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Full Length Article

Production and Nutritional Values of *Arthrospira platensis* Strain Aspi.MJU2 from Wastewater of Organic Cafeteria of Maejo University

Jongkon Promya*, Bunyat Montien-Art and Chanagun Chitmanat
Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiangmai 50290, Thailand
*For correspondence: jongkolp@mju.ac.th

Abstract

The research aimed to compare growth, nutrition values, pigments, and costs of *Arthrospira platensis* strainAspi.MJU2 cultured in wastewater. The CRD was applied; there were three treatments with 3 replications. Algal species was cultured in the raceway pond: T1 (control) *A. platensis* (Aspi.MJU2) cultured in Modified Zarrouk's Medium (MZm), T2 Aspi.MJU2 cultured in organic cafeteria wastewater (70% OCW) and T3 Aspi.MJU2 cultured in cafeteria wastewater (70% CW) of Maejo University, Thailand. The data were collected every 5 days for a 20-day culture period. The result showed that productions and carotenoids of Aspi.MJU2 in MZm and 70% OCW were significantly higher than other experiments. Cost and protein content of Aspi.MJU2 in 70% OCW were better than other treatments. The amounts of lead and mercury in Aspi.MJU2 from all experiments were below detection limit. It can be concluded that MZm and 70% OCW provided greater production and carotenoid than 70% CW but 70% OCW generated better production costs and protein than other treatments. The 70% OCW could be used as a cultured formula of Aspi.MJU2 to produce the safe fish feed. © 2018 Friends Science Publishers

Keywords: *Arthrospira;* Cafeteria wastewater; Production; Safe fish feed

Introduction

The *Arthrospira platensis* is a blue-green spiral coiled cyanobacterium. The spiral number and tightness vary among species (Promya *et al*., 2008). Their benefits are rich in nutrients including protein, vitamins, and valuable minerals. The *A. platensis* showed anti-cancers and possess antimicrobials (such as bacteria, fungi and viruses) because it contains phycocyanin, phycocyanobilin, allophycocyanin pigments and other precious nutrients (Nuhu, 2013). Generally, *A. platensis* is cultured for a healthy human food. The normal nutrients for *A. platensis* culture include $NaHCO_3$ 16.80 g L^{-1}, K_2HPO_4 0.50 g L^{-1}, $NaNO_3$ 2.50 g L^{-1}, NaCl 1.00 g L^{-1}, $MgSO_4$ 0.20 g L^{-1}, $FeSO_4$ 0.50 g L^{-1}, K_2SO_4 1.00 g L^{-1}, $CaCl_2$ 0.04 g L^{-1}, EDTA 0.08 g L^{-1}, while Modified Zarrouk's Medium (MZm) containing $NaHCO_3$ 6 g L^{-1}, $NaNO_3$ 1 g L^{-1}, NaCl 1 g L^{-1}, $MgSO_4$ 1 g L^{-1} and N:P:K (16:16:16) 0.6 g L^{-1} to be applied for *A. platensis* culture for animal feed (Promya *et al*., 2005). Normally, *A. platensis* are cultured in a raceway pond, which is relatively easy to be harvested (Milledge, 2010). To cut down the chemical fertilizer cost, wastewater rich in nitrogen could be used as culture medium. The 100% cafeteria wastewater was used to culture *A. platensis* and a dry weight of 0.70 g L^{-1} was harvested; its protein and fat amounts were 48–50% and 10–15%, respectively (Promya *et al*., 2006). In addition, *A. platensis* cultured in 50% effluent from pig farms gave 51% protein by dry weight (Promya, 2000). The culture of *A. platensis* in dormitory wastewater at concentration of 70% with NH_3-N 1.44±0.56 g L^{-1}, NO_3-N 0.68±0.07 g L^{-1} and PO_4-P 0.49±0.12 g L^{-1} produced alga with protein of 48–50%, fat 8–15% and carotenoid 187.89 mg g^{-1} by dry weight (Promya, 2002).

