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CULTIVATION OF *ARTHROSPIRA PLATENSIS* USING DISCHARGE WATER FROM THAI SHRIMP FARM FOR ALGAL PRODUCTION AND WASTEWATER TREATMENT

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ABSTRACT

The productivity of *Arthrospira platensis* culture is valuable due to its nutritive properties. This study sought to increase the productivity of algae and also reduce production costs during large-scale cultivation for industrial purposes.The experiments were conducted using wastewater taken from a shrimp farm at Kamphaengsaen, Nakhon Pathom province, Thailand, from August 2015 to April 2016. Algal culture from 100% wastewater with 60% $NaHCO_3$ produced the highest cell count (18 x 10^4 cells/ml). To produce a culture medium suitable for algal growth, non-diluted wastewater supplemented with 60% $NaHCO_3$, $NaNO_3$, and K_2HPO_4 under fluorescent light for 16 h/day had the highest yield (29 x 10^4 cells/ml). In terms of wastewater quality, the Biochemical Oxygen Demand $_5$ (BOD_5) value, as well as nitrogen, phosphorus, zinc, lead, and copper content were reduced after cultivating *A. platensis* in wastewater from a shrimp farm for 30 days. Therefore, adding *A. platensis* to the shrimp farm wastewater was very useful in keeping low N and P content and in improving water quality suitable for shrimp larvae. Nutrients from wastewater in a shrimp farm can be used for growing this algal species, while simultaneously treating the wastewater before discharging directly into waterways.

Key words: algae cultivation, Zarrouk medium

INTRODUCTION

Arthrospira platensis is a cyanobacterium that is being consumed because of its high nutritional value, as well as potential therapeutic benefits. It has a high protein content (50-70%), along with high amounts of essential vitamins, fatty acids, minerals, antioxidant pigments (phycobiliprotein and carotenoids), and amma-linolenic acid (GLA), a precursor for biologically-active compounds (prostaglandins, PGE1) (Habib et al. 2008). The regular consumption of *Arthrospira* sp.can enhance brain function, boost the immune system, regulate cholesterol, stimulate antibodies, and improve white blood cell count (Khanet al. 2005). C-phycocyanin extracted from *Arthrospira* sp. induced apoptosis in the human chronic myeloid leukemia cell line-K562 (Subhashini et al. 2004). Mice fed with an

Arthrospira extract exhibited enhanced activity of antitumor natural killer cells compared with those fed with the placebo (Akao et al. 2009). Thus, algal production is widely used in human nutritional supplements, animal feed, and pharmaceuticals. Globally, the production of *Arthrospira* sp. is 3,000 tons (dry weight) per year. At present, several companies produce *Arthrospira* sp., including Spirulina Mexicana (Sosa Texcoco) SA Mexico, Nippon Spirulina Co., Ltd., Japan, Earthrise Farms and Cyanotech Corporation, USA., and Siam Algae Co., Ltd., Thailand (Shimamatsu 2004). Techniques for *Arthrospira* sp. culture vary from place to place, based on the geographical location and producers' knowledge. Therefore, the productivity of algae culture has become a tremendous asset.

Zarrouk's medium, which is rich in bicarbonate, has successfully served as the standard medium for *Arthrospira* sp. culture (Zarrouk 1996). Cultivation of this alga is not complex, but incurs rather high costs because various chemicals are used for culture media. For example, sodium bicarbonate ($NaHCO_3$) can be used in large amounts and is relatively expensive (Becker and Venkataraman 1982). For large-scale cultivation for industrial purposes, it is important to produce *Arthrospira* sp. at reduced costs. Thus, there have been many attempts to develop a cheap and simple medium for *Arthrospira* sp. by substituting the nutrients present from Zarrouk's medium with cheaper and locally-available chemicals. It was reported that the effluent generated during the production of pickling vegetables could also serve as a medium for *Arthrospira* sp. cultivation (Duangsri and Satirapipathkul 2011).

In this study, wastewater from a shrimp farm, containing nitrate and phosphate (Shewin et al. 2013) essential for algal and phytoplankton growth, was used to substitute for some chemicals from the medium. The residues may be used as a medium to culture cyanobacteria. Moreover, overfeeding with shrimp diet often pollutes shrimp ponds, increasing N and P content markedly in the pond. Some algal species, such as *Tetraselmis tetrathele*, *Nannochloropsis oculata*, *Isochrysis* sp., and *Chaetoceros gracilis* can improve water quality for fish and shrimp production by taking up ammonium N and phosphate (Okauchi et al. 1995). Many algae, including *Spirogyra neglecta*, *Pitophora oedogonia*, and *Haslea ostrearia* have considerable potential to sequester some heavy metals, such as Ni, Cu, Co, Zn, and Cd, from waste water through different mechanisms depending on the alga, type of metal ions, and growth conditions. Some algae absorb heavy metal ions onto their cell surfaces through a process called 'biosorption'. Also, metals are accumulated intracellularly through active biological transport. Some algae have also been known to break down or degrade some toxic compounds. For example, *Dunaliella bioculata* has been reported to degrade the insecticide deltamethrin by producing esterase (Kumar 2014). In Iran, the impact of *A. platensis* growth on water treatment was examined. *A. platensis*is was capable of reducing total dissolved solids by 50%, BOD_5 by 82%, COD by 50%, and total hardness by 72%. This was because the cell wall of *A. platensis* is surrounded by a porous, three-dimensional, macromolecular network that can allow the passive binding of cations through a biosorption process (Dolatabadi and Hosseini 2016).

