Growth performance and biochemical profiles of *Arthrospira* spp. grown in a tubular Wuhan Friendship New Tech CO. LTD. photobioreactor

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Declaration

I certify that this thesis does not contain material which has been accepted for the award of any degree or diploma; and to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text of this thesis.

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Abbreviation

μm	micrometer
mg	milligram
mL	milliliter
nm	nanometer
cm	centimeter
g	gram
L	liter
s	second
h	hour
PUFAs	polyunsaturated fatty acids
AA	arachidonic acid
GLA	γ-linolenic acid
EPA	eicosapentaenoic acid
LA	linoleic acid
DHA	docosahexaenoic acid
HDL	high-density lipoprotein
IgA	immunoglobulin A
IgM	immunoglobulin M
ODA	Oregon Department of Agriculture
rcf	relative centrifugal force
PVC	Polyvinyl chloride
LED	Light emitting diode
PBR	Photobioreactor
RO water	Reverse osmosis water
DW	Dry weight
PC	Phycocyanin
RFT	Repeated freezing and thawing
OD	Optical density
SGR	Specific growth rate
H_3BO_3	Boric acid

$MnCl_2$	Manganese (II) chloride
$ZnSO_4$	Zinc sulphate
$CuSO_4$	Copper sulphate
$(NH_4)_6Mo_7O_{24}$	Ammonium molybdate tetrahydrate
Cu (NO_3)	Copper nitrate
$CaCl_2$. $2H_2O$	Calcium chloride dihydrate
FeSO4	Iron (II) sulphate
Na_2-EDTA. $2H_2O$	Disodium ethylenediaminetetraacetate dihydrate
$MgSO_4$	Magnesium sulphate
NaCl	Sodium chloride
K_2SO_4	Potassium sulphate
$NaNO_3$	Sodium nitrate
K_2HPO_4	Dipotassium hydrogen phosphate
$NaHCO_3$	Sodium bicarbonate
Asp	Aspartic acid
Thr	Threonine
Ser	Serine
Glu	Glutamic acid
Gly	Glycine
Ala	Alanine
Val	Valine
Cys	Cysteine
Met	Methionine
Ile	Isoleucine
Leu	Leucine
Tyr	Tyrosine
Phe	Phenylalanine
Lys	Lysine
His	Histidine
Arg	Arginine
Pro	Proline

Abstract

Arthrospira is a useful microalga with applications in nutraceuticals, cosmetics, agriculture and aquaculture. It has been cultivated for commercial purpose mainly in open pond systems due to cost consideration. Recently, cultivation in closed system photobioreactors has gained attention, as the health outcomes of fresh *Arthrospira* are predicted to be greater. A 120-litre tubular photobioreactor (PBR) has been designed specifically to produce fresh *Arthrospira* biomass. This project aimed to investigate basic growth performance and downstream effects on the biochemical profile of *Arthrospira* spp. in this tubular Wuhan Friendship New Tech CO. LTD. photobioreactor. The effect of batch and semi-continuous cultivation on total biomass production was also investigated. As a control system, a traditional 10 L bubble column inoculator was used and contrasted with the results obtained in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor. Environmental conditions (temperature, nutrients) between the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and the bubble column inoculator were kept identical, except for system-dependent differences in light intensity and the mixing process. Growth experiments were conducted with three independent replicates on time series conducted over 7 days. Growth performance was evaluated based on specific growth rate (μ) and biomass productivity (P_x). For biochemical characterization of the biomass, phycocyanin content was determined spectrophotometrically, total lipid gravimetrically, total protein as the sum of amino acids, and total carbohydrate determined by difference. The results revealed that the specific growth rate and biomass productivity of *Arthrospira* spp. were almost two-fold higher in the bubble column (μ=0.26 g day^{-1} and P_x=89.54 mg L^{-1} day^{-1}) than

in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor (μ=0.16 g day^{-1} and P_x=46.90 mg L^{-1} day^{-1}). Growth system also had a significant effect on phycocyanin content, which was 14% higher in the bubble column compared to the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor (95.5 vs 81.5 mg g^{-1} DW, respectively). In contrast, growth system had no significant effect on total lipid, protein, carbohydrate and ash content (bubble column: 17%, 64.17%, 12.43% and 6.4% DW respectively and tubular PBR: 14.16%, 61.09%, 19.4% and 5.3% DW respectively) ($P>0.05$ = not statistically different). Total biomass obtained from semi-continuous operation was higher than for batch operation with 58.6 and 48.5 g DW, respectively. A higher culture suspension efficiency in the bubble column inoculator are suspected to have led to effects of light and dissolved CO_2, triggering improved growth performance of *Arthrospira* spp. in the bubble column inoculator. Growth systems had a significant impact on phycocyanin content ($P<0.05$), which might be attributable to better light penetration in the bubble column inoculator. Although the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor underperformed compared to the bubble column under basic operational conditions, it may still be adequate for *Arthrospira*'s mass production, especially when semi-continuous harvesting is integrated in the production process. Results from this study can be used as base line data for studies aiming to optimize system performance and on developing fresh *Arthrospira* product.

Chapter 1. Literature Review

1.1. Introduction

Arthrospira is a filamentous cyanobacterium that can be found both in freshwater and seawater habitats (Ciferri, 1983). *Arthrospira* contains many bioactive compounds such as protein in high percentage, polyunsaturated fatty acids, vitamins, minerals and the blue pigment phycocyanin, a potent anti-oxidant (Borowitzka, 2013). Due to those valuable compounds, *A. platensis* has been produced commercially and used largely in nutraceutical, pharmaceutical and cosmetic industries (Ciferri and Tiboni, 1985).

The wide applications of *A. platensis* in industry result in a high demand of this blue-green alga in the market. In 2016, the global market size of *A. platensis* was more than US$ 700 million, and this figure is expected to increase to almost US$ 2,000 million by 2026. The robust market demand of *A. platensis* can be attributed to the increasing application of *A. platensis* in cosmetics, the launching of natural food colours produced from *A. platensis* and the recent production of ready-to-drink smoothies prepared from *A. platensis* (Persistence Market Research, 2017).

Currently, *A. platensis* is mainly cultivated using open pond systems. Open ponds are used more commonly for the commercial cultivation of algal biomass because these systems are simpler and cheaper than closed systems (photobioreactors (PBRs)). However, open pond cultivation has some drawbacks such as high water losses due to evaporation, a low production of biomass and contamination risks (Dębowski et al., 2012). Contamination in open ponds systems can compromise food standard-quality of *Arthrospira* biomass, especially when producing fresh living *Arthrospira* for human consumptionCultivation using PBRs, on the other

hand, is less prone to contamination and produce higher yields of biomass. However, the high operating costs for process control, cleaning, and maintenance are often a bottleneck for commercial-scale cultivation (Huang et al., 2017).

This chapter provides overview on (1) the characteristic of *Arthrospira* including its morphology, habitat and biochemical compositions, (2) the use of *Arthrospira* in several fields, (3) the commercial cultivation of *Arthrospira* and a brief information on the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor that was used in this study is also provided. In the end of this chapter, the summary of aims, hypotheses and biotechnological significances of this research project were also mentioned.

