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مروري مقاله

آزاده لاسرسی

په فوتوبیوریاکټر کې د (*Arthrospira* sp) نوعي مایکروالجي اصلاح کول

پوهنیار سید رحیم نیکمل*[1]، پوهنوال عبدالرحمن عثماني[۲]، پوهنمل محمد نعیم علیزاده[۲]
۱- بیولوژي دیپارتمنت، ښوونې او روزنې پوهنځی، وردګ د لوړو زده کړو مؤسسه، میدان وردګ، افغانستان
۲- بیولوژي دیپارتمنت، بیولوژي پوهنځی، کابل، پوهنتون کابل، افغانستان
۳- دیپارتمنت پاراکلینیک، پوهنځی علوم وترنری، پوهنتون کابل، کابل افغانستان
مسؤل لیکوال برېښنالیک nikmal1993@graduate.utm.my تلیفون شمېره: ۰۰۹۳۷۶۴۶۶۵۸۶۶

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دغه ژورنال د وردک پوهنتون په چوکاټ کې د لوړو زدکړو وزارت د رسمي جواز پر اساس فعالیت کوي.

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زمونږ سره اړیکی:
ایمیل: info@wu.edu.af
موبایل:
آدرس: سیدآباد ولسوالی، توپ دښته میدان وردک ولایت- افغانستان

لنډیز

د عصري اوسېدنې او روغتیايي ژوند په اړه د مصرفونکي پوهاوی په شدیده توګه د خوراکې توکو په صنعت، د خوړو بشپړونکي او خوراکي ارزښتونو له امله د آرتروسپیرا نوعي ته په پراخه کچه تقاضا لوړه کړې ده. د مایکروالجي کښت د ودې ښه فعالیت کولو لپاره د انوکولم مناسب پاشلو (Strain)، مناسبې میډیا او د کښت کنټرول شوي شرایط ته اړتیا لري. د دې څېړنې موخه دا ده چې په ۲ لیتره فوټوبیوریاکټر کې د بایوماس تولید اعظمي کچې ته ورسوي. په اوسنۍ څېړنه کې، د *Arthrospira platensis* هیلیکل (S1) او مستقیمې بڼې (S2) تر منځ د ودې فعالیت د ایرلینمیر فلاسکونو په کارولو سره مطالعه شوې دي. دې پایلې څرګنده کړه چې مورفولوژیکي توپیرونه د ودې په فعالیت باندې اغیزه نه کوي، دواړه بڼې د ودې په فعالیت کې ورته والی لري.

کلیدي کلمې: اصلاح کول، فوټوبیوریاکټر، مایکروالجي او آرتروسپیرا نوع

Assessment the Properties of postbiotics and their usage in medicine

Sayed Rahim Nikmal [1*] , Abdul Rahman Osmani [1*] , Mohammad Naiem Alizada [*1]
1.Biology Department, Education Faculty, Wardak Institute of Higher Education, Maidan Wardak, Afghanistan
3.Horticulture Department, Agriculture Faculty, Wardak University, Maidan Wardak, Afghanistan
3.Agronomy Department, Agriculture Faculty, Wardak University, Maidan Wardak, Afghanistan
[*1]*Corresponding Email:* nikmal1993@graduate.utm.my*Phone Number :* ۰۰۹۳۷۶۴۶۶۵۸۶۶

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Abstract

The influence of modern lifestyle along with enhanced consumerawareness about healthylifestyle has drastically increased the global demand of *Arthrospira* sp. as a food supplement in food industries due to its promisingtherapeuticandnutritivevalues.Microalgaecultivation requires suitable inoculum strain, appropriate medium and controlled cultivation conditions for enhanced growth performance. This study aimed to present an experimental approach to maximise biomass production in a 2 litres photobioreactor. In the present study, a comparative study of growth performance between helical (S1) and straight form (S2) *Arthrospira platensis* was conducted using Erlenmeyer flasks. The results revealed that morphological differences did not affect growth performance. Both forms shared similarities in growth performance.