The current research addresses the potential of waste water from a shrimp farm supplemented with $NaHCO_3$ to cultivate *A. platensis*. Algal yield from waste water were evaluated and compared with algal yield from Zarrouk medium. This approach aimed to evaluate the use of wastewater from shrimp farm as a potential source for algal production, to provide good quality water for shrimps, and to provide a means of treating wastewater before discharge.

MATERIALS AND METHODS

Inoculum development

A. platensis inoculum was maintained in the Department of Genetics, Kasetsart University Kampaengsaen campus, Thailand, which was originally obtained from the Thailand Institute of Scientific and Technological Research, Bangkok, Thailand. *A. platensis* was grown in Zarrouk medium (Zarrouk 1996) which consisted of (in g/l): $NaHCO_3$, 18.0; K_2HPO_4, 0.5; $NaNO_3$, 2.5; K_2SO_4, 1.0;

$MgSO_4$, 0.2; NaCl, 1.0; $CaCl_2$, 0.04; $FeSO_4$, 0.01; EDTA, 0.08; and 1 mL solution A5, containing (in g/l): H_3BO_3, 2.86; $MnCl_2$, 1.81; $ZnSO_4$, 0.22; $CuSO_4$, 0.08; and MoO_3, 0.01. *A. platensis*was cultured in Zarrouk medium until the optical density reached 1.0 [OD_{560}, ($1x10^6$ cell)], (Fig 1a). It was then filtered using a plankton net and washed with distilled water to remove the culture medium. A sample (30 g) of fresh *A. platensis* was mixed with water (1,000 ml), then 200 ml from the *A. platensis* mixture was added into the prepared culture medium.

Algal growth measurement

A. platensis cells were counted using a hemocytometer (BOECO, Germany) with a slide consisting of 2 small squares in the center. One drop of *A. platensis* sample was placed in the sample chamber of the hemocytometer slide and left for 1 min to allow the algae to sink to the bottom. A microscope with 100x magnification was used to inspect the slide. All *A. platensis* cells that appeared on the 2 squares were counted, and then the the mean total cell number was calculated using the following equation (Guillard and Sieracki 2005):

$$\text{Measured cell density} = \frac{\text{Average number of cells per small square x Dilution factor (if any)}}{\text{Volume of a small square (ml)}}$$

Algal growth in different concentrations of wastewater from a shrimp pond

The experiments were conducted using wastewater taken from a shrimp farm in Kamphaengsaen, Nakhon Pathom province, Thailand, from August 2015 - April 2016. The wastewater was diluted with distilled water to produce dilution percentages of 0, 10, 20, 40, 80, and 100. At each concentration level, $NaHCO_3$ at 20%, 40%, and 60% the amount contained in Zarrouk medium was added (Table1). A sample of*A. platensis* solution (200 ml) was inoculated in a 1litre flask containing 500 ml of prepared medium and incubated for 30 days at 25° C under 27 µmol m^{-2} s^{-1} intensity light (lux meter, ITandHome-TL-05, Thailand.) under an aeration system. The growth of algal culture in different media preparations was determined through cell count.

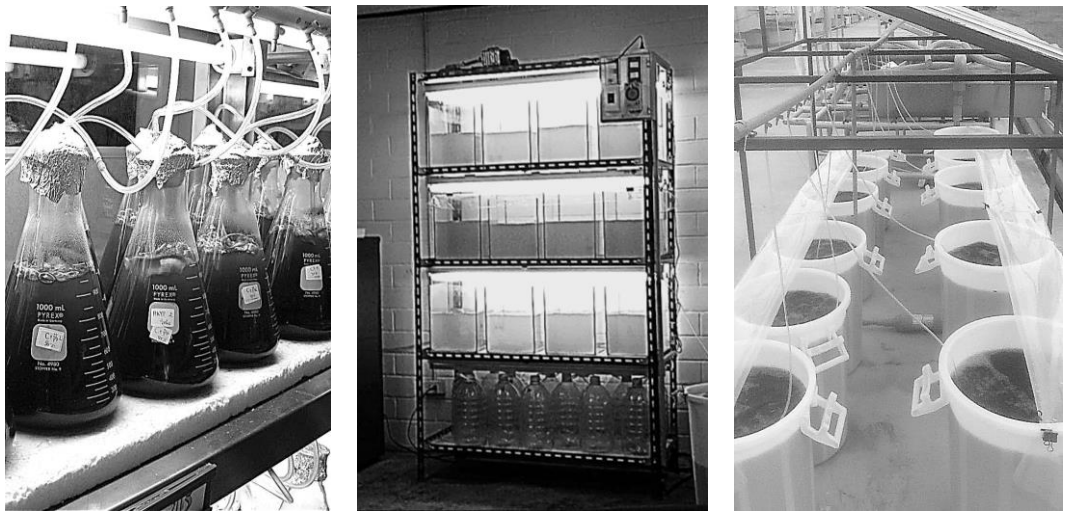
Table 1. Ingredients of media for microalgal culture in each experiment. All experiments were carried out in triplicate.

