Research Article

APPLIED FOOD BIOTECHNOLOGY, 2020, 7(4): 225-234
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

Photoautotrophic Cultivation of *Arthrospira maxima* for Protein Accumulation under Minimum Nutrient Availability

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Abstract

Background and Objective: *Arthrospira* cyanobacteria are important freshwater blue-green microalgae as food protein sources for humans. *Arthrospira maxima* phototrophic cultivation was carried out under various minimum media formulations to enhance biomass and protein productions. Macronutrients and micronutrients are vital for biomass production and protein accumulation of *Arthrospira maxima* for food supplemented uses.

Materials and Methods: Photoautotrophic cultivation of *Arthrospira maxima* IFRPD 1183 was carried out using various Zarrouk culture media and inoculum preparation conditions for biomass and protein productions. *Arthrospira maxima* IFRPD 1183 was cultivated using algal chamber under closed and open photobioreactor systems.

Results and Conclusion: Micronutrients of B_6 solution; NH_4VO_3, $K_3Cr_2(SO_4)_4.24H_2O$, $NiSO_4.7H_2O$, Na_2WO_3, $Co(NO_3)_2.6H_2O$ and $Ti_2(SO_4)_3$ did not affect cell growth and protein accumulation in absence of media for inoculum production. Inoculum preparation under various conditions of *Arthrospira maxima* IFRPD 1183 was studied using filtration of the old media before use (cell filtration inoculum) with no filtrations of the old media before use (cell non-filtration inoculum). Cell non-filtration inoculum preparation was reported appropriate for biomass production and protein accumulation. For large-scale productions, open pond system of *Arthrospira maxima* IFRPD 1183 resulted in maximum biomass and protein production at nearly 1 g l^{-1} and 64% (DW), respectively, for repeat batch photoautotrophic cultivation. Absence of micronutrients with cell non-filtration inoculum was reported as an easy process to achieve large biomass and protein productions for closed and open photoautotrophic cultivations of *Arthrospira maxima* at decreased costs.

Conflict of interest: The authors declare no conflict of interest.

Article Information

Article history:
Received 08 May 2020
Revised 02 June 2020
Accepted 10 July 2020

Keywords:
- *Arthrospira*
- Biomass
- Cyanobacteria
- Nutrients
- Photoautotrophic
- Protein

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How to cite this article

Pan-utai W, Poopat N, Parakulsuksatid P. Photoautotrophic Cultivation of Arthrospira maxima for Protein Accumulation under Minimum Nutrient Availability, Appl Food Biotechnol. 2020; 7(4): 225-234. http://dx.doi.org/10.22037/afb.v7i4.30353

1. Introduction

The *Arthrospira* genus includes a group of photosynthetic, filamentous multicellular cyanobacteria (blue-green microalgae) such as *Arthrospira platensis* and *Arthrospira* (*A.*) *maxima* [1,2]. *Arthrospira* spp. include rich sources of protein contents of 50-70% dry weight, vitamins and minerals, which depend on cultivation conditions [1,3]. Lack of cellulose in the cell wall of *Arthrospira* spp. facilitates digestion, compared to microalgae and macroalgae. Moreover, *Arthrospira* spp. have been reported to include therapeutic properties such as antivirus, antitumor, antioxidant, anti-inflammatory, antiallergic, antidiabetic and antibacterial properties with the ability to treat several diseases [4]. *Arthrospira* spp. have been used as edible nutrients and protein and amino acid sources for malnourished people in several regions of the world. Moreover, *Arthrospira* biomass can be used in foods as generally recognized as safe, granted by Food and Drug Administration [5]. *Arthrospira* spp. occur naturally under alkaline culture conditions. Developing trichomes of *Arthrospira* include a couple of millimeter in length and cylindrical cells of 3-12 µm in diameter. Trichome is typically masterminded as a tight helix and replicates by the

discontinuity of the developing trichome into shorter filaments through the breakdown of particular intercalary cells of necridia [6].

Arthrospira spp. can grow under various conditions, including photoautotrophically (using light as the energy and CO_2 as the carbon sources), heterotrophically (on organic carbon compounds) and mixotrophically (simultaneously in light and on organic carbon compounds). Photoautotrophic is an economically viable method for large-scale commercial cultivations of microalgae. Open outdoor autotrophic cultivation methods are widely carried out to minimize energy requirements [7]. Moreover, open raceway pond system was initially used for the commercial culture of *Arthrospira* and is now used for mass biochemical production of several other microalgal strains [8]. Open systems are widely used for large-scale microalgal cultures due to their low production costs, low power needs, appropriate scale ups and easy cleaning processes, compared to closed photobioreactors (PBRs) [9].

Several factors have been reported for *Arthrospira* spp. biomass and value-added productions, including light, temperature and nutrient availability [10]. Moreover, culture media of *Arthrospira* include high concentrations of hydrogen carbonate as inorganic salts with alkaline pH values of 9-10 [11]. *Arthrospira* spp. grow under high alkaline conditions leading to high hydrogen carbonate quantity and production costs of the culture media. Cultivation media include great effects on productivity of biomass and other compounds in cells [12]. Nitrogen concentrations in media importantly affect *Arthrospira* productivity [13]. Previous studies have been focused on other sources of nitrogen macronutrients such as urea, ammonium nitrate, ammonia and ammonium chloride to decrease production costs of *Arthrospira* spp. [14-18]. However, a few studies have investigated micronutrients for the cultivation of *Arthrospira* spp. *Arthrospira* media contain several nutrients that affect costs [16]. Therefore, phototrophic cultivation of *A. maxima* was carried out under various minimum media formulations to maximize biomass and protein productions in the current study.