1.2. Characteristics of *Arthrospira*

1.2.1. Morphology

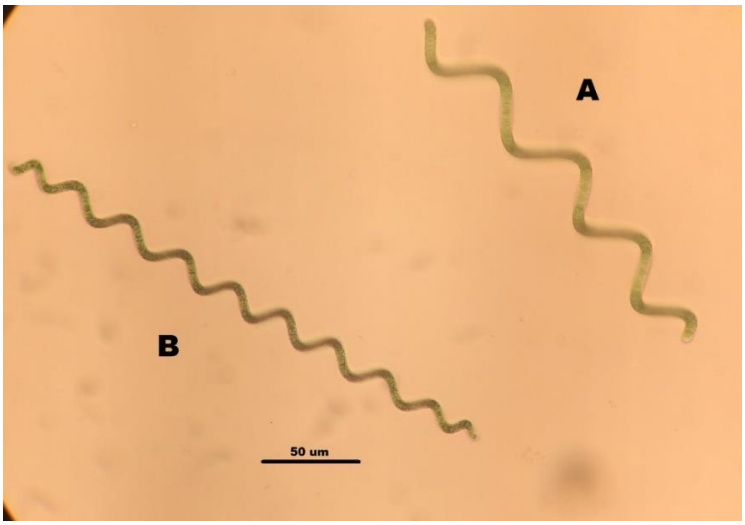


Figure 1.1. Micrograph of *Arthrospira platensis* (**A**) and *Arthrospira maxima* (**B**) under 100x magnification.

The main characteristic of *Arthrospira* is the helical shape of the filaments, which is only maintained in liquid medium. According to Tomaselli (1997), the length of

the filaments ranges from 50 to 500μm. The filament consists of vegetative cells and these cells divide by binary fission. Like other bacteria, *Arthrospira's* cell wall is composed by peptidoglycan.

The genus *Arthrospira* comprises around 15 species (Tompkins et al., 1995, Phang and Chu, 1999). Among those species, *Arthrospira platensis* and *Arthrospira maxima* are the most common species, which are cultivated worldwide.

Although filament shape of these two species is similar, the diameter of the filaments (or trichomes) in *S. maxima* are wider and the ends of the filaments are attenuated (**Fig. 1.1**) (Tomaselli, 1997).

1.2.2. Natural habitat and growth

Arthrospira can be found in a wide range of habitats including soil, freshwater, marshes, seawater, brackish water and thermal springs (Ciferri, 1983). However, unlike other cultivated microalgae, *Arthrospira* can grow in high alkaline conditions providing a competitive advantage. Waters with high pH (8.5 – 11) and moderately saline water (>30 g L^{-1}) favour good production of *Arthrospira* (Kebede and Ahlgren, 1996). In natural habitat, *Arthrospira* has been found growing in alkaline lakes such Lake Texcoco in Mexico and Lake Chad in Central Africa. *Arthrospira* is also a major microalga species found in the lake of the Rift Valley in Eastern Africa, where sodium carbonate is abundant and the pH value is close to 11 (Sasson, 1997).

Like many other cyanobacteria, *Arthrospira* is an obligate photoautotroph that cannot grow in the dark. Light is required mainly in the process of reducing carbon dioxide and assimilating nitrates (Cenci et al., 2013). The main carbohydrate storage product of *Arthrospira* is cyanophycean starch (Cenci et al., 2013). The

optimum growth temperature of *Arthrospira* is between 35 and 37˚C (indoors), whereas in outdoors, *Arthrospira* has been shown to tolerate an increase in temperature up to 39˚C for a few hours without adversely affecting photosynthetic performance. *Arthrospira* can also tolerate relatively low temperature especially at night and it has rather high resistance to ultraviolet light (Richmond, 1986).

1.2.3. Biochemical composition

Protein

The biochemical properties of *Arthrospira* have been analysed since 1970. *Arthrospira* contains considerable amounts of protein, between 60 to 70 percent of its dry weight (Pang et al., 2000).

Table 1.1. Protein content in *Arthrospira* and other foods (Koru, 2012)

Food Type	Crude Protein (%)
Arthrospira powder	65
Chicken egg	47
Beer yeast	45
Skimmed powdered milk	37
Cheese	36
Chicken meat	24
Beef	22
Fish	22

The protein content in *Arthrospira* is much higher compared to other sources of protein such as chicken egg, beef, fish, etc. (**Table 1.1**). *Arthrospira* protein also provides a complete amino acid nutrition, including leucine, isoleucine, valine, methionine, cystine, and lysine (Koru, 2012).

Lipid and polyunsaturated fatty acids

The crude lipid content of *Arthrospira* is approximately 5 – 7% on a dry weight basis (Falquet, 2006). So as a source of fatty acids, it is not a rich feedstock. In general, total lipids in *Arthrospira* can be divided into a saponifiable fraction (83%) and a non-saponifiable fraction (17%). The saponifiable fraction includes monogalactosyl and digalactosyl diglycerides, sulfoquinovosyl diglyceride, and phosphatidyl glycerol. While, the non-saponifiable fraction consists of pigments, sterol, essentially paraffin and terpene alcohol (Bujard et al., 1970, Santillan, 1974). According to Pascaud (1993), the dominant fatty acid in *A. maxima* is palmitic (63%), whereas γ-linolenic which is a type of ω-6 fatty acids, represents 40.1% of the total fatty acids in *A. platensis*. Overall, fatty acids content in *A. platensis* is higher than in *A. maxima*, as shown in **Table 1.2.**

Table 1.2. Distribution of fatty acids in two common strains of *Arthrospira* (Pascaud, 1993)

Fatty acids	***Arthrospira platensis*** (% of total fatty acids)	***Arthrospira maxima*** (% of total fatty acids)
Palmitic	25.8	63
γ-linolenic	40.1	13
Linoleic	12	9
Oleic	16.6	4
Palmitoleic	3.8	2
Stearic	1.7	1

Arthrospira contains 1.5-2.0% of polyunsaturated fatty acids (PUFAs) of total lipids. Like in other microalgae, essential omega-6 (ω-6) and (ω-3) fatty acids fatty acid contents can vary significantly in *Arthrospira* depending on growth conditions

and growth phase. For example, γ-linolenic acid (GLA) and linoleic acid (LA) contents have also been determined as 18% and 21% of total fatty acids, respectively (Chaiklahan et al., 2008).

Vitamins

Arthrospira can be considered as source of vitamins. Some vitamins such as vitamin A, vitamin B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_6 (pyridoxine), B_9 (folate), vitamin C, vitamin E (alpha-tocopherol) and vitamin K (phylloquinone) (Habib and Parvin, 2008).

Quantities of those vitamins vary in *Arthrospira*, with highest amounts being Vitamin B_3, followed by vitamin C and vitamin E (**Table 1.3**).