Experiment 1	Experiment 2	Experiment 3 (add new medium)
Wastwater 0% + $NaHCO_3$ 20% 40% 60%	Wastewater 100% $NaHCO_3$ 20% + $NaNO_3$, K_2HPO_4 50% of Zarrouk medium	Zarrouk
Wastewater 10% + $NaHCO_3$ 20% 40% 60%	Wastewater 100% $NaHCO_3$ 40% + $NaNO_3$, K_2HPO_4 50% of Zarrouk medium	Wastewater
Wastewater 20% + $NaHCO_3$ 20% 40% 60%	Wastewater 100 % $NaHCO_3$ 60% + $NaNO_3$, K_2HPO_4 50% of Zarrouk medium	Wastewater mixed with Zarrouk 1:1
Wastewater 40% + $NaHCO_3$ 20% 40% 60%	Zarrouk	
Wastewater 80% + $NaHCO_3$ 20% 40% 60%	Wastewater	
Wastewater 100% + $NaHCO_3$ 20% 40% 60%	Wastewater mixed with Zarrouk 1:1	
Zarrouk		

Culture medium and conditions

To determine the suitable medium preparation, wastewater at the concentration level wherein *A. platensis* grew best was selected from the previous experiment and supplemented with $NaHCO_3$ at 20%, 40% and 60% the amount in Zarrouk medium, and was also supplemented with $NaNO_3$ (1.5 g/l)

and K_2HPO_4 (0.5 g/l) at 50% the amount in Zarrouk medium (Table 1). $NaHCO_3$, $NaNO_3$, and K_2HPO_4 were added as carbon and nitrogen sources that could increase the growth of *Arthrospira* sp. *A. platensis* culture under fluorescent light (27 µmol m^{-2} s^{-1}) for 16 h/day and under natural light conditions (64.75 – 83.25 µmol m^{-2} s^{-1}) for 30 days were compared (Fig. 1b and c). Algal growth was determined by taking samples daily for cell counting.



(a) (b) (c)

Fig. 1. *A. platensis* cultured for inoculum development (a) under fluorescent light (27 µmol m^{-2} s^{-1}) for 16 h/day (b), and natural light conditions (64.75 – 83.25 µmol m^{-2} s^{-1}) (c) both for 30 days.

Algal growth under various culture modifications

The algae was cultured in three formulas with each containing 5,000 ml of culture medium: shrimp pond wastewater (100%), shrimp pond wastewater mixed with Zarrouk medium (1:1), and Zarrouk medium alone (Table 1). The algal cultivation samples were monitored for 30 days in a continuous culture, wherein fresh culture medium was added every 3 days into each treatment after cells have reached the end of the exponential phase and begin to enter retardation phase. The pH of the culture media was then adjusted to 10 ± 1 using 6 N NaOH.The best medium formula was determined based on whcih exhibited maximum algal growth.

Water quality measurement

The quality of wastewater prepared as a culture medium from the previous experiment was measured before and after cultivating *A. platensis*. The indicators were: BOD_5, ammonia concentration, nitrate concentration, phosphate concentration, as well as the concentration of heavy metals including copper, lead, and zinc. BOD_5 was measured using a DO meter (HACH Sension 6, HACH, USA.) while ammonia, nitrate, and phosphate concentration were measured using a HACH DR-2000 (HACH, USA.). The concentration of heavy metals (zinc, copper and lead) were measured using an atomic absorption spectrometer (Spectr AA 220FS, Varian, USA.)

Statistical analysis

All experiments were performed in triplicate with results presented as mean values ± standard deviation. Statistical analyses were performed using SPSS software pac kage (SPSS, Inc., Chicago, IL, USA). Multiple comparisons were conducted using Duncan's multiple range test with significance set at 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

Effects of different concentrations of shrimp pond wastewater on algal growth

A. platensis was grown in shrimp pond wastewater at 0%, 10%, 20%, 40%, 80%, and 100% concentrations for 30 days. At each percent concentration level, $NaHCO_3$ was added at 20%, 40%, and 60% the amount in Zarrouk medium. *A. platensis* grew best in non-diluted wastewater, together with $NaHCO_3$ at a concentration of 60% (Table 2). This was significantly different at the 95% confidence level compared to the growth observed from Zarrouk medium (Table 2 and Fig. 2). The effluent had values of 3.98 mg/l BOD_5, 3.40 mg/lNO_3, 0.13 mg/lPO_4, 2.60 mg/lNH_3, and 90 mg/l alkalinity, at tempratures raning from 29-33°C and pH from 7-9. This indicates that shrimp pond wastewater contained all the nutrients needed by *A. platensis*, including phosphorus and nitrate, but with lower amounts of phosphorus (0.13 mg/l) and higher amounts of nitrate (3.40 mg/l) than their respective amounts from Zarrouk medium (0.5 and 2.5 mg/l, respectively). Nonetheless, *A. platensis* still grew. It was reported that the optimal biomass production of *Arthrospira* sp. in Zarrouk medium was at pH 9 and at temperatures raning from 30-35° C (Pandey and Tiwari 2010). Therefore, the physico-chemical components of shrimp farm wastewatermay be suitable for algal growth perhaps because the algae had the ability to utilize inorganic nitrogen, ammonium, and phosphorus from the effluent for growth and to produce oxygen for reducing the effluent's BOD_5.