2. Materials and Methods

2.1. Microalgal strain and media maintenance

The *A. maxima* IFRPD 1183 microalga was provided by the Institute of Food Research and Product Development (IFRPD), Kasetsart University, Thailand. First, *A. maxima* IFRPD 1183 was cultured in Zarrouk liquid media, containing the following compounds (g l^{-1}): $NaHCO_3$ (16.8), $NaNO_3$ (2.5), K_2HPO_4 (0.5), K_2SO_4 (1.0), NaCl (1.0), $MgSO_4$. $7H_2O$ (0.2), $CaCl_2$. $7H_2O$ (0.04), $FeSO_4.7H_2O$ (0.01) and EDTA (0.08). Micronutrients as 1 ml of each A_5 and B_6 solutions were added into Zarrouk media. The A_5 solution contained compounds as follows (g l^{-1}): H_3BO_3 (2.86), $MnCl_2$. $4H_2O$ (1.81), $ZnSO_4$. $7H_2O$ (0.22), $CuSO_4$. $5H_2O$ (0.08), MoO_3 (0.01). The B_6 solution contained the following compounds (mg l^{-1}) of NH_4VO_3 (22.9), $K_3Cr_2(SO_4)_4$. $24H_2O$ (96.0), $NiSO_4$. $7H_2O$ (47.8), Na_2WO_3 (17.9), Co $(NO_3)_2$. $6H_2O$ (44.0) and $Ti_2(SO_4)_3$ (40). All chemicals were purchased from Ajax Finechem, NZ. Cell cultures were stored in Zarrouk liquid media at a controlled temperature of 25 °C [19].

2.2. Inoculum preparation

Inoculum preparation of *A. maxima* IFRPD 1183 was carried out using light-transparent glass bubble columns with 3.22 cm of internal diameter, 0.39 cm of thickness and 33.61 cm of height. The PBR was incubated in chamber equipment with temperature controlled at 30 °C. Light intensity included 162 µmol m^{-2} s^{-1} with 18-W daylight fluorescent lamps and 16:8 h light/dark cycles. Continuous air bubbles mixed with 2% CO_2 at a flow rate of 0.67 v v^{-1} m^{-1} were passed through PTFE membrane filters. Inoculums of *A. maxima* IFRPD 1183 were prepared in 50 ml of Zarrouk media and stored for three days. Then, inoculums were added with 100 ml of Zarrouk media into the culture column and stored for three days or until the optical density at 560 nm was 1. An *A. maxima* IFRPD 1183 preculture was used as inoculum. The inoculum density was 10% (v v^{-1}), corresponding to an OD_{560} of 0.1.

2.3. Photoautotrophic cultivation

2.3.1. Inoculum media

Various media were studied for the inoculum preparation and cell cultivation (Table 1). The *A. maxima* IFRPD 1183 was used as inoculum and adjusted to 10% (v v^{-1}) in working volume of photoautotrophic cultivation (OD_{560} 0.1) studied in a similar inoculum media. Initial pH of the media was 9.0 ±1.0. All experiments were carried out in triplicate.

Table 1. Media composition of *Arthrospira maxima* IFRPD 1183 under photoautotrophic cultivation

Component	Medium composition				
	ZM*	ZM1	ZM2	ZM3	ZM4
K_2HPO_4 (g l^{-1})	0.5	0.5	0.5	0.5	0.5
$NaNO_3$ (g l^{-1})	1.5	1.5	1.5	1.5	1.5
K_2SO_4 (g l^{-1})	1.0	1.0	1.0	1.0	1.0
NaCl (g l^{-1})	1.0	1.0	1.0	1.0	1.0
$MgSO_4.7H_2O$ (g l^{-1})	0.2	-	0.2	0.2	0.2
$CaCl_2.2H_2O$ (g l^{-1})	0.04	0.04	0.04	0.04	0.04
$FeSO_4.7H_2O$ (g l^{-1})	0.01	-	0.01	0.01	0.01
EDTA (g l^{-1})	0.08	0.08	0.08	0.08	0.08
A_5 (ml)	1	1	-	1	-
B_6 (ml)	1	1	1	-	-
$NaHCO_3$ (g l^{-1})	8.5	8.5	8.5	8.5	8.5

* Modified Zarrouk medium used as a standard medium

A_5 solution (g l^{-1}): H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.22; $CuSO_4.5H_2O$, 0.08; MoO_3, 0.01.

B_6 solution (mg l^{-1}): NH_4VO_3, 22.9; $K_3Cr_2(SO_4)_4.24H_2O$, 96.0; $NiSO_4.7H_2O$, 47.8; Na_2WO_3, 17.9; Co $(NO_3)_2.6H_2O$, 44.0; $Ti_2(SO_4)_3$, 40.

2.3.2. Growth media

Two various methods of inoculum preparation (10% v v^{-1}) were carried out, including 1) filtration of the media residues through 60-µm nylon membranes and washing cells with sterile water before use as inoculum (cell filtration, CF) and 2) cell with no filtrations (cell non-filtration, CNF). First, *A. maxima* IFRPD 1183 was cultured in culture media protein accumulation in cells, which was improved using various inoculum preparation methods and growth media. The *A. maxima* IFRPD 1183 photo-autotrophic cultivation was carried out in five various culture media compositions (Table 1). Various nutrient compositions were modified from Zarrouk media to minimum essential nutrients, while modified Zarrouk media (ZM) were used as control media. Cultures were incubated under similar conditions using chambers for CNF and CF inoculum preparations. Initial pH of the media was 9.0 ±1.0. All experiments were carried out in triplicate.