Keywords: Optimization, Photo bioreactor, Microalgae and *Arthrospira* sp

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INTRODUCTION

In recent years, there has been an exponentially increasing interest in the research area of microalgae biotechnology due to its unique characteristics that pose significant commercial values, particularly in food and feed industries for high-value chemicals (Garrido-Cardenas *et al.*, 2018). In this respect, spirulina (now named *Arthrospira*) is one of the most economically important genera in microalgae biotechnology due to its promising nutritional and therapeutic values for human health (Farag *et al.*, 2016). *Arthrospira* and *Spirulina* refer to two distinct genera of cyanobacteria that belong to the family Oscillatoraceae under the order Oscillatoriales 2001). It consists of various species of blue-green algae or cyanophytes (Ishida *et al.*, that naturally live in diverse aquatic environments, ranging from freshwater to brackish water. Some *Spirulina* species are now renamed as *Arthrospira* after the official recognition in 1989 of the cyanobacteria *Spirulina* and *Arthrospira* as two distinct genera by Castenholz (1989). Both *Arthrospira* and *Spirulina* are symbiotic, microscopic and filamentous cyanophytes that derive their names for its unique spiral appearance under the microscope (Houston, 2002). and helical filament-like Despite that, the term "spirulina" is commonly used as the trade name and vernacular name for the economically important species in *Arthrospira* genus such as *Arthrospira platensis* and *Arthrospira maxima.* Therefore, the word "spirulina" is commonly used .,2012). to refer to thesetwo distinct genera, *Spirulina* and *Arthrospira* (Sili *et al* Due to its unique properties, spirulina (*Arthrospira*) has played important roles in a wide area of applications and manufacturing in many industries such as pharmaceuticals, food, agriculture, medicine and research. It has been widely employed as a source of protein and vitamin supplement and is usually consumed in the form of tablets, capsules or powder (Valls *et al.*, 2013). Besides that, Spirulina has been incorporated in many processed food products such as noodles, biscuits and nutritional bars as the dietary supplements due to its high nutritional values and natural food colourant (Mathur, 2018; Priyadarshani and Rath, 2012). In the past, spirulina is normally cultivated and harvested from the natural lakes containing wild living algaespirulina population such as Lake Chad of the Republic of Chad (Abdulqader *et al.*, 2000). Today, the cultivation of spirulina is normally done in large outdoor ponds or lakes under the controlled conditions (Soni *et al.*, 2017). Nutritional studies have shown spirulina is a potent superfood containing a broad spectrum of nutrients with high digestibility. Also, clinical studies have proven spirulina has various possible health-promoting effects such as anticarcinogenic, hepaprotective, neuroprotective, anti-inflammatory properties, and hypocholesterolemic properties (Chaiklahan *et al.*, 2011; Reinehr and Costa, 2006).

As the result, spirulina has been marketed extensively as the food active ingredient in different variety of commercial products such as dietary supplements, functional food or formulated value-enhanced food products and even desserts (Agustini *et al.*, 2016; da Silva Vaz *et al.*, 2016; Vílchez *et al.*, 2011). The influence of modern lifestyle along with enhanced consumer awareness about healthy lifestyle has