This result was in line with Sungkanukit (2003) who grew *A. platensis* in layer chicken manure fermentation medium, which contained a small amount of nutrients. Nutrient content from the waste water was sufficient for *A. platensis* to grow under culture conditions. Furthermore, *A. platensis* cultured in waste water from a rubber factory, together with $NaHCO_3$, $NaNO_3$, and K_2HPO_4, grew algae just as well as was reported from Zarrouk medium.

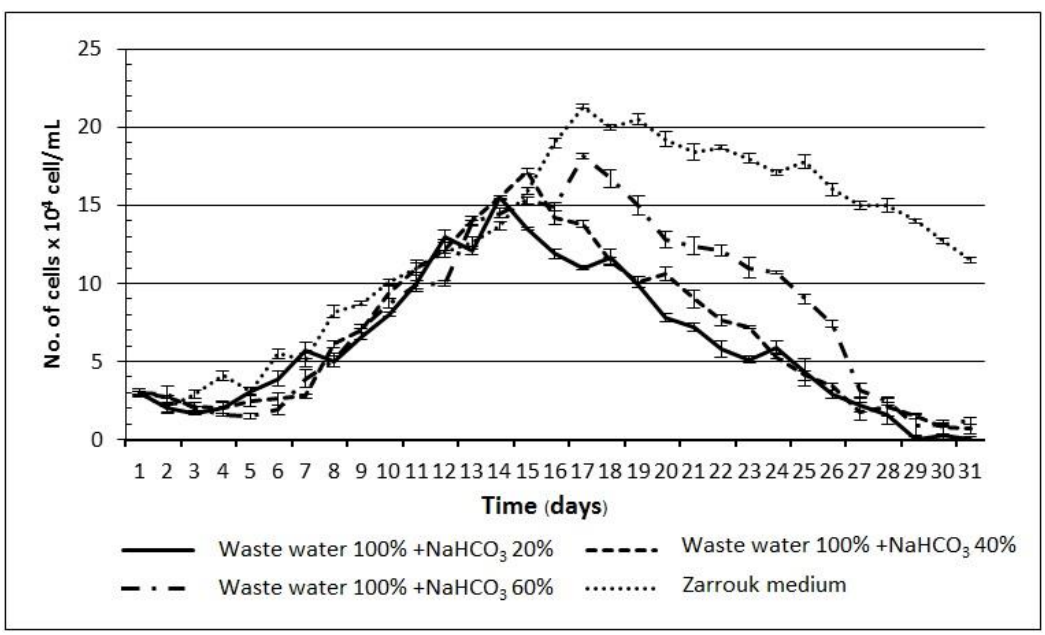


Fig. 2. *A. platensis* cell count in non-diluted shrimp pond wastewater (100%), together with $NaHCO_3$ at 20%, 40%, and 60% the amount from Zarrouk medium; and from Zarrouk medium alone.

Table 2. Highest growth of *A. platensis* based on cell number from 18 types of culture preparations compared with algal growth from the control (Zarrouk medium).

Culture medium		Highest algal cell number*(x 10^4 cells/ml)
Wastewater concentration	**$NaHCO_3$ concentration**	
0%	20%	3.50 ± 0.28^a
	40%	5.66 ± 0.33^b
	60%	7.33 ± 0.16^c
10%	20%	8.50 ± 0.00^d
	40%	10.16 ± 0.16^e
	60%	10.66 ± 0.16^e
20%	20%	10.33 ± 0.33^e
	40%	12.16 ± 0.16^{fg}
	60%	12.33 ± 0.16^g
40%	20%	11.50 ± 0.28^f
	40%	12.16 ± 0.16^{fg}
	60%	13.16 ± 0.16^h
80%	20%	13.66 ± 0.16^h
	40%	14.83 ± 0.44^i
	60%	16.33 ± 0.33^j
100%	20%	15.50 ± 0.28^i
	40%	17.16 ± 0.16^k
	60%	18.16 ± 0.16^l
Zarrouk		21.33 ± 0.16^m

* : mean (from 3 replicates) ± SD

The same lowercase letter indicates no significant difference at 95% confidence level using Duncan's multiple range test

