X is the final cell concentration. Yield is the final cell concentration per COD consumed.

A previous study showed that low light intensity can induce mixotrophy (Stoecker *et al.* 2006) and improve growth and nutrient removal in wastewater containing organic carbon and nitrogen (Pastore *et al.* 2018). At this point, the cells performed mixotrophic metabolism that provided better growth (Chojnacka & Noworyta 2004; Verma *et al.* 2020).

Comparing both control experiments, the growth rate at 4,300 lux was higher than the rate at 2,100 lux. This is consistent with the previous finding that shows the positive correlation between the specific growth rate and light intensity until the photoinhibition occurs at the intensity of 50 W/m^2 or 6,330 lux (Chojnacka & Noworyta 2004; Subashchandrabose *et al.* 2013). An increase in light intensity favors cell division, once optimal light intensity is attained, further increase in light intensity inhibits cell division (Ajayan *et al.* 2012). It was also reported that higher light intensity promoted cellular growth, but lower light intensity increased the cell chlorophyll content (Danesi *et al.* 2004).

When comparing the cultures with the addition of tempeh wastewater, we observed that higher light intensity favors higher cell concentrations. However, the positive correlation between the concentration of tempeh wastewater and the growth performances seen in the culture with 2,100 lux light intensity was not observed in the culture with 4,300 lux. The cell concentrations were higher at 4,300 lux however the specific growth rate decreased with the increase of tempeh wastewater. When tempeh wastewater at concentration higher than 0.5% v/v is used in this light intensity, the growth rates are lower than the rates from the 2,100 lux cultures. This particular result is similar to the previous study using *Chlorella protothecoides* for wastewater treatment, where the cell growth is lower at higher light intensity (Pastore *et al.* 2018). Photosynthetic metabolism is preferred at high light intensity. A lower growth performance in tempeh wastewater concentration above 0.5% v/v at a higher light intensity may indicate the inhibition of photosynthetic metabolism by certain chemicals in the wastewater. Further research on the related metabolic genes expression may provide a better understanding.

Table 4 | Specific growth rate constant at various concentrations of sodium nitrate (all with 1% v/v tempeh wastewater)

	Specific growth rate constant (day^{-1})	
Sodium nitrate (g/L)	**4,300 lux**	**2,100 lux**
control	0.24 ± 0.01	0.26 ± 0.01
0.75	0.26 ± 0.01	0.34 ± 0.01
1	0.28 ± 0.01	0.35 ± 0.01
2	0.30 ± 0.01	0.33 ± 0.01
2.5	0.31 ± 0.02	0.32 ± 0.02

Effect of nitrate concentration on the cell growth and COD degradation kinetics

The specific growth rate in various concentrations of sodium nitrate in the cultivation with 2,100 and 4,300 lux and 1% v/v tempeh wastewater can be seen in Table 4. *Arthrospira platensis* is a non-heterocyst alga, which does not have an ability for nitrogen fixation. Previous research shows that no nitrogenase genes were detected although several genes (*patU*, *hetR*, and *hetF*) important for heterocyst maturation and nitrogen fixation are conserved (Fujisawa *et al.* 2010; Furmaniak *et al.* 2017). The addition of nitrate in the medium induces ammonium formation, which increases the growth rate. This can be seen by comparing the growth rates in the media with nitrate addition to the growth rate from the control experiment where no nitrate was added.

Based on Equations (2)–(5), the kinetic of COD degradation follows pseudo-first-order reaction. The kinetics of COD degradation in wastewater treatment is also reported in a study using heterotroph microorganisms (Ray *et al.* 2019). We selected 1% v/v tempeh wastewater to analyze the kinetics because it gave a better COD removal under low light intensity. The rate reaction constants both without and with nitrate addition can be seen in Table 5. It shows that the COD degradation kinetic constants increased with the addition of nitrate.

Table 5 | COD degradation kinetics constant for 1% v/v tempeh wastewater, with and without addition of $NaNO_3$

Sodium nitrate [g/L]	Light intensity [lux]	Regression equation	R^2	k′ [day^{-1}]	k [ppm^{-1}. day^{-1}]
0	4,300	$y = -0.1818x + 5.4218$	0.9042	0.1818	0.0225
	2,100	$y = -0.2608x + 5.4167$	0.9302	0.2608	0.0323
2	4,300	$y = -0.2483x + 5.7729$	0.9205	0.2483	0.0308
	2,100	$y = -0.3748x + 5.8030$	0.9684	0.3748	0.0464

Table 6 shows the COD reductions and nitrate uptake rates at different sodium nitrate concentrations and 1% v/v tempeh wastewater. The highest COD reduction and nitrate uptake rate were obtained from the cultivation using 2 g/L sodium nitrate. We used the data from this nitrate concentration to analyze the kinetic of COD degradation using the pseudo-first-order assumption. Higher sodium nitrate (2.5 g/L) did not increase the COD reduction, a similar trend found on the growth rate data.