2.3.3. Photoautotrophic cultivation in open raceway

The *A. maxima* IFRPD 1183 was cultivated in 500-l raceway ponds with length of 2.08 m, width of 1.10 m and depth of 0.26 m. Open ponds were controlled using a paddle wheel speed of 15 rpm. The *A. maxima* IFRPD 1183 cultivation was carried out with added inoculums of 10% (v v^{-1}) in a working volume of 200 l in modified Zarrouk media (ZM). Cells were grown to exponential phase and then harvested and mixed with an equal volume of fresh modified Zarrouk media for repeat photoautotrophic cultivation. All experiments were carried out in triplicate.

2.4. Assessment of biomass cultivation

Biomass concentration was assessed using filtration of the sample through GF/C Whatman filter papers (Whatman, Maidstone, UK). Filter papers were dried to a constant weight at 105 ºC. The protein content was analyzed using Kjeldahl method with a conversion factor from nitrogen to protein of 6.25. Assessment of pH was carried out using *pH* meter (Model Lab850; Schott, Germany).

2.5. Kinetic parameters

Cell growth and protein production parameters were assessed and the kinetic parameters were calculated using modified method of Yang et al. [20]. The maximum specific growth rate (μ_m) was achieved from the calculation of cultivation parameters. The microalgal growth was calculated based on the Eq (1):

$$dX/dt = \mu_m(1 - X/X_m)X \qquad \text{Eq. (1)}$$

where $\frac{dX}{dt}$ was the rate of microalgal growth, μ_m was the maximum specific growth rate of microalgae, X was the concentration of microalgae in media and X_m was the maximum value of cell concentration. The volumetric rate of protein production (Q_p) was calculated as follows:

$$Q_p = P_m - P_0/dt \qquad \text{Eq (2)}$$

Protein productivity (Q_p) was calculated as a ratio of the corresponding variation in protein concentration ($P_m - P_0$) to cultivation time. The yield coefficient for the protein production was calculated based on the Eq (3):

$$Y_{P/X} = \Delta P/\Delta X \qquad \text{Eq (3)}$$

2.6. Statistical analysis

Triplicate data were statistically analyzed using one-way analysis of variance and SAS software v.8.1 (SAS Inst., Cary, NC, USA). Differences between the treatment means were analyzed using Fisher's least significant difference (LSD). Statistical significances were reported at $p \leq 0.05$.

3. Results and Discussion

Preliminary studies of various sodium hydrogen carbonate concentrations in Zarrouk media were carried out to investigate biomass concentrations of *A. maxima* IFRPD 1183 (Fig. 1). Sodium hydrogen carbonate at 4.2-16.8 g l^{-1} in culture media showed that biomass concentrations increased with increases in cultivation times. Sodium hydrogen carbonate at 8.5 and 12.6 g l^{-1} resulted in similar biomass growth rates. The maximum biomass concentration of 8.3 g l^{-1} was reported in culture media with a sodium hydrogen carbonate level of 8.5 g l^{-1}. Therefore, modified Zarrouk media at 8.5 g l^{-1} sodium hydrogen carbonate were used as control media in this study.

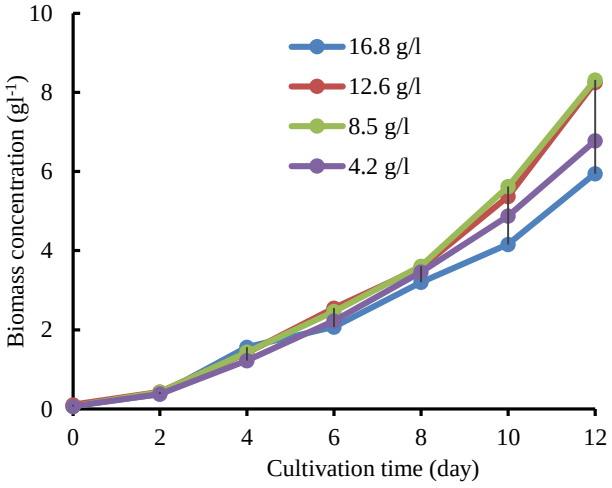

Figure 1. Biomass concentration of *Arthrospira maxima* IFRPD 1183 cultivated in Zarrouk media with various sodium hydrogen carbonate concentrations

3.1. Inoculum preparation and cultivation media

Biomass concentration and protein accumulation of *A. maxima* IFRPD 1183 in ZM for inoculum preparation and growth of culture media are shown in Fig. 2. Cell growth increased with cultivation time with the highest biomass concentration of 7 g l^{-1}. The highest protein content included 64% (DW), which then decreased with increases in time. Various media were studied for the inoculum preparation and growth cultivation (Fig. 3). The *A. maxima* IFRPD 1183 cell biomass concentrations in ZM1, ZM2, and ZM4 increased within 4-6 days of cultivation and then decreased rapidly into death phase, while cultivation of *A. maxima* IFRPD 1183 in ZM3 for inoculum and growth showed increases in biomass concentration with increases in time of cultivation. Therefore, ZM3 with no B_6 micronutrients for inoculum preparation and cultivation of *A. maxima* IFRPD 1183 for biomass and protein productions resulted in appropriate growth and protein accumulation, compared to control media. Furthermore, *A. maxima* IFRPD 1183 was cultivated in various media for inoculum preparation and cultivation. Parameters of X_m and μ_m from ZM3 were similar to those from ZM (control media) (Table 2). However, protein production and other parameters were lower than those in control media. Although ZM3 formulation with no B_6 was used in inoculum and production in this study, protein accumulation in cells decreased. Therefore, the best inoculum and production media of *A. maxima* IFRPD 1183 included ZM (modified Zarrouk media).