Table 1.3. Vitamin content in *Arthrospira* (Andrade et al., 2018)

Vitamins	Quantity (mg $100g^{-1}$ biomass dry weight)
Vitamin A	0.34
Vitamin B_1 (thiamine)	2.4
Vitamin B_2 (riboflavin)	3.7
Vitamin B_3 (niacin)	12.8
Vitamin B_6 (pyridoxine)	0.4
Vitamin C	10.1
Vitamin E	5.0

Pigments

Microalgae including *Arthrospira* contain many pigments. The pigments in microalgae can be classified into three major classes: chlorophylls, carotenoids and phycobilins. Chlorophylls and carotenoids are fat-soluble pigments, while phycobilins are water-soluble molecules (Begum et al., 2016). Chlorophylls consist of chlorophylls *a* only, whereas carotenoids are divided into two groups: carotenes

and xanthophylls (Vaz et al., 2016). Phycobiliproteins are sub-classified into four main classes: phycocyanin, phycoerythrin, phycoerythrocyanin and allophycocyanin. The maximum absorbance of each class as follows: phycocyanin λ_{Amax} 615-640 nm, phycoerythrin λ_{Amax} 565-575 nm, phycoerythrocyanin λ_{Amax} 577 nm and allophycocyanin λ_{Amax} 650-655 nm (Bryant et al., 1979). The major pigments in *Arthrospira* are β-carotene, phycobiliproteins (c-phycocyanin and allophycocyanin) and some xanthophylls (myxoxanthophyll, canthaxanthin, zeaxanthin, diatoxanthin) (Habib and Parvin, 2008).

The production of microalgal pigments can be affected by various environmental factors such as pH, temperature, photoperiods, irradiances, salinity, light quality, nutrient limitation, nitrogen supplements, heavy metal stress and pesticides (Hemlata and Fatma, 2009). Environmental and nutritional factors can also change phycobiliprotein composition in cyanobacterial cells (Grossman et al., 1993).

1.3. The use of *Arthrospira*

1.3.1. The use of *Arthrospira* in agriculture

Amongst other applications, *Arthrospira* is also used in agriculture as fertilizer. This blue-green alga acts as natural nitrogen source, which can replace chemical nitrogen in inorganic fertilizer. Dried *Arthrospira* is used by rice farmers in India and it increases the annual rice yield by 22%(Habib and Parvin, 2008). Zeenat et al. (1990) studied the effect of combined application of *Arthrospira* with chemical fertilizer on the yield of tomato, reporting a 522 and 977% increase in the number of fruits and the yield of tomato, respectively.

Arthrospira is not only used as fertilizer for plant, but it also used in poultry and livestock feeds as a protein supplement. Since *Arthrospira* contains high amount of

protein (**Table 1.1**), it can be used as protein supplement to partially replace soybean meal, fish meal, and groundnut meal which are usually used in fish, cattle, poultry and domestic animals diets (Venkataraman et al., 1994, El-Sayed, 1994, Britz, 1996).

1.3.2. The use of *Arthrospira* in aquaculture

The utilization of *Arthrospira* as a nutritional supplement in aquafeeds has been widely studied. Nakagawa and Gomez-Diaz (1975) studied the benefit of using *Arthrospira* as a feed additive for giant freshwater prawn farming, demonstrating significantly enhanced growth and survival. In addition, the cost of using *Arthrospira* as a feed ingredient is lower than for others feed additives.

In a comparison to feed of dried *Ecklonia maxima*, soybean meal and casein as feed ingredients, *Arthrospira* meal proved to significantly improve the growth performance of abalone (*Haliotis midae*) (Britz, 1996). It was further demonstrated that an *Arthrospira*-based diet affected the fatty acid profile of fish eggs. According to Lu and Takeuchi (2004), the eggs of Nile tilapia (*Oreochromis niloticus)* fed fresh unprocessed *A. platensis* contained more linoleic acid, eicosatrienoic acid, docosapentaenoic acid and γ-linolenic acid.

1.3.3. Human use of *Arthrospira*

1.3.3.1. *Arthrospira* as a food and nutritional supplement

Arthrospira has been consumed as food since ancient times. It was used as food by Kanembu people, who lived around Chad Lake and the Aztecs who had harvested *Arthrospira* from Texcoco Lake, near Mexico City more than a thousand years ago (Liang et al., 2004, Nicoletti, 2016). Nowadays, *Arthrospira* is produced commercially as a food supplements, either in the form of powder or tablets, alone

or in combination with other microalgae or plant extracts, and it is suitable for human and animal nutrition (Nicoletti, 2016). *Arthrospira* is also produced in liquid form, and, together with other ingredients, is incorporated into various food and drink products, including noodles, bread, biscuits, and green tea (Liang et al., 2004).

1.3.3.2. Potential application of *Arthrospira* in pharmaceuticals

Arthrospira has many health and pharmacological properties. *Arthrospira* positively affected cholesterol metabolism through increases in high-density lipoprotein (HDL) levels, required for healthy cardiovascular function (de Caire et al., 1995). *Arthrospira* capsules was shown to lower the level of blood lipid, as well as reducing the white blood corpuscles after chemotherapy and radiotherapy (Ruan et al., 1990). As an auxiliary medicine, *Arthrospira* enhanced immune system function by increasing immunoglobulin A (IgA) and immunoglobulin M (IgM) levels (Liu et al., 2000).

The pigment C-phycocyanin has anti-oxidant and anti-inflammatory functions (Romay et al., 1998). A recent study has also mentioned that *Arthrospira*, particularly *A. platensis*, has potential as a prebiotic owing its carbohydrates contents such as glucose, rhamnose, mannose, xylose and galactose (Gupta et al., 2017).

1.3.3.3. Food safety aspects of *Arthrospira* for human consumption

Food safety aspects relating to the use of *Arthrospira* for human consumption is very important to note. This is because there are many species of blue-green algae which produce toxin (microcystins). The specific species of *Arthrospira* used for human consumption need to be clarified as there is possibility of contamination of *Arthrospira* with other cyanobacteria. So far, only products from *Arthrospira*

platensis have been cleared by public health authorities for human consumption in many countries including Canada, Australia, and United State of America (Habib and Parvin, 2008).

There are several studies about the microcystin content in *Arthrospira* products. A study conducted in Canada found no microcystins in blue-green alga products containing *Arthrospira* only (Health Canada, 1999). Gilroy et al. (2000) analysed 15 *Arthrospira* samples from dietary supplements, documenting the presence of MCYST-LR in all samples. However, percentages of MCYST-LR present in those *Arthrospira* samples were below the regulatory level established by Oregon Department of Agriculture (ODA). Therefore, *Arthrospira* is considered safe, under specific conditions. *Arthrospira* used as ingredient in foods is recognized as safe at levels of 0.5 to 3.0 grams per daily intake.

1.4. Cultivation and production of *Arthrospira*

1.4.1. Laboratory cultivation

Cultivation of *Arthrospira* at laboratory-scale are usually conducted to optimise cultivation conditions required to increase the productivity of *Arthrospira*. The productivity of *Arthrospira* is influenced by many environmental factors. According to Ciferri (1983), there are eight major environmental factors which influence the productivity of *Arthrospira*: temperature, pH, light regimes or luminosity, inoculation size, the presence of macro and micronutrient in the media, dissolved solids and stirring speed.