Suitable medium and conditions of algal growth in the shrimp farm effluent

A. platensis was grown in shrimp pond wastewater (100%), together with $NaNO_3$ and K_2HPO_4 at 50% the amount from Zarrouk medium, and with $NaHCO_3$ at 20%, 40%, and 60% the amount from Zarrouk medium, either under natural light conditions or fluorescent light for 16 h/day. The nutritional effects of various media formulations and light sources markedly influenced final algal cell numbers. *A. platensis* grew best in the shrimp pond wastewater, together with $NaHCO_3$ at 60% the amount from Zarrouk medium, and also with $NaNO_3$ and K_2HPO_4 at 50% the amount from Zarrouk medium under fluorescent light (16 h/day). This treatment produced the highest algal cell number per day of 29 x 10^4 cells/ml (Table 3, Fig. 3 and 4). This may have been because both $NaHCO_3$ and $NaNO_3$ served as good sources of nitrogen for algal growth. $NaHCO_3$ also serves as a good source of carbon since it emits CO_2 which is needed for photosynthesis. It also produces NaOH which increases the pH value to 9 (Cheunbarn and Peerapornpisal 2010). The addition of $NaHCO_3$ at the appropriate level resulted in an improved content of algal cellular proteins, carbohydrates, lipids, chlorophyll, and beta carotenes (El-Kassas et al. 2015).

Table 3. Highest algae cell number observed from different culture media.

Culture medium	Highest algae cell number*(x 10^4 cells/ml)	
	Natural light	Fluorescent light for 16 h/day
Wastewater + $NaHCO_3$ 20% + $NaNO_3$and K_2HPO_4	18.16 ± 0.16[b]	24.33 ± 0.33[e]
Wastewater + $NaHCO_3$ 40% + $NaNO_3$and K_2HPO_4	22.50 ± 0.28[d]	27.16 ± 0.44[f]
Wastewater + $NaHCO_3$ 60% + $NaNO_3$and K_2HPO_4	24.50 ± 0.28[e]	29.16 ± 0.44[g]
Zarrouk	21.33 ± 0.16[c]	28.83 ± 0.16[g]
Wastewater	8.16 ± 0.16[a]	8.83 ± 0.16[a]

* : mean(from 3 replicates) ± SD
The same lowercase letter indicates no significant difference at 95% confidence level using Duncan's multiple range test

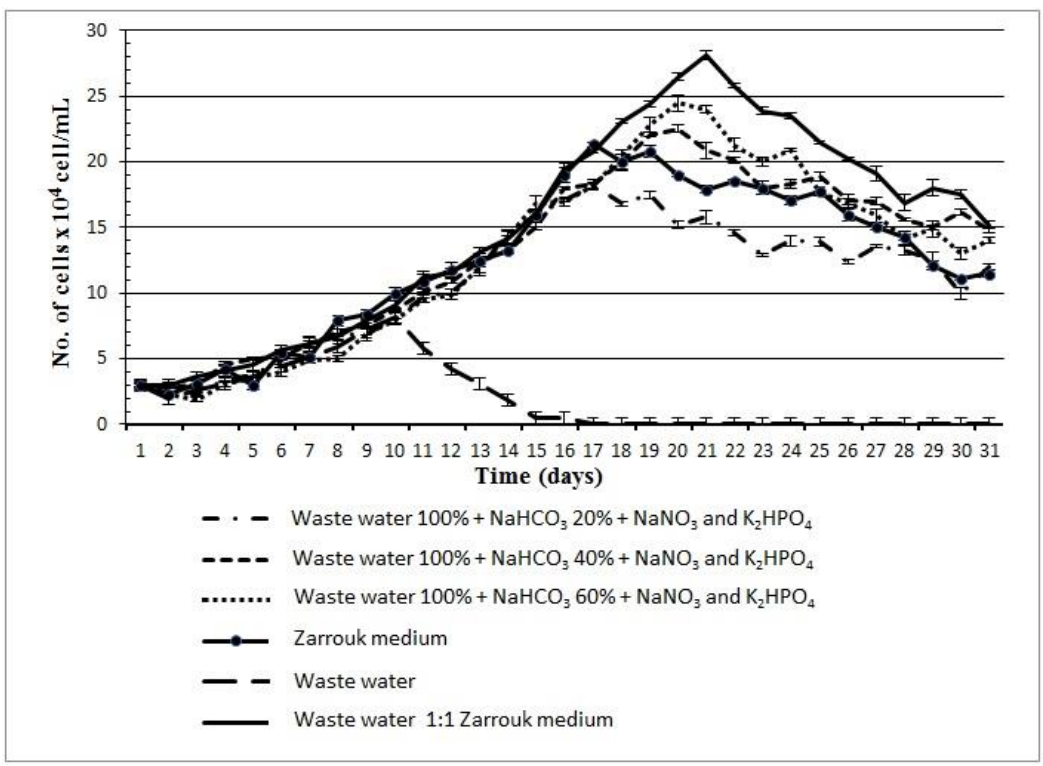


Fig. 3. Growth rates of *A. platensis* grown in wastewater, in wastewater together with $NaHCO_3$, $NaNO_3$, K_2HPO_4, and Zarrouk medium, and in Zarouk medium alone under natural light conditions.