Algae have been used as dietary supplements as well as a fishmeal replacement to stimulate growth and immunity in various aquatic animals. Red tilapia (*Oreochromis* sp.) fries fed with *A. platensis* had the better survival rate, increased red and white blood cells as well as enhanced immunity. (Promya *et al*., 2008). Moreover, the 5% *Cladophora*+basal diets feed could be used as African Sharptooth Catfish (*Clarias gariepinus*) feed in order to improve the carotenoid levels in the catfish, while the 5% *Arthrospira*+basal diets feed was more suitable for increasing the immunity of the catfish. Both *A. platensis* and *Cladophora* had the positive effects on growth performances, meat quality and immune stimulation of the African Sharptooth Catfish (Promya and Chitmanat, 2011).

There are now increase in the safety issues on fish products leading to more attention in an organic aquaculture. *A. platensis* production can be applied as an alternative protein source for organic aquaculture. In order to meet the standards of organic farming, every step including brood stock and fry management, feed and feeding, water treatment, disease prevention, capture,

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product transport, and post-harvest techniques must be environmental friendly practices. Therefore, the *A. platensis* strain Aspi.MJU2 was isolated in Chiang Mai and the effluent from the cafeteria of Maejo University was applied to culture this algal species. This research aimed to compare growth, nutrition values, pigments, and costs of Aspi.MJU2 cultured in wastewaters to produce a safety feed and to develop organic aquaculture according to the urgent policies worldwide not only in the Maejo University and Thailand.

Materials and Methods

A. platensis (Aspi.MJU2) Algae Preparation

The Aspi.MJU2 was cultured in Modified Zarrouk 's Medium (MZm) nutrients as: 6 g L^{-1} of $NaHCO_3$ (Qingdao Co., LTD, China), 0.5 g L^{-1} of $NaNO_3$ (Qingdao Co., LTD, China), 1 g L^{-1} of NaCl (Purity Salt Industry, Co., LTD, Thailand), 1 g L^{-1} of $MgSO_4$ (UTID Enterprise Co., LTD) and 0.6 g L^{-1} of N: P: K (16: 16: 16, YARA International ASA Co., LTD, Norway), adjusted to pH 10±0.5 using NaOH (Promya *et al*., 2005). Aspi.MJU2 was cultured in MZm in a 50 L aquarium, the initial optical density (OD) at 560 nm reached 0.30 (OD_{560}nm=0.30) before adding the inoculum of Aspi.MJU2. All cultures were continuously aerated and allowed to grow for 2 weeks until the optical density (OD) at 560 nm reached 1 (OD_{560}nm=1).

Preparation of Wastewater

The organic cafeteria wastewater (70% OCW) where raw materials for cooking are from organic farms and cafeteria wastewater (70% CW) of Maejo University were prepared. These concentrations were suitable for the growth of Aspi.MJU2 under previous laboratory research. Then, algae were cultured in outdoor ponds with MZm, 70% OCW and 70% CW for a period of one month.

Experiment Plan

The Aspi.MJU2 was cultured in raceway ponds under the completely randomized design (CRD) with the triplicate of three treatments. The experiment had been run for a period of two months as follows:

T1 (control): *A. platensis* (Aspi.MJU2) cultured in Modified Zarrouk 's Medium (MZm),

T2:Aspi.MJU2 cultured in organic cafeteria wastewater (70% OCW) of the Maejo University,

T3:Aspi.MJU2 cultured in cafeteria wastewater (70% CW) of the Maejo University.

Growth Analysis

The stocks of Aspi.MJU2 used in this experiment had the initial optical density (OD) at 560 nm of 0.30 (OD_{560}nm=0.30) before inoculum. All cultures were continuously aerated and allowed to grow up to 20 days. The optical density (OD) at 560 nm was measured every five days until it reached 0.8 (OD_{560}nm=0.8). To analyze the specific growth rate of cells, $\mu=\ln(N_2) - \ln(N_1)/t_2-t_1$, μ = specific growth rate of cells (units/ day); N_1 = concentration of cells at time t_1 (cells/mL); N2 = concentration of cells at time t_2 (cells/mL); t_1 = the first sampling time (day); t_2 = the second sampling time (day) at the time t_1, t_2 growing cell time in the Log phase (Kitprechawanich, 1996). The raw and dry Aspi.MJU2, during cultivation production cycle every 20 days were compared (Promya and Saetun, 2005).