To study whether the growth of *A. platensis* is nitrate limited or COD limited, the profiles of COD, sodium nitrate, and cell concentrations from the cultivation in 1% tempeh wastewater and 2 or 2.5 g/L sodium nitrate are presented in Figure 1. It shows that the growth curves reached stationary when the nitrate concentrations were still high, whereas the COD has decreased below 60 ppm. It can be proposed that using sodium nitrate at 2 g/L in the medium will not lead to nitrogen limitation. The effect of nitrogen limitation can accelerate the degradation of microbial pigments and protein important in supplying intracellular nitrogen to maintain high biomass productivity under mixotrophic cultivation. The photosynthesis performance will be negatively influenced by nitrogen limitation in mixotrophic cultivation compared to autotrophic or heterotrophic cultivations (Li *et al.* 2018).

We can calculate the size of the cultivation pond for tertiary treatment of 5 m^3/day of wastewater produced in the local tempeh industry based on kinetic data obtained from 1% tempeh wastewater, 2,100 lux, and 2 g/L nitrates. It requires 4 days batch times to reduce COD content from 400 to 100 ppm and needs a 20 m^3 cultivation pond (Equation (6)), which produces approximately 32.8 kg dry weight of microalgae. The amount of algae is calculated on the dry weight basis (1.64 g/L) produced for 4 days of cultivation at 1% w/w tempeh wastewater, 2,100 lux, and 2 g/L sodium nitrate.

Nitrate uptake rate

Sodium nitrate added to the culture dissociates to Na^+ and NO_3^-, as stated in the reaction:

$$NaNO_3 \rightarrow Na^+ + NO_3^- \tag{7}$$

The nitrate metabolism is nitrate reduction to nitrite by nitrate reductase followed by the assimilation process from nitrite to ammonium:

$$NO_3^- + NADH + H^+ \rightarrow NO_2^- + NAD^+ + H_2O \tag{8}$$

$$NO_2^- + 3H_2O + 2H^+ + light \rightarrow NH_4^+ + 0.5\,O_2 + 2H_2O \tag{9}$$

Ammonium is synthesized to glutamate and glutamine which later enter the central nitrogen metabolic pathway in A. *platensis* (Fariduddin *et al.* 2018). Table 6 shows nitrate uptake rate increased with the increase in sodium nitrate concentration, which demonstrates that nitrate could stimulate the growth of *Arthrospira platensis*. However, the uptake rates were relatively constant when the sodium nitrate was 2 g/L and above. The nitrate uptake rates obtained are comparable to values reported from the research using immobilized *Chlorella vulgaris* cyanobacterium that gives the uptake rate of 0.011 g/L-day (Jeanfils *et al.* 1993) and from the cyanobacterium *Synechococcus* cultivation (0.077 g/L-day) (Hu *et al.* 2000). The lower nitrogen uptake rate in the higher light intensity cultivation is due to the competition of mixotrophic-autotrophic pathways when both light and organic compounds are provided sufficiently in the system (Pastore *et al.* 2018) This contrasts with the previous study in autotrophic culture (Hu *et al.* 2000), probably due to the mixotrophic nature of the cultivation in this research.

In addition, the residual nitrate concentration after 7 days of cultivation was about 40% of the initial concentrations for initial sodium nitrate of 2 g/L, which was

Table 6 | COD reductions and nitrate uptake rates at various concentration of $NaNO_3$ from the cultivation with 1% v/v tempeh wastewater

	COD_0 (ppm)		**COD_7 (ppm)**		**%reduction**		**average Q_N (g/L-day)**	
Sodium nitrate [g/L]	**4,300 lux**	**2,100 lux**	**4,300 lux**	**2,100 lux**	**4,300 lux**	**2,100 lux**	**4,300 lux**	**2,100 lux**
0.75	353.27 ± 6.74	368.51 ± 13.10	88.41 ± 13.00	62.43 ± 9.52	74.97 ± 4.69	83.06 ± 2.51	0.029	0.042
1	342.79 ± 20.74	347.25 ± 10.22	71.26 ± 17.62	39.56 ± 3.35	79.21 ± 7.85	88.61 ± 0.78	0.048	0.075
2	338.98 ± 8.20	357.08 ± 16.24	66.50 ± 24.73	27.82 ± 1.86	80.38 ± 9.33	92.21 ± 0.61	0.065	0.122
2.5	321.83 ± 7.50	357.08 ± 21.03	79.84 ± 24.36	52.07 ± 5.93	75.19 ± 9.72	85.42 ± 1.00	0.056	0.115

COD_7 is obtained after 7 days.