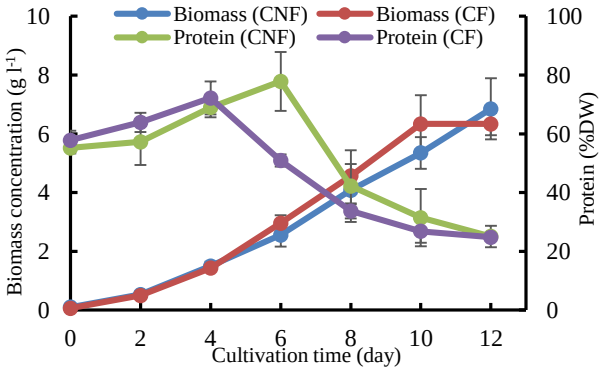

Figure 2. Biomass concentrations and protein contents of *Arthrospira maxima* IFRPD 1183 cultivated in modified Zarrouk media for inoculum and growth cultivation (CNF, non-filtration; CF, cell filtration)

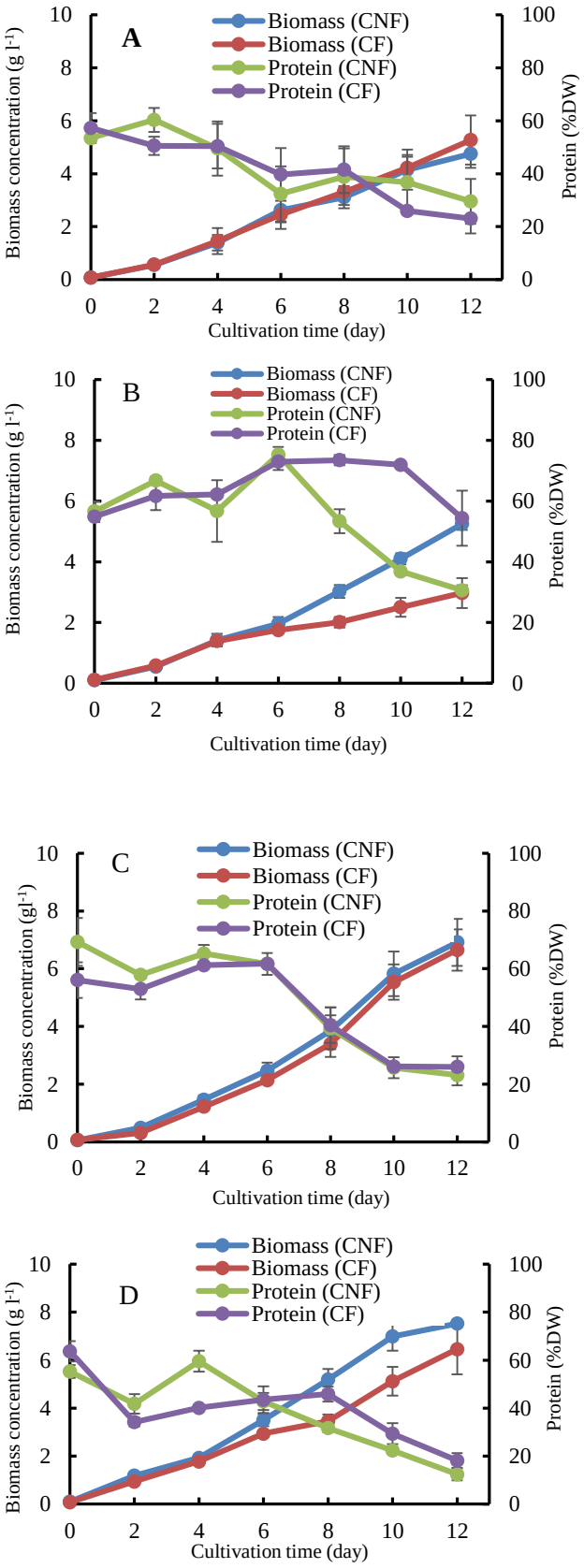

Figure 3. Biomass concentrations and protein contents of *Arthrospira maxima* IFRPD 1183 cultivated in various media of inoculum and growth cultivation, including (A) ZM1, (B) ZM2, (C) ZM3 and (D) ZM4 (CNF, non-filtration; CF, cell filtration)

Table 2. Kinetic parameters of *Arthrospira maxima* IFRPD 1183 cultivated using various inoculum and growth media conditions

Medium*	X_m (g l^{-1})	μ_m (d^{-1})	Protein (% DW)	P_m (g l^{-1})	Q_P (g l^{-1} d^{-1})	$Y_{P/X}$ (g g^{-1})
ZM (control)	7.02±0.67^{a}	0.470±0.02^{a}	64.43^{a}	1.57±0.12^{a}	0.245±0.02^{a}	0.463±0.08^{a}
ZM1	1.36±0.14^{bc}	0.374±0.00^{c}	29.80^{c}	0.28±0.03^{c}	0.040±0.00^{cd}	0.197±0.00^{b}
ZM2	0.81±0.15^{c}	0.397±0.04^{bc}	39.53^{b}	0.17±0.02^{d}	0.026±0.00^{d}	0.165±0.01^{b}
ZM3	6.85±0.14^{a}	0.478±0.01^{a}	39.51^{b}	0.88±0.03^{b}	0.104±0.00^{b}	0.218±0.02^{b}
ZM4	1.66±0.11^{b}	0.417±0.00^{b}	39.83^{b}	0.32±0.02^{c}	0.045±0.00^{c}	0.176±0.00^{b}

* Medium composition shows in Table 1.