Nutritional Value Analysis

The raw Aspi.MJU2 was baked at a temperature of 60ºC for 48 h and then protein, fat, fiber, ash, moisture, carbohydrates, carotenoid, C-phycocyanin, lead and mercury were analyzed (Berns *et al*., 1963; Jauncey and Ross, 1982; AOAC, 1990).

Physical and Chemical of the Water Quality Analysis

The physical and chemical parameters of the water quality before, during and after the trial were determined. In each treatments, their physical and chemical parameters (Traichaiyaporn, 2000) were measured every five days until the completion of the trial and water parameters: water temperature using a thermometer, pH using pH meter (Schott-Gerate CG 840), dissolved oxygen (DO) by Azide modification method, ammonia-N by Direct Nesslerization method, Nitrate - nitrogen by Phenol disulfonic acid method and orthophosphate - phosphorus by Stannous chloride method.

Statistical Analysis

Fish growth performances, nutritional values, carotenoid, C-phycocyanin, production costs, lead and mercury and water quality in each trial were compared. Data were presented as mean values±standard deviation. Comparison of mean were made by one way-analysis of variance (ANOVA) treatment of the difference between the average and compared by Duncan's Multiple Range Test (DMRT) at a significance level of $p<0.05$ (Steel and Torrie, 1980).

Results

Growth Performances

The highest specific growth rates of Aspi.MJU2 cells found after five days of culture; Aspi.MJU2 cultured in MZm day and 70% OCW were 10±0.14 units/and 9.38±0.13 units/day, respectively. But Aspi.MJU2 cultured in 70% CW and 70% OCW at ten days had greater specific growth rates of cells than MZm (Fig. 1).

Table 1: Production and cost of Aspi.MJU2 each treatment after 20-day cultivation

Treatments	Wet weight of algae ($g\ L^{-1}$)	Dry weight of algae ($g\ L^{-1}$)	Variable costs (Baht kg^{-1})
Modified Zarrouk 's Medium (MZm)	3.52 ± 0.25^{a}	0.44 ± 0.03^{a}	420.67 ± 20.43^{a}
organic cafeteria wastewater; 70%OCW	3.18 ± 0.20^{b}	0.40 ± 0.03^{b}	327.67 ± 21.22^{b}
cafeteria wastewater; 70%CW	2.55 ± 0.13^{c}	0.32 ± 0.02^{c}	410.33 ± 18.55^{a}

Note: Different letters (a,b,c,d) show statistically significant differences ($p<0.05$)

Table 2: Average levels of nutritional values in Aspi.MJU2 (%dry weight) of each treatment

Treatments	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)	Fiber (%)
Modified Zarrouk 's Medium (MZm)	6.74 ± 0.31^{b}	50.16 ± 3.74^{b}	0.76 ± 0.13^{b}	22.20 ± 1.31^{a}	17.59 ± 2.93^{a}	2.55 ± 0.43^{c}
Organic cafeteria wastewater;70%OCW	6.53 ± 0.25^{b}	56.25 ± 1.14^{a}	1.27 ± 0.14^{a}	17.03 ± 2.63^{b}	14.87 ± 2.47^{b}	4.05 ± 0.91^{a}
Cafeteria wastewater; 70%CW	7.67 ± 0.06^{a}	51.62 ± 0.96^{b}	0.73 ± 0.16^{b}	21.72 ± 0.51^{a}	15.33 ± 0.66^{b}	2.93 ± 0.39^{b}

Note: Different letters (a,b,c,d) show statistically significant differences ($p<0.05$)

Table 3: Average of carotenoids, C-phycocyanin, lead and mercuryin Aspi.MJU2 of each treatment

Treatments	Carotenoids (mg g^{-1})	C-phycocyanin (mg g^{-1})	Lead (Pb) (mg g^{-1})	Mercury (Hg) (mg g^{-1})
Modified Zarrouk 's Medium (MZm)	5.86 ± 1.10^{a}	6.18 ± 1.46^{ns}	ND	ND
Organic cafeteria wastewater; 70%OCW	3.89 ± 0.55^{b}	6.12 ± 0.50^{ns}	ND	ND
Cafeteria wastewater; 70%CW	2.80 ± 0.65^{c}	5.92 ± 0.20^{ns}	ND	ND