The effect of light regimes and nutritional media on the growth performance of *Arthrospira* have been evaluated in many studies. Based on the evaluation of chlorophyll content and optical density of *S. platensis*, the optimum photoperiod

for growth and yields was 16 h day:8 h night (Pareek and Srivastava, 2001). In contrast, intermittent addition of urea did not result in significant differences on the yield of *A. platensis* compared to continuous feeding (Sanchez-Luna et al., 2004). Although, light should be taken into consideration for optimising growth of *Arthrospira*, this factor cannot be controlled in large-scale outdoor cultivation.

1.4.2. Commercial cultivation

The cultivation of microalgae for commercial purpose is now over 50 years old. The large-scale cultivation of microalgae was first established in Japan in the early 1960s with *Chlorella* as the first species of microalgae to be cultivated commercially (Tsukada et al., 1977). It was followed by the mass cultivation of *Arthrospira* at Lake Texcoco, Mexico in the early 1970s. Leaders in the commercial production of *Arthrospira* are Hainan DIC Marketing in China, Earthrise Nutritional LLC in USA, Cyanotech Corporation in Hawaii, and Pary Nutraceutical in India. Today, it is estimated that 80% of the world's *Arthrospira* production comes from China. The total industrial production of *Arthrospira* in 2014 was estimated to be 8,000 tons of dry powder produced by 65 registered *Arthrospira* growers. The major producer of *Arthrospira* is China, followed by the United State of America, Thailand and Taiwan Province of China (Habib and Parvin, 2008).

1.4.2.1. Cultivation of *Arthrospira* in open pond systems

Currently, open ponds systems are the most commonly used systems to cultivate *Arthrospira* commercially due to favourable economics. Compared to closed systems, open ponds have simpler designs, are cheaper in construction and easier to operate. Open ponds are usually built in the form of a racetrack or round pond with up to 0.5 m in depth. Suspension is mechanical using a paddlewheel and CO_2

is required to avoid carbon limitation of photosynthesis. CO_2 can be obtained directly from the atmosphere through mixing and macro- and micro-nutrients required for growth are added directly into the ponds (Dębowski et al., 2012). The most common design of open ponds are raceway ponds. It is typically designed in a closed loop recirculation duct, and can be made of clay, PVC or concrete ranging from 1,000 to 5,000 m^2 (Molina-Grima et al., 2003).

(a) **(b)**

Figure 1.2. Examples of *Arthrospira* cultivation in open pond systems. *Arthrospira* farm in Boonsom, Thailand (a), and *Arthrospira* ponds in India owned by Parry Nutraceutical (b).
(Henrikson, 2011)

However, there are some variables that influence the production of *Arthrospira* grown in open ponds systems or outdoor cultivation. These factors include the oxygen concentration, light, temperature, photoinhibition, and contamination (Vonshak, 1997). Light relates to the seasonal differences in the length of day and night and light intensities. In the summer, the growth of *Arthrospira* in open systems can be photo-inhibited. In contrast, the main limiting factor for *Arthrospira* growth in winter is temperature (Vonshak, 1997).

1.4.2.2. Cultivation of *Arthrospira* in closed systems or photobioreactors

Mass cultivation of photosynthetic microalgae such as *Arthrospira* in photobioreactors (PBRs) is an attractive approach to produce high value products. Compared to open pond systems, many PBRs offer higher photosynthetic efficiency, low contamination, a controlled environment and prevent water quality changes due to evaporative water loss (Wang et al., 2012). The productivity of microalgae grown in different cultivation systems is shown in **Table 1.4**. With regards to *S. platensis* biomass productivity, open pond cultivation was as effective as flat-plate PBR, but performance in tubular PBRs was low.

Various types of PBRs have been developed to produce high value products from microalgae, including airlift reactors (Ranjbar et al., 2008), tubular PBRs (Richmond et al., 1993), flat-plate PBRs (Hu et al., 1996), bubble column reactors (Oncel and Vardar, 2008), conical reactors (Watanabe and Saiki, 1997), torus PBRs (Pruvost et al., 2006), stirred-tank reactors (Zhang, 2013), and seaweed-type PBR (Chetsumon et al., 1998).

Table 1.4. Productivities of some microalgal strains in different cultivation systems (Hulst, 2012, Xu et al., 2009)

Culture system	Microalgae strain	Productivity (g L^{-1} day^{-1})
Open pond	*Spirulina platensis*	2.1
	Chlorella	0.11-0.32
Tubular PBR	*Spirulina*	0.62
	Nannochloropsis	0.16-0.73
	Isochysis galbana	0.32
	Haematococcus pluvialis	0.06
	Chlorella sorokiniana	0.3-1.47
Flat plate PBR	*Spirulina platensis*	2.1
	Nannochloropsis	0.3-0.36

Although many different types of PBRs have been developed, only few of them can be utilized for mass cultivation, due to high capital and operating costs, propensity to biofouling, accumulation of harmful metabolites and oxygen build up, nutrient and carbon limitation – the latter three factors are of particular concern in long vertical tubular PBRs. In addition, the complexity of PBRs is still a challenge in designing more efficient types (Huang et al., 2017).

Figure 1.3. Different types of photobioreactors (a) A tubular photobioreactor designated PBR 4000 G IGV Biotech, and (b) plastic plate photobioreactor PBR 500 P IGV Biotech (Alaswad et al., 2015).

Wuhan Friendship New Tech. CO. LTD. recently designed a novel three in one tubular photobioreactor (a system that incorporates an inoculator, a tubular growth compartment and a harvester), specifically for the cultivation of food-grade *Arthrospira* (**Chapter 2.4**), for which growth performance needed to be evaluated. Therefore, this study investigated the growth performance, productivity and biochemical composition of *Arthrospira* spp. grown in this novel PBR under baseline conditions to provide data as a starting point for further research relating to mass production of fresh live *Arthrospira* as a direct food source for human consumption.

1.5. Summary of aims, hypotheses and biotechnological significances

1.5.1. Aims

The aims of this research project were as follows:

1. To investigate the growth performance of *Arthrospira* spp. cultivated in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and the bubble column inoculator.
2. To analyse the biochemical profiles of *Arthrospira* spp. biomass produced in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and the bubble column inoculator.
3. To compare total *Arthrospira* spp. biomass yields under batch and semi-continuous harvesting operations in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor

1.5.2. Hypotheses

The specific hypotheses to be tested in the current work were:

1. Differences in culture mixing will result in differences in growth performance and biochemical profiles of *Arthrospira* spp. between the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and the bubble column inoculator.
2. Running the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor under semi-continuous harvesting mode will result in higher biomass yields compared to the batch harvesting mode.

1.5.3. Biotechnological significance

The tubular Wuhan Friendship New Tech CO. LTD. photobioreactor tested in this study is a hitherto not evaluated microalgal cultivation system. Information related to the performance of this tubular Wuhan Friendship New Tech CO. LTD. photobioreactor is very limited and until today there has been no study regarding growth performance of any microalga produced in this system. The results of this research project provide baseline data on growth performance under standard non-optimised conditions and the impact on biochemical properties of *Arthrospira* spp. In addition, the data from this work will be useful for further research and investigations on determining optimized PBR operational parameters. Moreover, information on the productivity of *Arthrospira* spp. grown in this tubular Wuhan Friendship New Tech CO. LTD. photobioreactor under standard operating conditions can be used for consideration of production of *Arthrospira* for commercial purpose.