X_m**:** Maximum biomass concentration, μ_m**:** Maximum specific growth rate, P_m: Maximum protein concentration, Q_P: Volumetric rates of protein production and $Y_{P/X}$**:** Protein yield coefficient

$^{a,b,\cdots}$Means in the same column with different letters are significantly different ($P < 0.05$). Data were calculated from triplicate experiments ±standard deviation**.**

3.2. Inoculum and cultivation media

Biomass concentration and protein production of *A. maxima* IFRPD 1183 were studied under various treatments using inoculum preparation methods as CNF and CF in various culture media of ZM and ZM1–ZM4. Results of ZM and ZM1–ZM4 are shown in Figs. 4 and 5, respectively. Figure 4 shows *A. maxima* biomass concentration and protein production in ZM culture media. Biomass concentration increased for the two inoculum preparation methods with increases in cultivation time, including similar trends in various inoculum preparation methods. Protein production increased with increases in cultivation time until Days 4 and 6 of cultivation and then decreased. The highest protein content from CNF included 75% (DW), compared to CF inoculum methods. The *A. maxima* was cultivated under various photoautotrophic conditions in various culture media (Fig. 5). Treatments showed that biomass concentration increased with increases in cultivation time, whereas protein content increased to a maximum of 43-78% (DW) and then decreased with continuous cultivation. The CNF inoculum method achieved protein contents higher than those the CF method did. Various inoculum preparation methods, the highest biomass concentration and protein content revealed that the CNF condition was better than the CF condition.

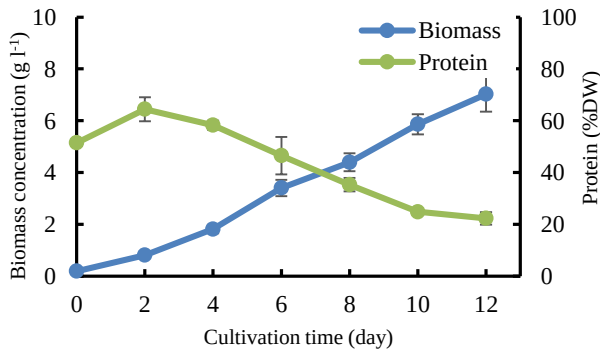

Figure 4. Biomass concentrations and protein contents of *Arthrospira maxima* IFRPD 1183 cultivated in modified Zarrouk media using various inoculum preparation methods

Parameters of *A. maxima* IFRPD 1183 photoautotrophic cultivation in various culture media conditions for the highest biomass production and protein accumulation are shown in Tables 3 and 4 as CNF and CF inoculum preparation, respectively. The maximum biomass concentration (X_m) in various culture media ranged 4.8-7.5 g l^{-1} with the highest concentration recorded for ZM4 media with no significant differences from ZM and ZM3 (Table 3). In contrast, low maximum protein contents were achieved from ZM4. Maximum protein contents under various media ranged 56-78% (DW) with no significant differences between ZM to ZM3. Moreover, maximum specific growth rate (μ_m), maximum protein concentration (P_m) and protei productivity (Q_P) did not significantly vary within various culture media. Yield of protein production from cell biomass ($Y_{P/X}$) ranged 0.3-0.8 g g^{-1}, which was not similar to that from control media (ZM).

Table 4 shows the parameters of *A. maxima* IFRPD 1183 with CF inoculum preparation in various culture media. The X_m value of cultivation in various media treatments ranged 3-6.6 g l^{-1} with no significant differences for ZM2, whereas μ_m showed the highest value in ZM control media. Protein productions in ZM2 and ZM3 were similar to that in ZM, whereas ZM1 and ZM4 showed lower protein productions in terms of dry weight ($p \leq 0.05$). However, no significant differences of P_m and Q_P were seen between the culture and control media. These ranged 1.4-1.7 g l^{-1} and 0.14-0.19 g l^{-1} d^{-1}, respectively. Protein yield from cell $Y_{P/X}$ was the highest in ZM2, with a low biomass concentration and high protein production. The ZM control media showed $Y_{P/X}$, similar to ZM3 culture media. Various media and inoculum preparation conditions of *A. maxima* IFRPD 1183 achieved various kinetic results; however, inoculum preparation with no CF showed increased cell growth and protein accumulation during cultivation.