drastically changed the commercial dynamics of spirulina products. Today, consumers always look for functional food or nutraceuticals incorporated with high-value ingredients in the hope to promote their health conditions as well as disease prevention (Bimbo *et al.*, 2017; Ferrão *et al.*, 2019). As a result, the production of spirulina as a nutrient supplement and functional food has popularly gained the attention from food and pharmaceuticals industries for its promising capabilities and potential health benefits. According to the market research, the global spirulina market has garnered USD 346 million in 2018 and is anticipated to reach USD 779 million by 2026, followed by the demand of phytopigments with anestimated market of USD 722 million by 2025 (Mitra and Mishra, 2019). Therefore, the spirulina cultivation needs to be effective and efficient to satisfy the high demand of the global market in the future. The most common method to promote the yield of spirulina is the optimization of its cultivation condition. Like other cultures, microalgae culture needs to grow at an optimum environmental condition to ensure its high biomass and productivity for mass production. Moreover, the bioactive compounds composition of the bioactive compounds of spirulina also varies based on the changes of different environmental parameters (Can *et al.*, 2017; Dewi *et al.*, 2016; Pandey *et al.*, 2010). For instance, a study has shown that among the production of phytopigment, the production of chlorophyll is higher when the temperature is 35°C, while the production of carotenoids is higher when it is 25°C (Kumar *et al.*, 2013). Besides that, the selection of the optimised parameters could be chosen depending on the type of required final product from the spirulina as reported by Marrez *et al.*(2013) whose study shows that modified BG-11 medium is best for phytopigments production, while Zarrouk's medium is best for biomass production. Since different parameter changes influence the spirulina cultivation variously, choosing an appropriate experimental design for the optimisation of selected environmental factors can help in enhancing the productivity of the biomass of spirulina for the commercial purposes.

The Morphology of *Arthrospira*

In term of morphology, *Arthrospira* consists of blue-green unbranched and non-heterocystous filaments that composed of vegetative cells that undergo binaryfission in a single plane, perpendicular to the main axis (Ali and Saleh, 2012) The intercalary cell division of the vegetative cells along the filaments contribute to the elongation of the total length of filamentous spirulina. Typically, the cells are 3-5 µm in length and the filaments are around 500 µm long which are too small to see with the naked eye. The formation of blue-green colour is because its main phytopigment in the vegetative cells is a blue colour photosynthetic pigment known as phycocyanin. spirulina also contain other accessory pigments such as chlorophyll-a, carotenoid and phycoerythrin which bestow these bacteria on red or pink colour. In the aquatic environment, these vegetative filaments are typically free-floating and displaying gliding motility (Koru, 2012). Among the myriads of cyanophytes, spirulina has a very distinctive characteristic that is distinguished from other oscillatoriacean genera, which is its

unique helical shaped and regularly coiled trichome (filaments) morphology (Castenholz, 1989). This distinguishable feature is the unique characteristic of this genus and it can only be maintained in a liquid environment or culture medium as in Figure 1. These helical trichomes, however, can lose their original helical forms and convert to abnormal morphologies under the adverse condit ions of temperature and pressure. This morphological change is commonly irreversible and considered as a permanent degeneration for many spirulina species except *A. platensis* which has reversible linear filaments (Sili *et al.*, 2012). These filaments generally coiled in spirals with varying tightness and number of turns based on the spirulina species. Likewise, the length (50-500 µm) and width (3-4 µm) of the trichomes also vary according to the strain as well as the growth conditions (Habib, 2008). Due to the presence of helical nature of filaments and gas-filled vacuoles in the vegetative cells, many spirulina species form a floating algae mat near to the surface of aquatic environments. Therefore, the water is covered with their blue-green colour (Habib, 2008). Unlike other unicellular algae used in food production, *Chlorella* which certainly is a eukaryotic plant, spirulina is a group of photosynthetic microalgae that fall under the category of prokaryotic cyanobacteria. Thus, they share many main characteristics and features with the eubacteria, particularly gram-negative bacteria: both contain cell wall comprised of a lysozyme-sensitive heteropolymer known as peptidoglycan and other non-lysozyme-sensitive components. Since spirulina lacks a cellulose cell wall, it can be easily digested by the body and thus, making it a suitable food and feed additives for both humans and livestock (Mathur, 2018).