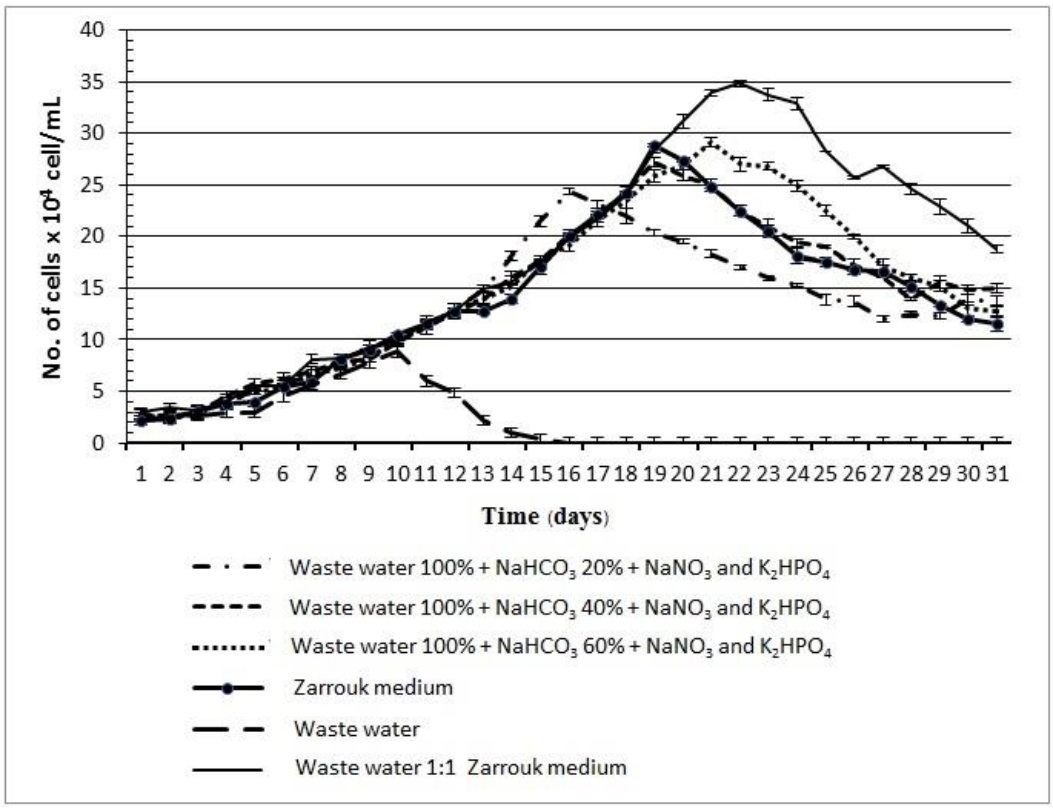


Fig. 4. Growth rate of *A. platensis* grown in shrimp pond wastewater, in wastewater together with $NaHCO_3$, $NaNO_3$, K_2HPO_4 and Zarrouk medium, and in Zarouk medium alone under fluorescent light for 16 h/day**.**

Another important factor affecting algal culture is light intensity because it influences the synthesis of various algal components (Pandey and Tiwari 2010). Because *A. platensis* was cultured under semi-natural conditions in a building with a light intensity of 64.75 – 83.25 µmol m^{-2} s^{-1}, it did not receive adequate amounts of light (6-8 h/day). However, algae exposed to high light intensity may also cause photolysis and cell division may also be inhibited. *A. platensis* grown under fluorescent white light with a moderate light intensity of 27 µmol m^{-2} s^{-1} received sufficient light over a long period (16 h/day) and so the rate of photosynthesis was high, leading to a better growth rate. The optimum light intensity for the growth of *Arthrospira* sp. in Madhya Pradesh, India was only of moderate light intensity (Pandey and Tiwari 2010). Nevertheless, *A. platensis* cultured under the two conditions could grow in shrimp pond wastewater containing sufficient nutrients. Pink and red light-emitting diodes (LEDs) had the highest biomass productivity for *A. platensis* growth (Markou 2014).

Effects of various modifications of the culture on algal growth

The algae was cultured in three different medium formulations for 30 days. These formulations were: shrimp pond wastewater (100%), shrimp pond wastewater and Zarrouk medium at a ratio of 1:1, and Zarrouk medium alone. Each formula had a final volume of 5,000 ml, wherein new medium was added every 3 days. Daily cell counts showed that during the first 5 days, the growth rate of *A. platensis* among the three culture media were not significantly different among each other. The best growth rate was from wastewater mixed with Zarrouk medium (42.74 x 10^4 cells/ml) on the 26th day of culture. The highest cell number (18.37 x 10^4 cells/ml) was from shrimp pond wastewater during the 26th day,