Note: Different letters (a,b,c,d) show statistically significant differences ($p<0.05$), ns=no significant difference, ND= not detected

Table 4: Average water quality of cultured Aspi.MJU2 raceway pond systems

Treatments	Water Tem.(°C)	pH(Units)	DO (mg L^{-1})	NH_3-N(mg L^{-1})	NO_3-N(mg L^{-1})	PO_4-P(mg L^{-1})
Modified Zarrouk 's Medium (MZm)	30.20 ± 0.00^{ns}	10.16 ± 0.05^{ns}	8.05 ± 1.06^{ns}	0.14 ± 0.02^{ns}	11.30 ± 0.86^{ns}	13.01 ± 1.95^{ns}
Organic cafeteria wastewater; 70%OCW	30.40 ± 0.00^{ns}	10.17 ± 0.02^{ns}	7.75 ± 1.67^{ns}	0.15 ± 0.03^{ns}	10.61 ± 0.31^{ns}	12.58 ± 3.18^{ns}
Cafeteria wastewater; 70%CW	30.40 ± 0.00^{ns}	9.93 ± 0.06^{ns}	6.93 ± 0.67^{ns}	0.19 ± 0.04^{ns}	12.67 ± 2.03^{ns}	12.11 ± 1.54^{ns}

Note: Different letters (a,b,c,d) show statistically significant differences ($p<0.05$), ns = no significant difference

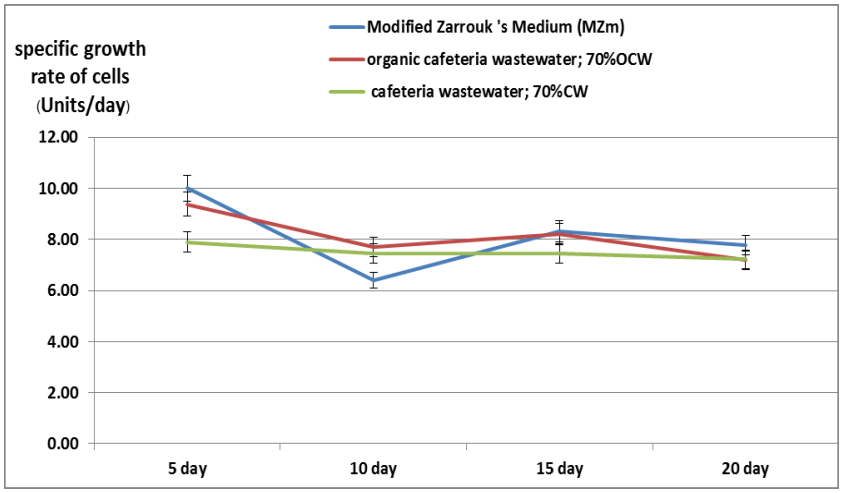

Fig. 1: Specific growth rates of Aspi.MJU2 cells in each treatment

Production and Costs

The raw and dry production of Aspi.MJU2 cultured for 20 days were determined. The production of raw Aspi.MJU2 in MZm was 3.52 ± 0.25 g L^{-1} while dried Aspi.MJU2 was 0.44 ± 0.03 g L^{-1}, which was more than cultured Aspi.MJU2 in 70% OCW and 70% CW. The lowest production cost of dry Aspi.MJU2 found in 70% OCW was 327.67 ± 21.22 Baht kg^{-1} which was cheaper than Aspi.MJU2 cultured in MZm and 70% CW (Table 1).

Nutritional Value Analysis

The protein, fat and fiber levels of Aspi.MJU2 cultured in

70% OCW was significantly ($p < 0.05$) higher than ones cultured in 70% CW and MZm. The carbohydrate content of Aspi.MJU2 cultured in MZm and 70% CW was significantly ($p < 0.05$) higher than the one cultured in 70% OCW. The Ash levels of Aspi.MJU2 cultured in MZm was significantly ($p < 0.05$) higher than in the ones cultured in 70% CW and 70% OCW. The Moisture levels of Aspi.MJU2 cultured in 70% CW was significantly ($p < 0.05$) higher than in the ones cultured in MZm and 70% OCW (Table 2).