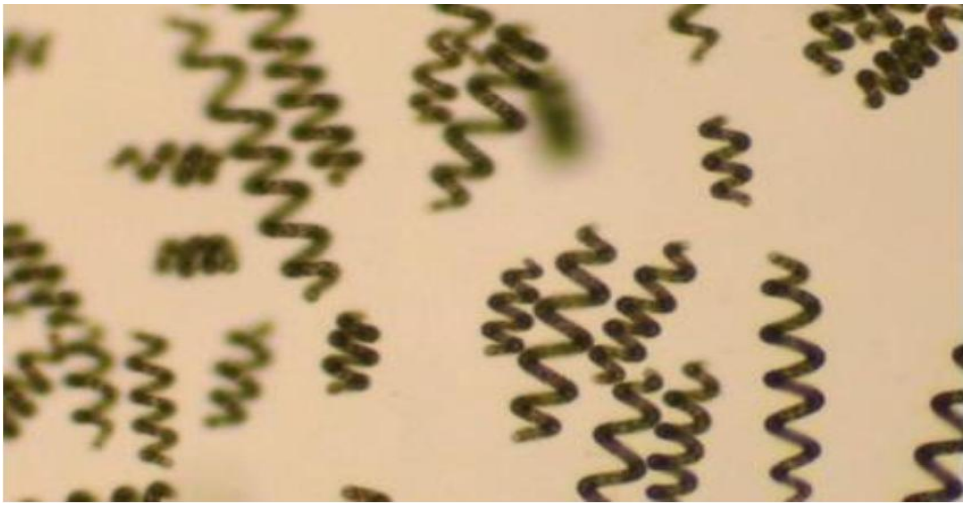


Figure 1. The unique helical and coiled morphology of microalgae spirulina (Arthrospira) under the view of a microscope (Koru, 2012).

Problem Statement

Today, the increasing awareness on health and risks associated with the synthetic ingredients and additives in food has led to the increase of consumer's demand for natural dietary supplement and formulated foods as well as the food industry's demand for natural colourant (Premkumar and Vasudevan, 2018). Therefore, the global demand of spirulina products has been increasing significantly

due to its high nutritive values and health-promoting properties. In fact, the global spirulina market is anticipated to grow at compound annual growth rate (CAGR) of 10.6% from 2019 to 2026 (Mitra and Mishra, 2019).

However, several factors such as climate change and limited land area have hindered the large-scale production of spirulina through conventional open pond system (Andrade *et al.*, 2018). This has commercially affected many major countries involving in industrial spirulina manufacturing such as Australia, India, Japan, and Myanmar (Furmaniak *et al.*, 2017; Raja *et al.*, 2018). This is particularly relevant to Malaysia as the unpredictable weather pattern in Malaysia significantly influences the growth parameters of the open pond system (Almahrouqi *et al.*, 2015). As a result, a closed bioreactor system with better-controlled parameters, optimisation platform and a productive cultivation medium is needed (Almahrouqi *et al.*, 2015). Although many studies have been conducted for the biomass production of spirulina using closed bioreactor systems (Kumar *et al.*, 2013; Marrez *et al.*, 2013; Thirumala, 2012), there is little attention about the optimisation of spirulina strains with morphological differences.

Objectives

The purposes of conducting this proposed study are as follows:

1. To study the growth performance of coiled-form and straight-form *Arthrospira* strains in Erlenmeyer flask.
2. To study the effect on biomass production of microalgae in modified Zarrouk's medium with different nitrogen sources and concentration

Scope of Study

The scope of this study was to study and compare the growth and biomass performance between coiled form strain and straight form strain in Erlenmeyer flask. Then, the effect of a reformulated Zarrouk's medium with different nitrogen sources and levels for biomass production was investigated.