Chapter 2. Materials and Methods

2.1. Microorganism

The non-axenic culture of *Arthrospira* spp. used in this research was provided by the Centre for Marine Bioproduct Development in the College of Medicine and Public Health at Flinders University, South Australia, Australia.

2.2. Culture medium

The *Arthrospira* spp. was grown photoautotrophically in culture medium used at the industry partners premises (**Table 2.1**). The trace metal solution was prepared as a stock solution. Each litre of trace metals solution contained the following compounds: 2g H_3BO_3, 1g $MnCl_2$, 0.2g $ZnSO_4$, 0.05g $CuSO_4$, 0.01g $(NH_4)_6Mo_7O_{24}$, and 0.005g $Cu(NO_3)$. To prepare 1 L medium, compounds 1-9 were weighed into 900 mL of reverse osmosis (RO) water and 1 mL of the trace metals solution was added. The medium was made to 1 L with RO water.

Table 2.1. Composition of the culture medium (based on Zarrouk's medium)

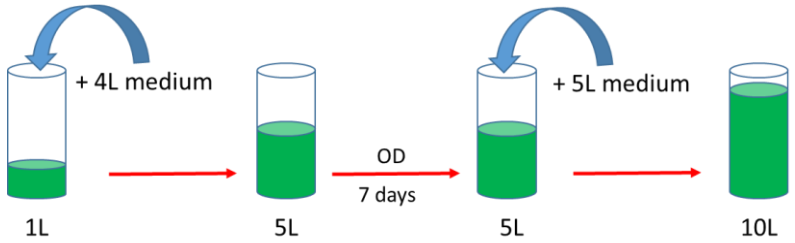
No	Component	Quantity (g L^{-1})
1	$CaCl_2$. $2H_2O$	0.02
2	$FeSO_4$	0.01
3	Na_2-EDTA. $2H_2O$	0.01
4	$MgSO_4$	0.2
5	NaCl	0.5
6	K_2SO_4	1
7	$NaNO_3$	2.5
8	K_2HPO_4	0.5
9	$NaHCO_3$	20
10	Trace metals solution	1 mL

2.3. Culture condition and inoculum preparation

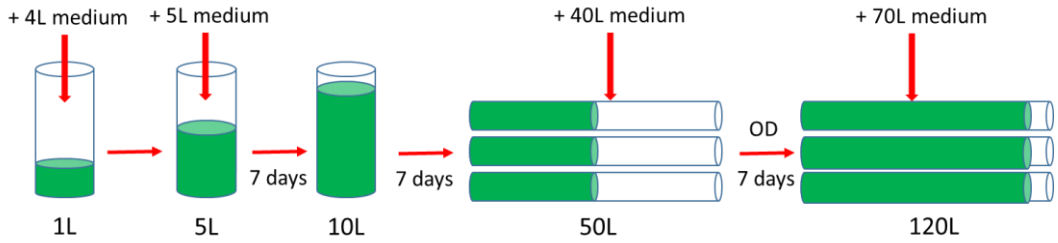
Arthrospira spp. cultures were grown in 250 mL Erlenmeyer flasks containing 100 mL of culture medium. These flasks were maintained on a reciprocating shaker at 84 rpm and placed in the plant cell culture room at 25±1˚C. These cultures were illuminated continuously with 84 μmol photons m^{-2} s^{-1}. Every three weeks, the cultures were sub-cultured into fresh medium at a ratio of inoculum to medium of 1: 10.

To raise the culture for the inoculation of larger scale cultivation systems, 100 mL culture was scaled up stepwise to 500 mL and then to 1 L. The 1 L culture was then used to inoculate 4 L medium in the 10 L bubble column inoculator, giving a total of 5 L culture with a starting optical density (OD) of 0.2 (ratio of inoculum to medium = 1:4). Once the OD reached 0.8, the culture was up-scaled to 10 L, starting with an OD of 0.4 in the bubble column inoculator (**Figure 2.1a**). OD and pH in the bubble column inoculator was now monitored daily for 7 days. This was repeated three times, to give three independent runs.

For the 120 L tubular Wuhan Friendship New Tech. CO. LTD photobioreactor, scaling up of culture from 1 L to 10 L was done in the bubble column inoculator, and the 10 L culture was then used to inoculate the tubular reactor. The 10 L inoculum (OD = 1) was transferred into the tubular reactor and 40 L medium was added (ratio of inoculum to medium = 1:4) to obtain 50 L culture with OD = 0.2. The 50 L culture was then allowed to grow until the OD become 0.96, so that after adding 70 L of medium, the OD of the final volume (120 L) become 0.4 (**Figure 2.1b)**. OD and pH measurements were taken daily for 7 days. This scaling up procedure was repeated three times to obtain three independent runs.



(a)



(b)

Figure 2.1. Schematic of inoculum preparation for the 10 L bubble column inoculator (a) and the tubular Wuhan Friendship New Tech CO., LTD. photobioreactor (b)

2.4. Growth experiment set up

The cultivation system used in this study was developed and manufactured in China by Wuhan Friendship New Tech CO., LTD. This system is designed to produce fresh, food-grade *Arthrospira* biomass for human consumption. Initially, this system was designed for one of the China Space projects aiming to supply fresh *Arthrospira* biomass as sustainable nutrients for future space stations.

This cultivation system consists of three major components:

- The in-line inoculator, a 10-litre bubble column inoculator that can be connected or separated from the main cultivation system. Inoculum is firstly grown in the in-line inoculator by applying bubbling air for suspension. Once the culture grows to a certain density, it can be directly inoculated into the main

cultivation system. To provide light for the culture, LED lights are equipped on one side of the cylinder tank.

- The main cultivation system is a 120-litre tubular reactor made from PVC (Polyvinyl chloride) with an outer diameter of 90 mm. Circulation in this system is driven by negative pressure, therefore, under vacuum, a natural wave is created to mix the culture designed to eliminate damage to *Arthrospira* filaments. LED lights sources are equipped on one side of the tubes. The reactor is equipped with temperature control and in-line pH monitoring.
- The PBR is equipped with a simple filtration-based harvesting system. This filtration unit is added at the end of the tubular next to the inoculator.

The experiments were carried out in the 120 L tubular Wuhan Friendship New Tech CO. LTD. photobioreactor (**Fig. 2.2b**) and in the 10 L bubble column inoculator (**Fig. 2.2a**). The tubular Wuhan Friendship New Tech CO. LTD. photobioreactor was set to default settings with a light intensity of 254 μmol photons m^{-2} s^{-1} and the vacuum was 0.1 bar or equal to 0.01MPa. The applied vacuum stopped every 20 min and started again after 5 min of rest.