Carotenoid, C-Phycocyanin, Lead and Mercury Analysis

The carotenoid of Aspi.MJU2 cultured in MZm 70% OCW was significantly ($p < 0.05$) higher than carotenoids in 70% CW and 70% OCW. The C-phycocyanin contents were not significantly different among three treatments. The amounts of lead and mercury in Aspi.MJU2 from all three treatments were under detection limits (Table 3).

The Water Quality of Cultured Aspi.MJU2 Raceway Pond Systems

The water temperature, pH, DO, NH_3–N, NO_3-N and PO_4–P were not significantly different among three treatments (Table 4).

Discussion

Referring to the literatures, there are many studies on the *Arthrospira* cultured in different wastes like kitchen wastewater (Promya *et al*., 2008), swine wastewater (Mezzomo *et al.*, 2010), and confectionary industry effluent (Hala *et al*., 2015). This work aimed to treat the wastewater from on-campus cafeteria by growing *A. platensis*.

The specific growth rates of Aspi.MJU2 cultured in MZm was 10±0.14 units/day and the one in 70% OCW was 9.38±0.13 units/day, which were more than 70% CW. Similar to the experiment of Susanna *et al.* (1995), the specific growth rate of Aspi.MJU2 cultured in water containing organic materials was 8-10 units/day. The alternative use of chemical fertilizers to grow Aspi.MJU2 is to employ organic wastewaters. Because cafeteria wastewater is rich in nitrogen, algae can be used either in the form of organic matter (Chuntapa *et al*., 2003). Blue green algae can be used to reduce inorganic and organic pollutants from agro-industrial wastewater and subsequently their products possibly generate biodiesel and animal feeds (Markou and Georgakakis, 2011).

Raw Aspi.MJU2 cultured in MZm was 3.52±0.25 g L^{-1} while dried production was 0.44 ± 0.03 g L^{-1} which was higher than Aspi.MJU2 cultured in 70% OCW and 70% CW sources. Similar to the cultivation of Aspi.MJU2 in Modified Zarrouk 's Medium (MZm) conducting by Promya and Chitmanat (2013) the yield of dried Aspi.MJU2 was 0.42 ± 0.03 g L^{-1}. The dilution of wastewater is one of the most important factors that need to be considered during algae cultivation. Laliberté *et al.* (1997) reported the digested waste must be diluted before culturing *Arthrospira* due to the ammonia toxicity.

Dried Aspi. MJU2 cultured in 70% OCW was 327.67± 21.22 baht kg^{-1} which was better than Aspi.MJU2 cultured in MZm and 70% CW. The production cost of Aspi.MJU2 was less than *A. platensis* production cost of Promya and Chitmanat (2013) cultured in Modified Zarrouk 's Medium (MZm), 517.13 ± 27.73 baht kg^{-1}. It was possible that the water from the organic cafeteria wastewater (70% OCW) had suitable nitrogen concentration for algae growth and provided a lower operating cost (Traichaiyaporn, 1994).

The protein of Aspi.MJU2 cultured in 70% OCW was 56.25±1.14% dry weight, which was more than other treatments. The *A. platensis* protein cultured in 25% medium mixed with 0.1% fish fermented water was 53.48±0.02% (Thaiudomsap, 2009). The fat and crude fiber in Aspi.MJU2 cultured in 70% OCW were 1.27± 0.14% and 4.05±0.91%, respectively which were more than other treatments. It could be noticed that algae cultured in the cafeteria wastewater had higher fat and fiber than ones in the MZm due to the cafeteria wastewater had carbohydrate, fat and oil as a carbon source, while MZm has sodium bicarbonate as a carbon source (Promya and Traichaiyaporn, 2010).