Significance of Study

This study is significant by discovering the possibility of linearised spirulina strain that may be used as an additional resource or even alternative to the imported coiled - form spirulina inoculum that is commonly used in the commercial production. This is particularly relevant for Malaysia which has been working closely together with Japan for the commercial development and cultivation of spirulina (David, 2018, August 28). Since only just a small fraction of discovered algal species are cultivated for producing commercial products (Mobin and Alam, 2017), the investigation of the basic growth requirement and factors affecting the linearised spirulina cultivation may help in providing referenced information to interested production industries for the use of applications (Singh *et al.*, 2017). Apart from satisfying the global demand, the development of growth and production optimisation platform for spirulina using closed bioreactor system showed the potential of using closed bioreactor systems as a better sustainable alternative to conventional open pond system as the main platform for mass spirulina cultivation, giving benefits to the global economy, environment as well as human health. Lastly, the development of a productive cultivation medium will further help in

curtailing the time required to produce good quality spirulina biomass in large scale cultivation.

RESEARCH METHODOLOGY

Outlines the general steps for the experiment with the detail of the concept and method of the experiment. The study is divided into three different parts with different objectives respectively. The first part of this work was to study and compare the growth performance between coiled and linearised spirulina strain in the 500 mL Erlenmeyer flask. Next, the growth of *Arthrospira platensis* in the ReformulatedNitrogen Media was evaluated using different nitrogen sources.

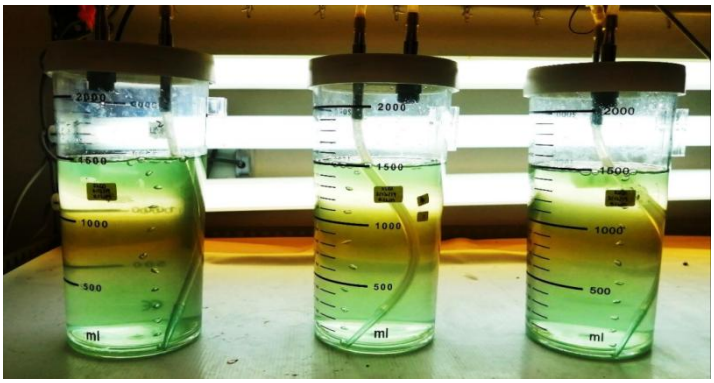
Zarrouk's Medium

In this study, a Zarrouk's medium (ZM) based on the formulation of Amara and Steinbüchel (2013) was used as the main cultivation medium for both *Arthrospira* sp. strains. The media was prepared beforehand by weighing the macro- and micronutrients respectively based on chemical constituents listed in Appendix A. The pH of the medium was adjusted to pH 9 using concentrated sodium hydroxide. It was then sterilized in an autoclave before inoculation. The container was covered with aluminium foil and sealed with parafilm to prevent any evaporation. Meanwhile, agarised Zarrouk's medium (1.5% w/v) was prepared on the sterilised Petri dishes and stored at refrigerator until needed.

Bioreactor Set-up

To study the influence of each factor on the biomass production, *A. platensi* culture was cultivated using 2L photobioreactor (bubble column) with 1.5 L of Zarrouk's medium as shown in Figure 2. Similarly, inoculum (10% v/v) was inoculated into the working medium and the samples were grown at room temperature with aeration of 0.5 L/min using air pump under the irradiance of 3000 Lux (42 µmol photons $m^{-2}s^{-1}$) with 0 h dark and 24 h light cycle for 18 days. Same laboratory condition was used for each experiment unless otherwise stated. Likewise, al experiments were triplicated and the mean of the triplicate was analysed.

Figure. 2 Liters macro bubble column photo bioreactor for *Arthrospira platensis* cultivation.



Determination of Biomass Dry Cell Weight Using Standard Curve

The amount of microalgal biomass concentration of the experimental set was obtained by direct spectrophotometric measurement of optical density at 680 nm. A series of microalgae standards with different cell concentrations were prepared by mixing the maturely grown *A. platensis* cultures with distilled water with the following microalgae: distilled water (1:9, 2:8, 3:7, 4:6, 5:5 6:4, 7:3, 8:2, 9:1 and 10:0 v/v). Then, the optical density of each standard was spectrophotometrically determined using the wavelength at 680 nm. At the same time,

another 1 mL of each standard was taken for off-line measurement of biomass concentration. The samples were taken from the well mixed, homogenous standards and filtered through a preweighed cellulose acetate membrane filter 0.45 µm. The samples were rinsed with distilled water several times to remove any medium component coupled with the vacuum filtering system. The samples were then air-dried in the drying oven at 80 °C for 48 hours until a constant weight was obtained (Vonshak, 1997). The dry cell weight was then calculated as g/L using the equations (A) and (B).