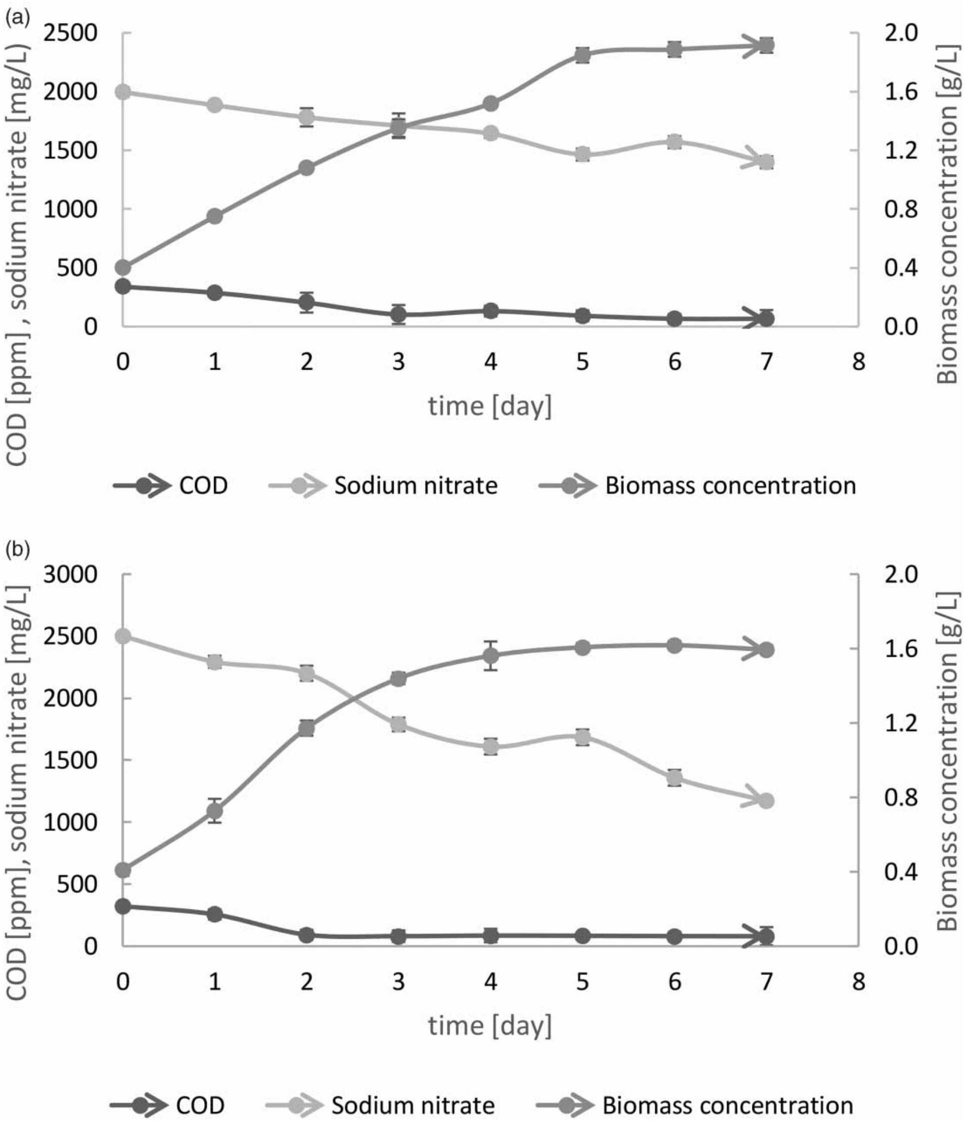

Figure 1 | COD, biomass concentration, and sodium nitrate profiles versus time at a system with (a) 2 g/L $NaNO_3$ 1% v/v wastewater and 4,300 lux and (b) 2.5 g/L $NaNO_3$, 1% v/v wastewater and 4,300 lux.

around 580 ppm nitrate ions. The WHO standard for nitrate concentration in drinking water is 50 ppm (Fan 2011). In order to reduce the nitrate level prior to discharge, we propose recycling the medium for the following microalgae cultivation after harvesting the algae and adjusting the substrate and other components to the concentration level needed for algae cultivation.