The carbohydrates of Aspi.MJU2 cultured in MZm and 70% CW were 22.20±1.31% and 22.20±1.3%, which were more than ones in 100% OCW. The carbohydrates in *A. platensis* cultured with 0.1% mackerel fermented water was 24.15±0.29% (Reungsomborn *et al*., 2010). The ash of Aspi.MJU2 cultured in MZm was 17.59 ± 2.93% which was more than other treatments. However, Thaiudomsap (2009) reported that the ash in *A. platensis* cultured in organic nutrients was 11.07± 0.01% because the MZm containing phosphorus as an ingredient results in high ash in the algae (Promya and Traichaiyaporn, 2010). Moisture of Aspi.MJU2 cultured in 70% CW was 7.67±0.06% which was more than other treatments. This moisture content was similar to *A. platensis* cultured in 100% cafeteria wastewater, 7.72±0.24% (Promya and Traichaiyaporn, 2010). The disadvantage of applying wastewaters as algae culture medium is the variation in their composition (Markou and Georgakakis, 2011).

The carotenoid of Aspi.MJU2 cultured in MZm was 5.86±1.10 mg g^{-1} which was more than other treatments; a similar to experiments of Promya (2002), carotenoids in *A. platensis* cultured in dormitory wastewater at Maejo University was 7.89 mg g^{-1} dry weight.

The C-phycocyanin of Aspi.MJU2 was between 5.92±0.0.20-6.18 ± 1.46 mg g^{-1}, which was not significant differences among three treatments. A similar experiments of Promya and Chitmanat (2013), *A. platensis* cultured in Modified Zarrouk's Medium (MZm) provided 5.10 mg g^{-1} of C-phycocyanin.

The amount of lead and mercury in Aspi.MJU2 from

three treatments were below detection limits. The lead (Pb) content in Aspi.MJU2 was less than 1 mg kg^{-1} and the amount of mercury in Aspi.MJU2 was not more than 0.5 mg kg^{-1} which both were safe for consumption (FAO, 2004).

The water temperature of Aspi.MJU2 culture was 30.20±0.00-30.40±0.00ºC which was not significant difference among treatments. The similar water temperatures were found when *A. platensis* cultured in 90% and 100% cafeteria wastewaters, 30.90-30.97°C (Promya and Traichaiyaporn, 2010) and temperature in the range of 30-35°C (Somasekaran, 1987). The pH of Aspi.MJU2 culture was 9.93±0.06-10.17±0.02 units, which was not significant difference among treatments. The similar pH of *A. platensis* cultured in the palm oil wastewater, 9.71-10.24 (Kaewkeuix and Ariyadech, 2008) and *Arthrospira* grew well at pH values between 9–11 (Vincent and Silvester, 1979). The dissolved oxygen of Aspi.MJU2 culture was 6.93±0.67–8.05±1.06 mg L^{-1}, which was not significant difference among three treatments, similar dissolved oxygen was observed in *A. platensis* cultured in 100% cafeteria wastewater, 6.82± 0.12 mg L^{-1} (Promya and Traichaiyaporn, 2010). And similar dissolved oxygen to the *A. platensis* cultured in noodles factory wastewater, 6.57 mg L^{-1} (Boonnakn, 2010).

The ammonia-N of water in Aspi.MJU2 culture was 0.14± 0.00–0.19± 0.004 mg L^{-1}, which was no significant differences among treatments. The similar to the *A. platensis* cultured in 0.1% mackerel fermented mixed 0.025% acacia leaves fermented was 0.18±0.02 mg L^{-1} (Thaiudomsap, 2009). The nitrate-N of water in Aspi.MJU2 culture was 10.61± 0.31–12.67±2.03 mg L^{-1}, which was no significant differences among treatments. The similar to the *A. platensis* cultured in shrimp wastewater treatment was 12–16 mg L^{-1} (Chuntapa *et al*., 2003). The orthophosphate-P of water in Aspi.MJU2 culture was 12.11±1.54–13.58±3.18 mg L^{-1}, which was no significant differences among treatments. The similar to the *A. platensis* cultured in wastewater from noodles factory was 11.80 mg L^{-1} (Boonnakn, 2010).

Conclusion

Aspi.MJU2 cultured in MZm and 70% OCW provided greater production and carotenoid than the one cultured in 70% CW but the application of 70% OCW had lower production costs and higher protein than other treatments. The 70% OCW could be used as a culture medium of Aspi.MJU2 to reduce the variable cost of the *A. platensis* supplement in aquaculture feed.

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