Cell Weight (g) = [Weight of Filter Paper + Aluminium Foil + Cell (g)]
-[Weight of Filter Paper + Aluminium Foil (g)] ..... (A)

Dry cell weight(g/L) = Cell weight (g)/Sample volume (mL) × 1000 mL ..... (B)

Next, a standard linear regression with a good linear correlation (r_2>0.95) was constructed based on the observed optical density readings and biomass dry cell weight of microalgae standards (Appendix C and Appendix D) and it was used to analyse and characterise the dynamic growth curves of microalgae as described by Chen *et al.* (2016) .

RESULTS AND DISCUSSION

The growth performance between *A. platensis* strains with coiled trichome (S1) and with straight trichome (S2) were studied and the inocula of both strains (10% v/v) were introduced and cultivated in the respective 500 mL Erlenmeyer Flask with 250 mL working medium for 18 days. All inocula were standardized spectrophotometrically before inoculation to ensure that every flask started with the same amount of inoculum concentration. The growth of both *Arthrospira* strains was determined by measuring the optical density at wavelength 680 nm along the culture period.

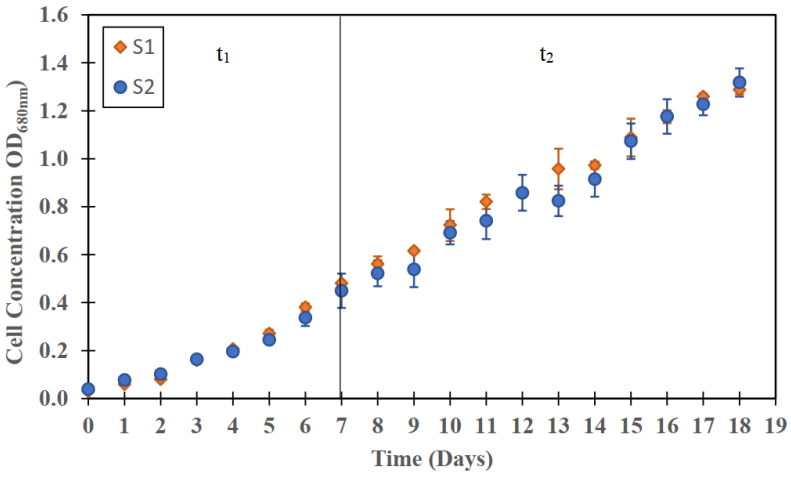


Figure 3. Growth profile (OD_{680nm}) variation of *A. platensis* in batch culture without aeration: S1 (orange diamonds) and S2 (blue circles), in triplicates. Markers and bars represent mean ± standard deviation.

The results of the growth profile variation of strains S1 and S2 reveals that both strains exhibited similar growth profile and performance pattern in the unaerated batch culture for 18 days of cultivation. Additionally, the growth profile of *A. platensis* microalgae was observably differentiated into two phases throughout these 18 days of cultivation: lag phase or early exponential phase (t_1); and log phase or late exponential phase (t_2). From Figure 3, it can be seen that early exponential phase occurred between 0 to 7 days (t_1), while late exponential phase started from 7 days to the 18 days which was at the end of experiment event. The maximum cell concentrations of both strains were only observed at the end of the experiment in which the exponential phase was still ongoing. The maximum cell concentration (OD_{680nm}) for S1 was found to be 1.287 ± 0.019 for S1. On the contrary, S2 had its maximum cell concentration with 1.318 ± 0.059. The results obtained from the growth performance study suggested that to obtain the complete growth profile of *A. platensis*, the cultivation period should be prolonged regardless of morphological differences.