The bubble column inoculator used is a component of the system which could be operated independently. While the tubular part of the system is made from clear UV-stabilized PVC, the bubble column inoculator is made from glass with outer diameter of 18 cm. Continuous air supply to the bubble column inoculator ensured culture mixing. LED lights (15W/DC12V) were fitted to one side of the bubble column inoculator to illuminate the culture. However, in this experiment, we added more light to the bubble column by providing 4 panel of 20-Watt LED lights, resulting in a light intensity of 347 μmol photons m^{-2} s^{-1}. The light intensity was

measured on four sides of the surface of the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and the bubble column inoculator (LiCor light meter). The average of the four side measurements per system is reported as the starting light intensity for the respective systems.

(a) **(b)**

Figure 2.2. The bubble column inoculator **(a)** with a maximal capacity of 10 L and the tubular compartments **(b)** with maximal capacity of 120 L.

Arthrospira spp. batch cultures of each system were grown for 7 days with three independent cultivation runs (inoculi) with the same starting cell density of 0.4 $OD_{560\ nm}$. At the end of each cultivation, cultures were harvested by filtering *Arthrospira* spp. cells using a nylon mesh with a pore size of 20 µm. **Table 2.2.** summarises the cultivation conditions of both systems.

Table 2.2. The cultivation condition of the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and the bubble column inoculator

Cultivation condition	Bubble column inoculator	The tubular Wuhan Friendship New Tech CO. LTD. photobioreactor
Temperature	25±1˚C	25±1˚C
Starting light intensity	347 μmol m^{-2} s^{-1}	254 μmol m^{-2} s^{-1}
Photoperiod	Continuous	Continuous
Stirring	Air bubbles	+/- pressure
Pressure	0.01 MPa	0.01 MPa
Volume	10 L	120 L

2.5.Growth analysis

2.5.1. Determination of biomass concentration

Biomass concentration of *Arthrospira* spp. was measured spectrophotometrically using a UV/VIS Spectrometer (T70 UV/VIS Spectrometer, John Morris Scientific Pty. Ltd., Suite 17, 22 Greenhill Road Wayville SA, 5034). Samples from each culture were taken daily to determine optical density at 560 nm (OD_{560nm})with the medium serving as a blank (Saeid and Chojnacka, 2016). Samples with an (OD_{560nm})> 1.0 were diluted with the medium to < 1.0. The pH of each culture was also measured daily using a pH meter (TPS 901-PH pH-mV-Temperature, Stennick Scientific, Melrose Park SA).

2.5.2. Determination of dry weight (DW) and biomass ash content

Arthrospira spp. dry weight was determined from (OD_{560nm}) using a calibration curve (Leduy and Therien, 1977) following regression equation 1 (eq. 1) with an R^2 of 0.9972 (**Appendix 1**).

$$y = 0.517x + 0.0993 \quad \text{(eq. 1)}$$

To establish the calibration curve, a culture dilution approach, using a stationary phase culture, was performed resulting in 20 mL of 0%, 20%, 30%, 40%, 50%, 60%, 80% and 100% of culture (n = 3). Following (OD_{560nm}) reads, samples were filtered through pre-ashed and pre-weighed MN GF 47mm filter paper (Macherey-Nagel GmbH & Co. KG, Germany) and dried at 60˚C overnight and then weighed using a 4-digit balance (Mettler Toledo AB204-S, Southern Cross Science PTY LTD., Melrose Park SA 5039).

To determine total ash content, the dry biomass samples from each culture were ignited in a muffle furnace (LABEC Laboratory Equipment P/L, Marrickville, NSW) at 550˚C overnight (AOAC, 2000). The weight of the samples before and after combustion was recorded on a 4-digit balance. The ash content was calculated following the equation 2 (eq. 2):

$$Ash\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample}\ x\ 100 \qquad \text{(eq. 2)}$$

2.5.3. Specific growth rate (μ) and biomass productivity

Specific growth rate (μ), expressed in mg day^{-1}, was measured during the exponential phase only and calculated using equation 3 (eq. 3) (Goksan et al., 2007):

$$\mu = \frac{lnX2 - lnX1}{t2 - t1} \qquad \text{(eq. 3)}$$

where X2 and X1 represent the biomass concentration at the times t2 and t1, respectively.

Biomass productivity (Px) was calculated as the ratio between the variation in dry weight (DW2 – DW1) and the cultivation time (Tc) according to equation 4 (eq. 4) (von Alvensleben et al., 2016):

$$Px = \frac{(DW2 - DW1)}{Tc} \quad \text{(eq. 4)}$$

Where: Px = biomass productivity (mg L^{-1} day^{-1})

DW1 = initial dry weight (mg L^{-1})

DW2 = final dry weight (mg L^{-1})

Tc = cultivation time (days)

2.6. Semi-continuous harvesting in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor

Semi-continuous harvesting was carried out in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor for 7 days. Culture was partially removed by harvesting *Arthrospira* spp. every day. Prior to harvesting, the optical density (OD) of the culture was measured and the amount of culture harvested was adjusted so that the OD of the remaining culture was 1 (equal to biomass concentration = 0.62 g DW L^{-1}). The harvested volume was replaced with fresh medium. Total biomass yield was calculated by adding up total biomass harvested each day.

2.7. Biochemical analysis

For biochemical analysis, filtered culture samples (culture paste) were freeze-dried in a freeze dryer (VirTis®BenchTop™ K; Labconco Corporation, Kansas City USA) to remove frozen water. Before freeze-drying, culture paste was rinsed with the RO water, then frozen at -80˚C for 1 or 2 h. The dried *Arthrospira* samples were homogenised into a powder using mortar and pestle which was stored at -20˚C prior to use. Three replicate samples were analysed per independent culture run. Data are presented as the mean of all replicates for the three independent runs.

2.7.1. Determination of phycocyanin (PC) content

Phycocyanin was extracted from freeze-dried *Arthrospira* biomass samples using repeated freezing and thawing (RFT) (Bennett and Bogorad, 1973). Samples were mixed with 0.1 M sodium phosphate buffer containing NaH_2PO_4 and Na_2HPO_4 (**Appendix 2**), followed by freezing at -20˚C for 24 h, thawing at 4˚C for 24 h. After that, phycocyanin was separated from the cell by centrifugation in the centrifuge (Select Spin 21; AdeLab Scientific, Thebarton, SA) at 9400rcf for 15 min.

Phycocyanin content was calculated according to Bennett and Bogorad (1973) (eq. 5):

$$PC = \frac{[OD620 - 0.47xOD652]}{5.34} \quad \text{(eq. 5)}$$

Where PC is the phycocyanin content (mg mL^{-1}), OD_{620} and OD_{652} are the optical densities of the samples at 620 and 652 nm, respectively.

Samples were diluted 100x with MQ water prior to absorbance readings, and the results were multiplied by the dilution factor.

2.7.2. Determination of total lipid

Total lipid content of *Arthrospira* biomass was determined gravimetrically. Lipid was extracted using a single-step procedure following Axelsson and Gentili (2014). Briefly, 20 mg of dried *Arthrospira* were rehydrated and resuspended with chloroform:methanol (2:1) and shaking for 20 min in a nutator. Samples were then centrifuged at 5300rcf (Select Spin 21; AdeLab Scientific, Thebarton, SA) for 15 min. The supernatant was collected to a pre-weighed glass tube and allowed to dry in the fume hood.