CONCLUSIONS

The alga *Arthrospira platensis* grows in media containing diluted tempeh wastewater with the COD <400 ppm and successfully reduces the COD level well below the required limit by the legislation. These cyanobacterium cells grow faster at higher light intensity when there is no wastewater. The addition of wastewater improves the cell growth compared with the control experiment that indicates that mixotrophic growth is preferred. The growth in tempeh wastewater at low light intensity increases with the increase in the wastewater concentration. The opposite result is seen in the wastewater cultivation at the high light intensity that indicates that while the photosynthetic metabolism is preferred, the growth is inhibited by the wastewater. The results are similar to a previous study using the *Chlorella* cyanobacterium for wastewater treatment. Further research is needed for confirmation.

From the cultures with nitrate addition, the highest percentage of COD removal is 92.2% in a 1% v/v tempeh wastewater culture with 2 g/L $NaNO_3$ at 2,100 lux. The COD degradation follows a pseudo-first-order kinetic model with the kinetic constant of 0.3748 day^{-1} and the nitrate uptake rate of 0.122 g/L-day. The results obtained can be applied for designing a pilot-scale tempeh wastewater treatment facility in a batch system. The cultivation reactor volume required is 20 m^3 for 5 m^3/day wastewater treated, which needs 4 days for cultivation and COD degradation. This culture method can produce approximately 32.8 kg dry weight of *A. platensis*. The cells' ability to reduce the COD at lower light intensity may be beneficial in reducing the energy requirement during a large-scale operation. A response surface study for the optimization of the light intensity and nitrate concentration may be conducted in the future.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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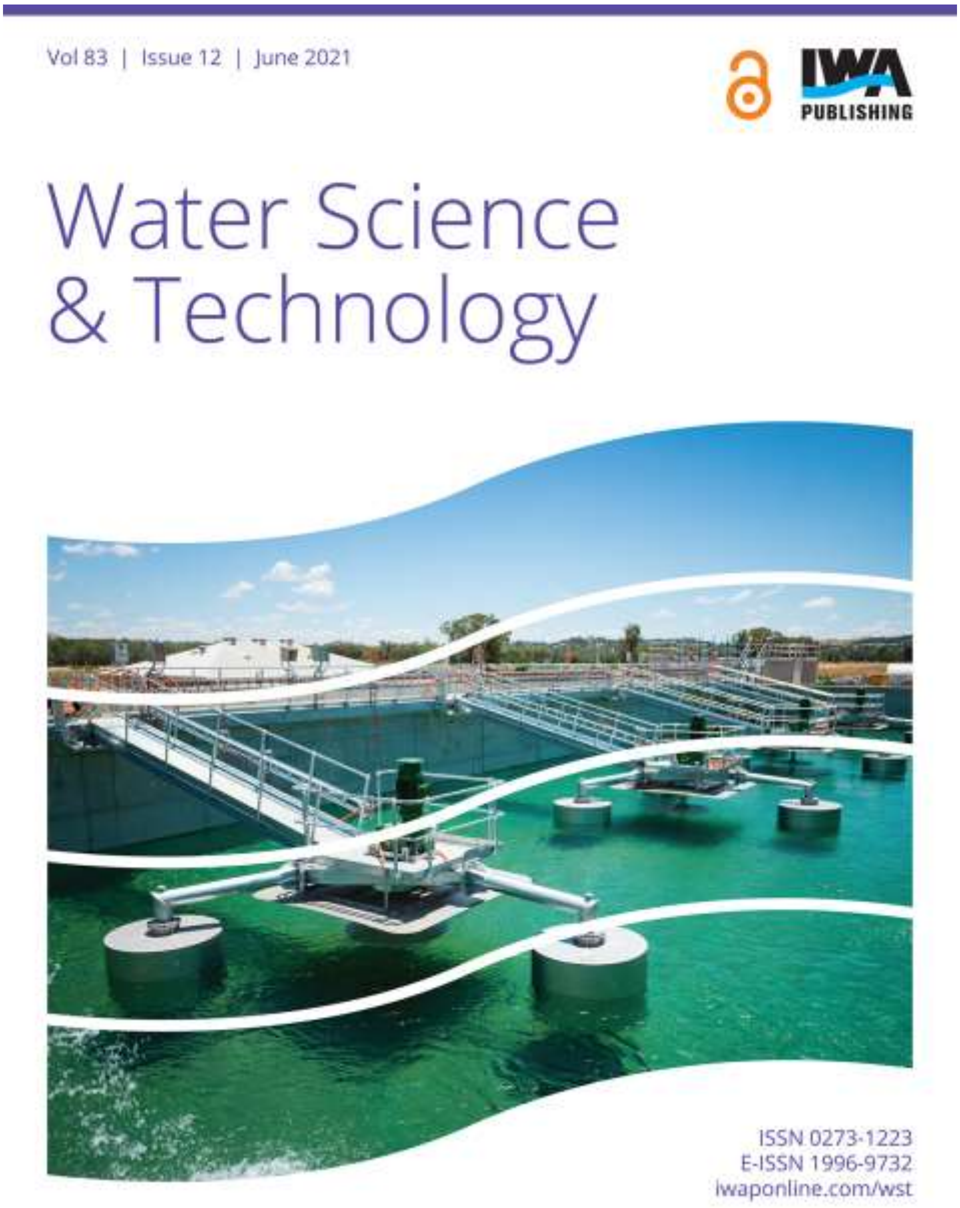
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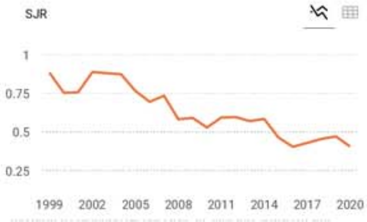