which was significantly different from the cell number observed from Zarrouk medium alone, then growth became stationary. On the 28th day, the highest cell number of 38.35 x 10^4 cells/ml was observed from Zarrouk medium alone (Table 4 and Fig 5). Thus, shrimp pond wastewater combined with Zarrouk medium at a ratio of 1:1 was the best medium formula for algal growth. Shrimp pond wastewater also contained important nutrients, including $NaHCO_3$ (2.6 g/l), $NaNO_3$ (3.4 g/l), and K_2HPO_4 (0.13 g/l), which *A. platensis* needs; however, only a relatively small amount of these nutrients were present. The growth period for *A. platensis* growth was shortened because the nutrients were not sufficient enough to sustain prolonged algal growth. Therefore, it was necessary to add some nutrients (from the effluent or from Zarrouk medium) to enhance algal growth. Similarly, when $NaHCO_3$(8 g/l), $NaNO_3$ (1.5 g/l), and K_2HPO_4(0.1 g/l), were added into the medium to serve as a nutrient source, the algae cell number was higher than the control (Cheunbarn and Peerapornpisal 2010). Notwithstanding, shrimp pond wastewater can be used for *A. platensis* culture, only that more wastewater should be added during the middle or towards the end of the exponential phase. In another study, *A. platensis* was grown by adding culture medium every 4 days and it was subsequently found that *A. platensis* could grow well (Sarttarapan 1993).

Table 4. Highest cell number observed in different culture media.

Culture media	Highest cell number * (x 10^4 cells/ml)
Zarrouk	38.35 ±0.44[b]
Wastewater	18.37 ±0.16[a]
Wastewater mixed with Zarrouk (1:1)	42.74 ±0.16[c]

* : mean(from 3 replicates) ± SD
The same lowercase letter indicates no significant difference at 95% confidence level using Duncan's multiple range test

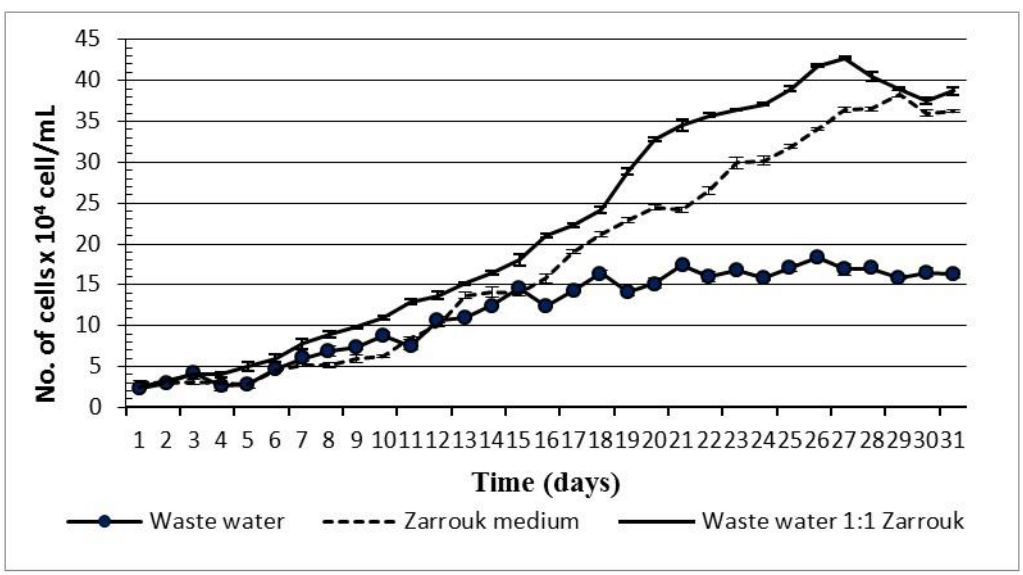


Fig. 5. Growth rate of *A. platensis* grown in shrimp pond wastewater, shrimp pond wastewater mixed with Zarrouk medium (1:1), and Zarrouk medium alone, with culture media added every 3 days.

Water quality measurement

Several physico-chemical parameters were measured, namely: BOD_5, PO_4, NH_3, and NO_3 content, and the heavy metal content of Zn, Pb, and Cu. Analysis of shrimp pond wastewater before and after algal treatment showed a decrease in BOD_5, heavy metal concentrations, and PO_4, NH_3, and NO_3 content in the water samples (Table 5).

Table 5. Water chemical properties of shrimp pond wastewater before and after algal treatment. The experiments were carried out in triplicate.

Parameter	Concentration (mg/l)		
	0 day*	30 days*	Standards**
BOD_5	3.98± 0.43	1.67± 0.23	< 20
PO_4-P	0.13± 0.12	0.02± 0.26	< 0.4
NH_3-N	2.60± 0.34	1.38± 0.17	< 1.1
NO_3-N	3.40± 0.08	2.49± 0.33	< 4
Zn	0.075± 0.22	0.039± 0.26	-
Pb	0.062± 0.45	0.036± 0.11	-
Cu	0.041± 0.03	0.018± 0.14	-

* : mean(from 3 replicates) ± SD

** Notification of the Ministry of Natural Resources and Environment in accordance with the regulations for the control of effluent quality standards of aquaculture ponds of brackish aquatic animals (The Royal Government Gazette 2007).