The lipid content was calculated using equation 6 (eq. 6) (Pohndorf et al., 2016):

$$Lipid\ (\%) = \frac{m1}{m2} \text{ x } 100 \quad \text{(eq. 6)}$$

Where $m1$ is the weight of extracted lipid (mg) and $m2$ is the weight of dried *Arthrospira* biomass (mg) used for extraction.

2.7.3. Determination of total protein

Analysis of protein of the dry *Arthrospira* biomass was outsourced to Shanghai Jiao Tong University for amino acid analysis, as the laboratory is not set up to conduct this analysis. Total protein content was determined by the sum of amino acids of the samples.

2.7.4. Determination of total carbohydrate

Total carbohydrate of the dried *Arthrospira* biomass was calculated by difference, following equation 7 (eq. 7) (Merrill and Watt, 1973).

Total carbohydrate (%) = 100% - (% protein + % lipid + % ash) (eq. 7)

2.8. Statistical analysis

To determine significance of differences between the means of two groups, data were analysed by Independent-Samples T-test using IBM SPSS Statistics 22. Observed differences of means were considered significant when $P < 0.05$. The degree of confidence was 95%.

Chapter 3. Results

3.1. Growth of *Arthrospira* spp.

Mean biomass growth of *Arthrospira* spp. cultivated in two different growth systems is shown in **Fig. 3.1a**. Over a cultivation period of 7 days, the increase in biomass concentration of *Arthrospira* spp. grown in the bubble column inoculator was higher than in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor, resulting in final biomass concentrations of 1.10 and 0.52 g DW L^{-1} in the bubble column inoculator and in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor, (respectively).

Semi-logarithmic transformation of the growth data showed that exponential growth of *Arthrospira* spp. occurred from day 0-4, entering late logarithmic phase thereafter (**Fig. 3.1a**). In contrast, in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor, the exponential phase was longer (day 1-7), but daily biomass increase was ~half of that of the bubble column inoculator.

This is reflected in the productivity (Px) and the specific growth rate (μ). Highest specific growth rate (μ) derived from the exponential (log) phase of the culture growth was 0.25 g DW day^{-1} and biomass productivity was also significantly higher (independent-samples T-test: $P<0.05$), reaching a maximum of 89.5 mg DW L^{-1} day^{-1} (**Figs. 3b-c**).

The pH of *Arthrospira* spp. culture increased gradually during cultivation in both growth systems **(Fig. 3.1d)**. The starting pH (day 0) was slightly lower in the bubble column inoculator (8.91) than in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor (9.13). After a cultivation period of 7 days, however, it was

similar to the pH in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor (9.8 vs. 9.7, respectively).

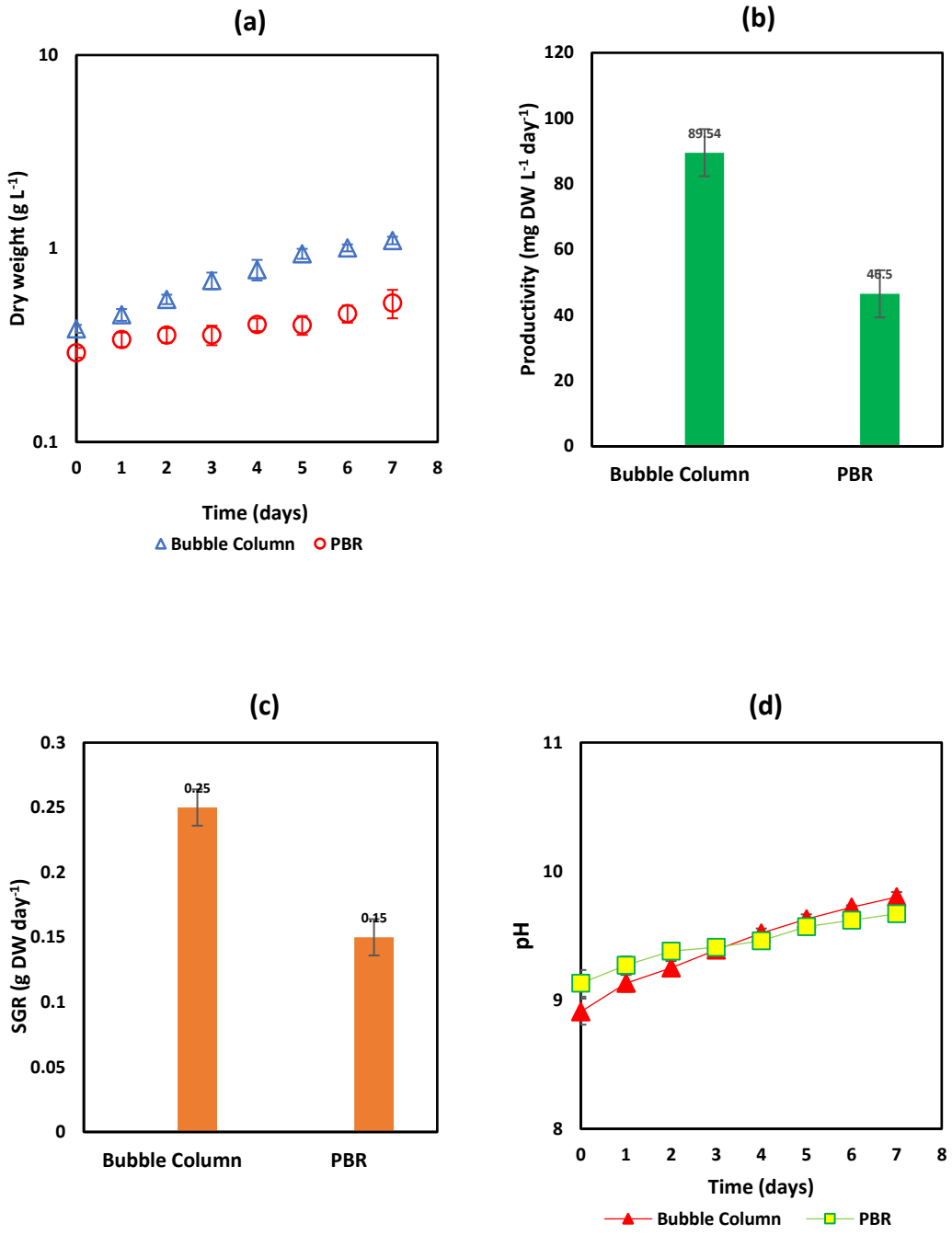


Figure 3.1. Biomass growth characteristic of *Arthrospira* spp. and culture pH development in the 10 L bubble column inoculator and the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor. **(a)** Mean biomass growth (g DW L^{-1}); **(b)** Mean biomass productivity (mg DW L^{-1} day^{-1}) and **(c)** Mean specific growth rate (μ) (g DW day^{-1}); **(d)** Mean culture pH development . *n=3*. Standard error is shown.