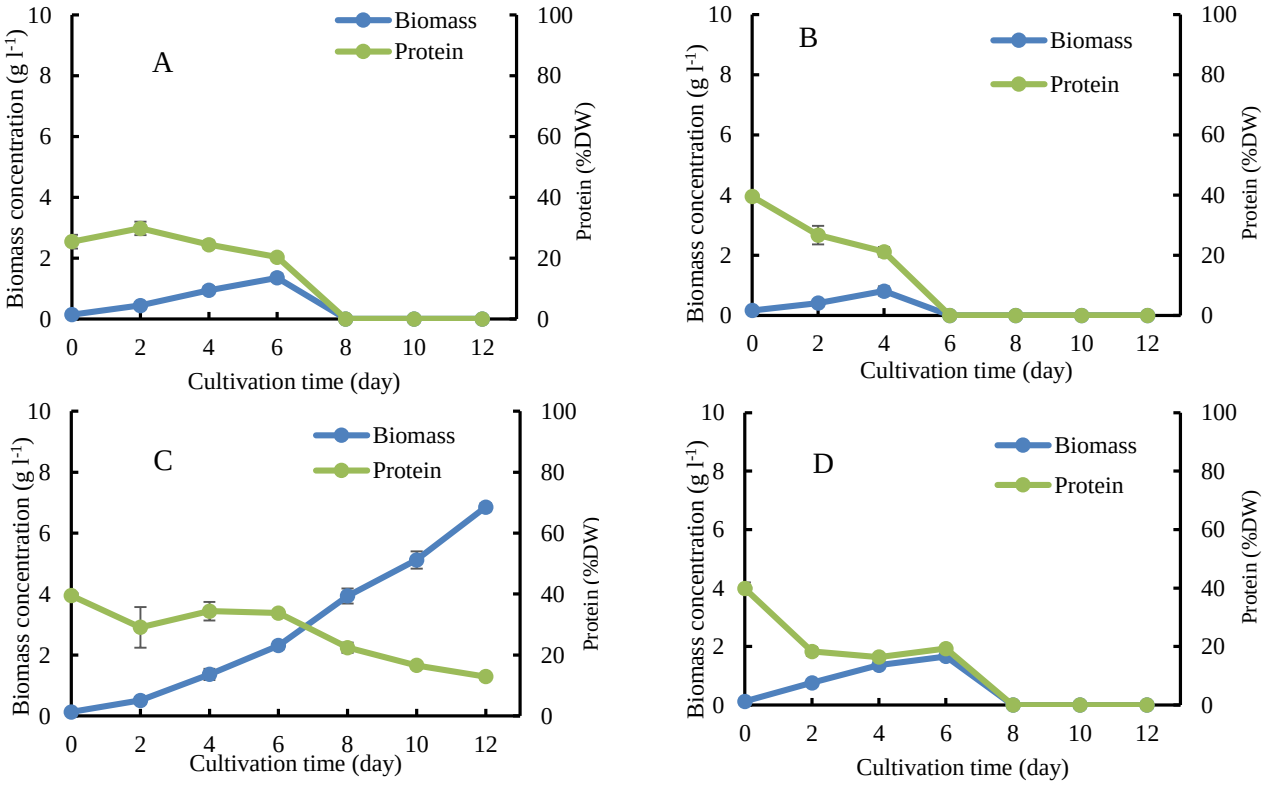

Figure 5. Biomass concentrations and protein contents of *Arthrospira maxima* IFRPD 1183 cultivated in various media using various inoculum preparation methods, including (A) ZM1, (B) ZM2, (C) ZM3 and (D) ZM4

Table 3. Kinetic parameters of *Arthrospira maxima* IFRPD 1183 cultivated using various conditions and cell non-filtered inoculum preparation methods (CNF)

Medium*	X_m (g l^{-1})	μ_m (d^{-1})	Protein (% DW)	P_m (g l^{-1})	Q_P (g l^{-1} d^{-1})	$Y_{P/X}$ (g g^{-1})
ZM (control)	6.85±1.04^{a}	0.539±0.02^{a}	77.83^{a}	1.96±0.49^{a}	0.318±0.08^{a}	0.788±0.22^{a}
ZM1	4.75±0.54^{b}	0.564±0.05^{a}	60.34^{ab}	1.50±0.32^{a}	0.243±0.05^{a}	0.365±0.10^{bcd}
ZM2	5.25±0.20^{b}	0.493±0.02^{a}	78.24^{ab}	1.61±0.02^{a}	0.258±0.00^{a}	0.539±0.03^{b}
ZM3	6.91±0.81^{a}	0.614±0.02^{a}	66.65^{ab}	1.58±0.06^{a}	0.257±0.01^{a}	0.228±0.03^{d}
ZM4	7.53±0.06^{a}	0.465±0.18^{a}	55.59^{b}	1.64±0.06^{a}	0.265±0.01^{a}	0.312±0.02^{cd}

* Medium composition shows in Table 1.
X_m: Maximum biomass concentration, μ_m: Maximum specific growth rate, P_m: Maximum protein concentration, Q_P: Volumetric rates of protein production and $Y_{P/X}$: Protein yield coefficient
$^{a,b,\cdots}$Means in the same column with different letters are significantly different ($P < 0.05$). Data were calculated from triplicate experiments ±standard deviation.

Table 4. Kinetic parameters of *Arthrospira maxima* IFRPD 1183 cultivated using various conditions and cell filtered inoculum preparation methods (CF)

Medium*	X_m (g l^{-1})	μ_m (d^{-1})	Protein (% DW)	P_m (g l^{-1})	Q_P (g l^{-1} d^{-1})	$Y_{P/X}$ (g g^{-1})
ZM (control)	6.34±0.98^{a}	0.641±0.02^{a}	72.19^{a}	1.68±0.22^{a}	0.166±0.02^{ab}	0.266±0.04^{c}
ZM1	5.27±0.93^{a}	0.574±0.03^{b}	57.30^{b}	1.37±0.43^{a}	0.166±0.05^{ab}	0.412±0.14^{b}
ZM2	2.97±0.50^{b}	0.454±0.01^{c}	73.47^{a}	1.58±0.00^{a}	0.151±0.00^{ab}	0.727±0.00^{a}
ZM3	6.65±0.71^{a}	0.588±0.03^{b}	61.72^{ab}	1.73±0.28^{a}	0.141±0.02^{b}	0.257±0.04^{c}
ZM4	6.46±1.05^{a}	0.585±0.02^{b}	43.49^{c}	1.58±0.04^{a}	0.191±0.01^{a}	0.455±0.03^{b}

* Medium composition shows in Table 1.
X_m: Maximum biomass concentration, μ_m: Maximum specific growth rate, P_m: Maximum protein concentration, Q_P: Volumetric rates of protein production and $Y_{P/X}$: Protein yield coefficient
$^{a,b,\cdots}$Means in the same column with different letters are significantly different ($P < 0.05$). Data were calculated from triplicate experiments ±standard deviation.