Heavy metal content was reduced after algal cultivation in wastewater from the shrimp farm for 30 days. This may have been because these heavy metals were absorbed by the algal cell membrane through ion exchange between the heavy metal and functional groups on the algal surface membrane (Sulaymon 2012). This may have caused the media to have a slightly lowered pH. These were similar to Palaniswamy and Veluchamy (2017) who reported that the cyanobacterium, *A. platensis* had the ability to absorb heavy metals, such as zinc, through biosorption. Thus, *A. platensis* culture from shrimp pond wastewater provides not only the yield, but also generates a by-product in terms of wastewater treatment. The biomass of *Acanthopora spicefera* can be efficiently used for the biosorbtion of heavy metals, such as Cr (VI), Cr (III), Hg (II), Cd (II), and Pb (II), from environmental sources (Tamilselvan et al. 2011). The metal-uptake ability of cyanobacteria means that it can used as an absorbent agent in the removal of heavy metals, such as chromium, cadmium, and lead (Anjana et al. 2007). The binding mechanism is further complicated by which of these elements can interact with microbial cells and can be accumulated as a result of different mechanisms (Gadd 2011). For example, the binding mechanism of lead to the algal biomass includes a combination of ion exchange, chelation, and reduction reactions, accompanied by metallic lead precipitation on the cell walls. The cell wall constituents of *Arthospira* sp., such as carboxyl, hydroxyl, sulphate, and other charged groups, which interact with positively-charged heavy metal ions are chiefly responsible for adsorbing heavy metals on their surface (Rai and Tripathi 2007, Palaniswamy and Veluchamy 2017). Cyanobacteria, including *Oscillatoria* sp., *Phormidium* sp., *Aphanocapsa* sp., *Westiellopsis* sp., and *Anabaena* sp., are capable of absorbing heavy metals at a high rate and also provide favorable conditions for the removal of heavy metals from the effluent. This is because their interior pH is higher than the surrounding water, so it provides resistance to the mass transfer of products out of the biofilm (Vijayakumar 2012). However, *Spirulina* grown in an uncontrolled environment has the potential to absorb heavy metals and other toxins so it is important to ensure that it is toxin-free. The concentration of six typical heavy metals/minerals (Ni, Zn, Hg, Pt, Mg, and Mn) from 25 Spirulina products for direct human consumption were examined by Coupled Plasma Mass Spectroscopy (ICP–MS). They were found not to exceed present regulation levels and can be considered as safe (Al-Dhabi 2013).

During the beginning of the study, organic substances were present in the shrimp pond wastewater. After *A. platensis* culture, the organic substances were decomposed by bacteria. The organic substances were also used by *A. platensis* during photosynthesis and for their metabolic processes. This helped improve wastewater quality leading to a lower BOD_5. The results were supported Chuntapa et al. (2003), who reported that *A. platensis* could effectively utilize nitrogen in the form of nitrate, ammonia, and nitrite, as it could reduce nitrate concentration in water from 16-18 mg/l to 4mg/l, and ammonia and nitrite to 0.0-0.15 mg/l in one day, depending on the number of shrimps in the pond. Nitrogen derived from sodium nitrate (20.7 g/l) can be used to produce 1.992 g of algae (Costa et al. 2001). A sudden drop in nitrogen content from waste water treated with *Arthrospira* sp. was observed since it used nitrogen for photosynthesis and algal growth. Nitrogen is one of the important components of the algal genetic material, some amino acids (glycine, serine, alanine, glutamic acid, and aspartic acid) and some pigments, such as chlorophyll. Therefore, limited amounts of nitrogen can result in lowered protein and chlorophyll content (Richardson et al. 1969). Replacing nitrate with urea in *Arthrospira* sp. culture resulted in an increased growth of *Arthrospira* sp. but no change in final chlorophyll content (Denesi 2002). This finding was consistent with Feng and Wu (2006) wherein algae successfully grew in diluted human urine, with the highest biomass production at urine dilution ratios of 140-240. PO_4-P played an important role in maintaining high algal productivity. Mostert and Grobbelaar (1987) reported that algal productivity increased with an increase P supply to 2 mg/l. Our current findings showed that *A. platensis* reduced PO_4 from 0.13 g/l to 0.02 g/l, which indicated that microalgae could remove 84% PO_4 from the effluent. Similarly, in another study, *A. platensis* decreased PO_4 content by 81.49% while *Chlorella vulgaris* decreased PO_4 content by 88%, with a treatment of 0.45 g/l (Sayadi et al. 2016).

CONCLUSION

Our results suggest that the waste water discharged from a shrimp farm in Nakhon Pathom province, Thailand was a powerful stimulant for algal culture. The best formula for algal production was wastewater, together with $NaHCO_3$ at 60% the amount from Zarrouk medium, as well as $NaNO_3$and K_2HPO_4, under fluorescent light for 16 h/day. The highest cell number was observed during the 21st day of culture. The mass culture of *Arthrospira* sp. can provide as a source for dietary supplements, whole food, livestock and fish feed, as well as for pharmaceuticals.

Application of *A. platensis* for shrimp farm waste water treatment has many advanctages. It is low-cost, low-energy, maintains low N and P content, removes heavy metals, and lowers BOD_5 levels. Thus, waste water discharged from a shrimp farm in Nakhon Pathom province, Thailand, can be used as a cheap nutrient source for algal production, while at the same time providing a natural and cost-effective waste water treatment process.

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