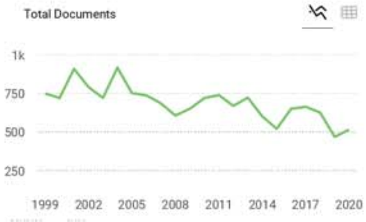

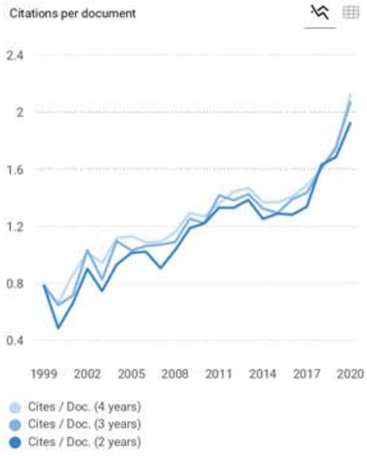

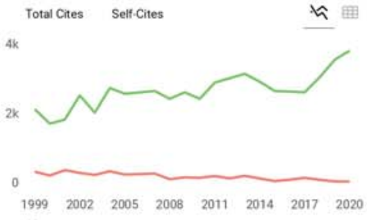

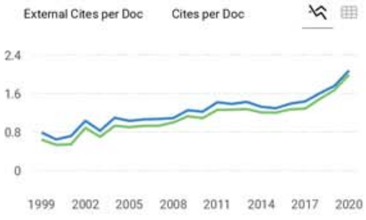

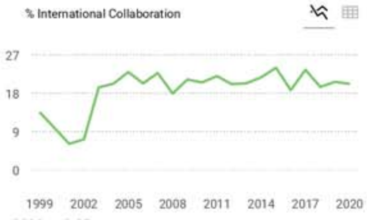

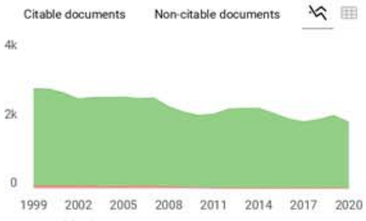

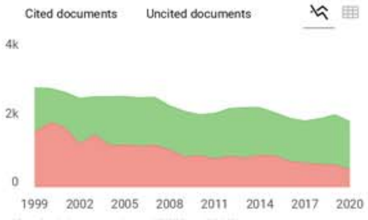


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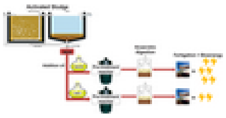

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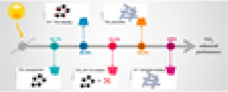

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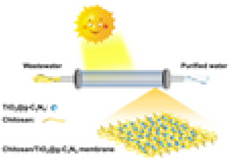

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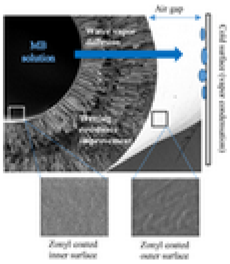

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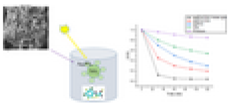

Photocatalytic degradation of 2,4-dichlorophenoxyacetic acid from aqueous solutions by Ag_3PO_4/TiO_2 nanoparticles under visible light: kinetic and thermodynamic studies

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