3.3 Open raceway cultivation

Open photoautotrophic raceway cultivations of *A. maxima* IFRPD 1183 for biomass production and protein accumulation were carried out in modified Zarrouk media for inoculum preparations of CNF and culture media. Open pond systems of *A. maxima* IFRPD 1183 cultivation are open to the environment; therefore, nutrient formulation of the culture media is the only factor controlled. Biomass and protein productions are shown in Fig. 6. A period of one month *A. maxima* IFRPD 1183 cultivation was studied in a raceway pond with a working volume of 200 l. Biomass concentration and protein content of the cells were assessed. The *A. maxima* cells showed increased growth until the early stationary phase within 10-14 days of cultivation at nearly 0.8 g l^{-1}. Then, 100 l of the cells were harvested as Batch I and mixed with 100 l of fresh culture media. Cells were harvested as Batch II and a fresh culture media was added for production up to 28 days. In fact, *A. maxima* IFRPD 1183 achieved the maximum biomass concentration of 0.9 g l^{-1}. This was lower than that of lab-scales because the environmental factors could not be controlled precisely. However, cells rapidly grew after harvesting. Moreover, accumulation of protein contents during cultivation was observed with a stable protein accumulation and the highest protein content of 64% (DW) with an average of nearly 53% (DW). The average pH, light intensity and temperature values included 9.6, 471 µmol m^{-2} s^{-1} and 37 °C, respectively, during open pond cultivation. Therefore, decrease of modified Zarrouk media is appropriate for biomass and protein production from *A. maxima* IFRPD 1183.

Arthrospira spp. are microalgal photoautotrophic filamentous cyanobacteria, commonly used as sources of human foods, animal feeds and cosmetic colorants [21]. The most accessible commercial products are primarily derived from *A. platensis* and *A. maxima* with high protein contents [22]. Furthermore, proteins from *Arthrospira* spp. include various amino acid and protein qualities, which are principally assessed by the concentration, proportion and availability of its amino acids. Optimization of *Arthrospira* growth conditions for mass production targets progress of production efficiency and decrease of costs. Several factors affect biomass production and biochemical accumulation in cells such as light intensity and temperature. However, environmental factors limit control in open systems in commercial productions. The economically significant cyanobacteria, *Arthrospira*, generally appear in large production ponds under sunlight sources. Moreover, nutrients in culture media play important roles in costs of massive productions. Therefore, nutrient formulations in cell productions are significant options for cost decreases.

Various nutrient formulations in culture media composition change the biochemical compositions of microalgal accumulation for valuable products such as proteins, carbohydrates, lipids and pigments.

Macronutrients and micronutrients are essential for media cultivation of *Arthrospira* biomass and protein production. *Arthrospira* spp. include a high hydrogen carbonate requirement, not only as a carbon source but also to maintain alkaline conditions that are favorable for their growth [25].

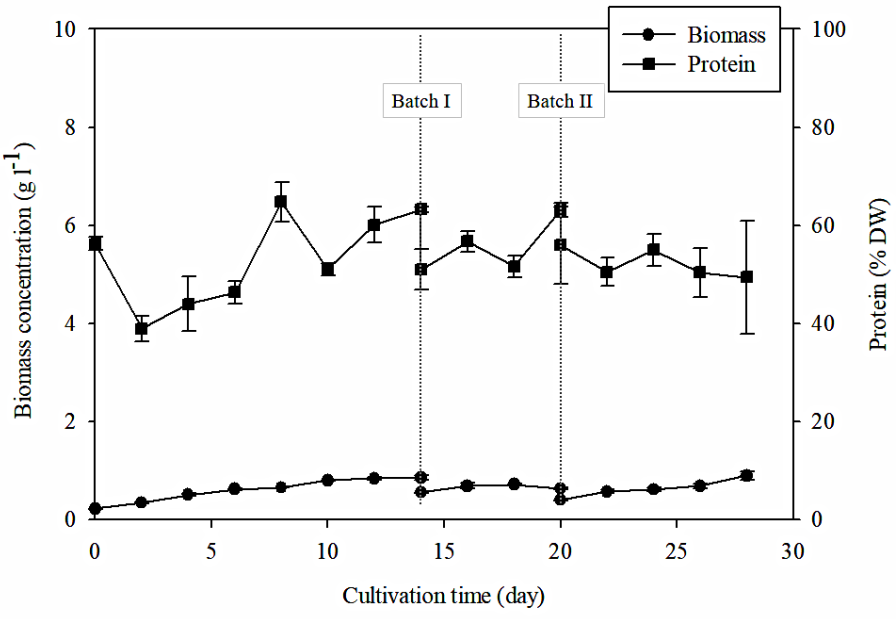

Figure 6. Biomass concentrations and protein contents of *Arthrospira maxima* IFRPD 1183 cultivated in open photoautotrophic raceway