As mentioned by many researchers, *Arthrospira* can undergo a morphological transformation. The occurrence of morphological variation observed from *Arthrospira* sp. is proposed to be a protective mechanism to the changes in environmental variables such as temperature, light intensity and salinity (Kaggwa *et al.*, 2013; Ogato and Kifle,2014). This phenomenon is frequently observed under laboratory and outdoor mass cultivation conditions and the transformation is often irreversible. Despite the morphological changes, the biochemical composition, nutritional properties and growth rate is not affected by the shape of the filaments as suggested by Noor *et a l.*

(2008) since the morphological changes are mainly due to the genetic mutation in cell shape determination proteins (Hongsthong *et al.*, 2007). This finding fits the current research findings and also confirms the previous idea that morphological differences do not play an important role in growth profile nor growth performance.

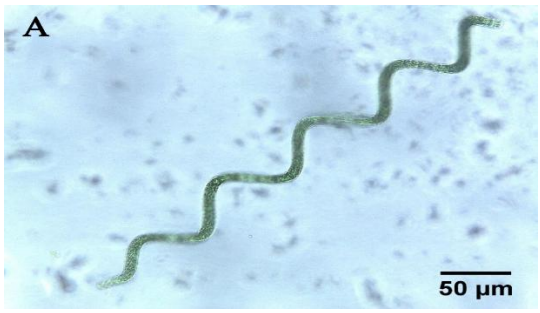


Figure 4 (A). Demonstrates the morphology of S1 and S2 under 400X magnification using a light microscope.

Figure 4 (B). Morphology of laboratory culture of *Arthrospira* sp., under 400X magnification (A: coiled trichome forms; B: straight trichome forms). Both cultures were obtained from UTEX).

In the present study, *A. platensis* with straight trichome (S2) was grown in 500 mL sterilised Erlenmeyer flasks containing 250 mL Zarrouk's medium, supplemented with three different nitrogen sources with various grades of nitrogen concentrations under fully controlled conditions. The growth rates of S2, expressed as dry cell weight (g/L), in the standard control media (ZM) and the reformulated nitrogen media (Treatments 1-9) are illustrated in Figure 4.5a, b and c. As shown in the figures, the growth curves lacked a lag phase for both ZM and the reformulated nitrogen media (except for Treatments 5 and 6 which showed restricted growth). This was probably because the cells used for the inoculum were harvested in the exponential phase, therefore, *A, platensis* grew immediately in all nitrogen sources. Besides that, similar growth

conditions during the inoculum and the cultivations may likely bring about the absence of lag phase (Rodrigues *et al.*, 2010). In ZM culture, the *A. platensis* biomass (g/L) increased gradually with increasing incubation time, giving the maximum biomass values (X_m) at 0.919 ± 0.041g/L on the 18th day.

CONCLUSIONS

In the present study, the comparative study on the growth performance of coiled-form (S1) and straight-form (S2) *Arthrospira platensis* in Erlenmeyer flask, the effect of reformulated nitrogen medium on microalgal cultivation, and optimization of microalgae cultivation in 2 litres photobioreactor have been developed and evaluated. The comparative study between helical and straight form indicated that morphological differences between S1 and S2 did not significantly affect their growth performance. The comparisons between the two forms showed similarity in growth pattern, growth characteristics and growth kinetics. The result also revealed that both strains did not complete their growth phase cycle within 18 days of cultivation, where both S1 and S2 were still in the exponential phase at the end of the cultivation period. However, S2 appeared to grow faster on agarised Zarrouk's medium (1.5 % w/v) than S1 due to its better adaptability, better gliding motility and performance on a solid surface. Its high mechanically rigidity and no coiling nature also allowed it a good candidate involved in large-scale microalgal farming.

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