Nitrogen deprivation debilitates protein accumulation and thus stimulates depletion of adenosine diphosphate (ADP) and nicotinamide adenine dinucleotide phosphate, supporting dysfunction of cell growth [26]. Phosphorus is an important element for microalgae, which is used in energy metabolism [27]. Phosphorus supplementation offers an effective procedure to resolve nitrogen limitation and is utilized to improve microalgal biomass production under nitrogen limitation conditions [28]. Moreover, micronutrients are important components in media to support cell growth and biochemical composition. Zarrouk medium is a complete medium that affects *Arthrospira* growth at limited costs. However, lack of micronutrients including magnesium, iron and A_5 and B_6 solutions in inoculum and cultivation media adversely affect cell expansion and protein production. Micronutrients such as magnesium, selenium, sulfur, iron and calcium and trace elements such as boron, copper, manganese, zinc and molybdenum are important in media, mostly used in enzymatic reactions [24]. These micro-nutrients are found in growth media at low levels; however, they are essential for the microalgal growth [29]. However, metals such as iron, magnesium and copper are toxic at high concentrations, while magnesium is an important cofactor in chlorophyll synthesis [30]. Therefore, lack of these micronutrients in media for a long time of growth culture induces cell weakness, chlorophyll synthesis decreases and rapid death phase. Indeed, *A. maxima* showed growth and protein production in media lacking B_6 (ZM3). Inoculum preparation of *A. maxima* IFRPD 1183 with CNF resulted in cell growth and protein accumulation. The residual nutrients from the old inoculum assisted cells to preserve and produce protein accumulation. Moreover, cells with no filtrations of the old inoculum media are appropriate for the process of large-scale productions.

4. Conclusion

Arthrospira spp. are significant protein sources, which are used as food supplements. In this study, optimization of biomass and protein productions by *Arthrospira* spp. in decreased nutrient concentrations was carried out. The *A. maxima* IFRPD 1183 was cultivated under various media of inoculums and productions as well as inoculum preparations. Modified Zarrouk media with no B_6 micronutrients can be used for inoculum preparation and culture media. The complete media of inoculum preparation with no filtrations in production stages were optimized to enhance cell growth and protein accumulation. The *A. maxima* photoautotrophic cultivation was carried out in modified Zarrouk media as the inoculum and production media using open systems.

5. Acknowledgements

This research was financially supported by the Kasetsart University Research and Development Institute (KURDI), No. [P-T(D)101.55], Kasetsart University, Thailand.

6. Conflict of Interest

The authors declare no conflict of interest.

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Research Article

APPLIED FOOD BIOTECHNOLOGY, 2020, 7 (4): 225-234
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

کشت نورخودپرورد[1] *آرتروسپیرا ماکسیما* به منظور تجمع پروتئین در شرایط کمینه دسترسی به مواد مغذی

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تاریخچه مقاله

دریافت ۸ می ۲۰۲۰
داوری ۲ ژوئن ۲۰۲۰
پذیرش ۱۰جولای ۲۰۲۰

واژگان کلیدی

- *آرتروسپیرا*
- زی‌توده
- سیانوباکتر
- مواد مغذی
- نورخودپرورد
- پروتئین

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تلفن ۳۵-۲۹۴۸۶۲۹-۶۶۰+
دورنگار: ۲۹۴۰۶۴۵۵-۶۶۰+
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چکیده

سابقه و هدف: سیانوباکترهای آرتروسپیرا ریزجلبک‌های سبز-آبی آب شیرین به عنوان منبع پروتئین غذایی برای انسان اهمیت دارند. به منظور افزایش زی‌توده[2] و تولید پروتئین، کشت نورخودپرورد *آرتروسپیرا ماکسیما* تحت فرمولاسیون‌های گوناگون کمینه محیط کشت انجام شد. برای تولید زی‌توده و تجمع پروتئین *آرتروسپیرا ماکسیما*، به منظور کاربردهای مکمل غذایی، کلان مغذی‌ها[3] و خرد مغذی‌ها[4] حیاتی می‌باشند.

مواد و روش ها: برای تولید زی‌توده و پروتئین، با استفاده از محیط کشت‌های گوناگون زاروک و شرایط تهیه تلقیح، کشت نورخودپرورد *آرتروسپیرا ماکسیما* IFRPD 1183 انجام شد. *آرتروسپیرا ماکسیما* IFRPD 1183 با استفاده از اتاقک جلبک تحت سامانه‌های روباز و بسته بیوراکتورهای نوری کشت داده شد.

یافته‌ها و نتیجه‌گیری: خردمغذی‌های محلول B_6 شامل NH_4VO_3، $K_3Cr_2(SO_4)_4\ 24H_2O$، $NiSO_4.\ 7H_2O$، Na_2WO_3، $Co(NO_3)_2.6H_2O$ و $Ti_2(SO_4)_3$ بر رشد سلول و تجمع پروتئین در غیاب محیط کشت برای تولید مایه تلقیح تاثیر نداشتند. تهیه مایه تلقیح در شرایط گوناگون *آرتروسپیرا ماکسیما* IFRPD 1183 با فیلتراسیون محیط کشت قدیمی قبل از استفاده (تلقیح فیلتراسیون سلولی) مورد مطالعه قرار گرفت. تهیه مایه تلقیح بدون فیلتراسیون سلول برای تولید زی‌توده و تجمع پروتئین مناسب تشخیص داده شد. در مقیاس بزرگتر، سامانه حوضچه روباز *آرتروسپیرا ماکسیما* IFRPD 1183، در تکرار کشت نورخودپرورد ناپیوسته، بیشینه تولید زی‌توده و پروتئین به ترتیب حدود $1\ g\ l^{-1}$ و ۶۴٪ (DW) بود. نبود خردمغذی‌ها در تلقیح سلول فیلتر نشده به عنوان فرایندی آسان به منظور حصول تولید بالای زی‌توده و پروتئین در کشت‌های روباز و بسته *آرتروسپیرا ماکسیما* IFRPD 1183 و با هزینه کاهش یافته گزارش شد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[1]- Photoautotrophic : ویژگی اندامگانی که برای تأمین انرژی و رفع نیازهای غذایی خود با استفاده از مواد ساده معدنی، مواد آلی پیچیده می‌سازند و انرژی خود را از نور به دست می‌آورند.

2 Biomass
3 Macronutrients
4 Micronutrients