3.2. Biomass yields from batch and semi-continuous harvested growth system

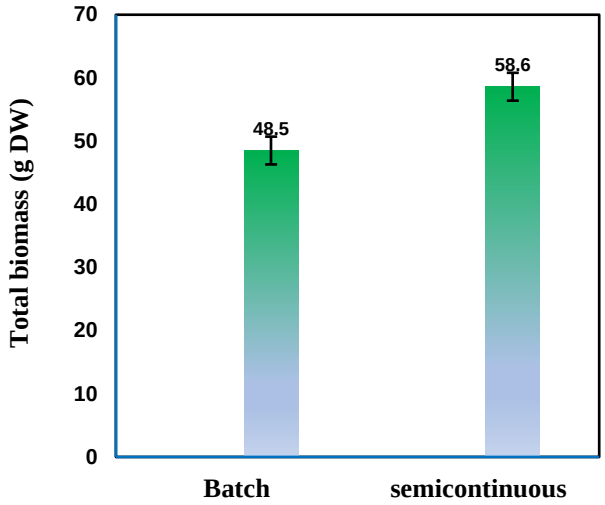


Figure 3.2. Total *Arthrospira* spp. biomass yields (g DW) obtained from batch and semi-continuously harvested growth systems. n=3. Standard error is shown.

Total dry biomass yields of *Arthrospira* spp. produced in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor, harvested in batch and semi-continuous mode, were higher under semi-continuous harvest operations (58.6 vs. 48.5 g DW), but differences were not significant ($P > 0.05$).

3.3. Biochemical profiles of *Arthrospira* spp.

In the present study, the biochemical profiles of *Arthrospira* spp. (phycocyanin -, lipid -, protein -, carbohydrate - and ash contents) cultivated in the bubble column inoculator and a tubular Wuhan Friendship New Tech CO. LTD. photobioreactor were compared. The phycocyanin content of *Arthrospira* spp. in the bubble column inoculator was 14% higher (95.5 mg g^{-1} $DW_{biomass}$) which was significant ($P<0.05$), compared to the content in the biomass from the tubular Wuhan Friendship New Tech CO. LTD. (81.5 mg g^{-1} $DW_{biomass}$).

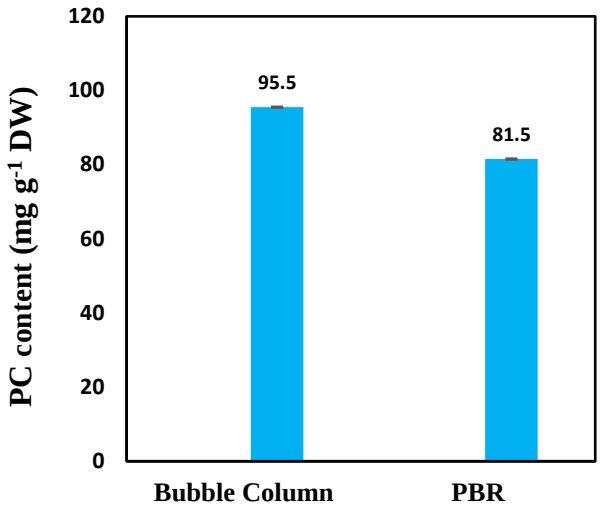


Figure 3.3. Phycocyanin content (mg g^{-1} DW) of *Arthrospira* spp. grown in the bubble column inoculator and the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor. n=3. Standard error is shown.

The lipid and protein contents of *Arthrospira* spp. cultivated in the bubble column inoculator were also higher than in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor (17 and 64.2% DW vs. 14.2 and 61.1% DW, respectively) (**Table 3.1**). In contrast, carbohydrate content of *Arthrospira* spp. cultured in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor was ~7% higher than in the bubble column inoculator (**Table 3.1**). There was no significant difference ($P > 0.05$) in ash contents between *Arthrospira* spp. cultures grown in the bubble column inoculator and in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor (6.4% and 5.3%, respectively; **Table 3.1**).

Table 3.1. Comparison of biochemical contents (% w/w DW) of *Arthrospira* spp. cultivated in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and in the bubble column inoculator. Data shown as Mean ± SE (n=3).

Biochemical content	Bubble column inoculator	Tubular Wuhan Friendship New Tech CO. LTD. photobioreactor
Lipid	17 ± 0.3	14.2 ± 0.2
Protein	64.2 ± 0.7	61.1 ± 4.4
Carbohydrate	12.4 ± 1.2	19.4 ± 4.1
Ash	6.4 ± 0.9	5.3 ± 0.5

The amino acid profile of *Arthrospira* spp. i is shown in **Table 3.2**. Glutamic acid (Glu) counted for the highest proportion both in *Arthrospira* spp. biomass produced in the bubble column and in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor, with 90.6 and 87 mg g^{-1} $DW_{biomass}$ respectively, followed by asparagine, leucine and alanine. Cysteine was present in the smallest amounts of ~4.9 and 4.7 mg g^{-1} $DW_{biomass}$.

Table 3.2. Amino acids composition and content of *Arthrospira* spp. cultivated in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and in the bubble column inoculator. Data shown as mean ± SE (n=3).

Amino Acids	Bubble column (mg g^{-1} $DW_{biomass}$)	Tubular Wuhan Friendship New Tech CO. LTD. (mg g^{-1} $DW_{biomass}$)
Asp	62.9 ± 0.2	60.2 ± 3.9
Thr	33 ± 0.3	31.2 ± 2.2
Ser	33.1 ± 0.6	31.5 ± 2.2
Glu	90.6 ± 1.9	86.9 ± 6.2
Gly	33.2 ± 0.2	31.8 ± 2.2
Ala	51.3 ± 0.8	49 ± 3.4
Val	40.7 ± 0.4	39.5 ± 2.6
Cys	4.9 ± 0.2	4.7 ± 0.3

Met	15.3 ± 0.5	14.4 ± 1.1
Ile	37.0 ± 0.5	35.8 ± 2.5
Leu	61.4 ± 0.7	59.3 ± 4.1
Tyr	32.1 ± 0.7	30.7 ± 2.3
Phe	30.7 ± 0.4	29.3 ± 2.1
Lys	31.6 ± 0.4	30 ± 2.4
His	10.6 ± 0.1	10.2 ± 0.7
Arg	45.4 ± 0.4	43.4 ± 3.5
Pro	24.7 ± 0.8	23.4 ± 1.9

Chapter 4. Discussion

In the present study, the growth performance in 7-day time course experiments and the biochemical profiles of *Arthrospira* spp. cultivated in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and a bubble column inoculator were compared.

4.1. Growth of *Arthrospira* spp.

Specific growth rates and biomass productivity of *Arthrospira* spp. were significantly better in the bubble column inoculator than in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor. The influence of operational parameters such as temperature, culture medium and pH (Shi et al., 2016, Ndjouondo et al., 2017) can be eliminated, as these factors were kept identical between the two growth systems. Hence, the better growth performance of *Arthrospira* spp. in the bubble column inoculator was more likely due to the system-dependent differences in light and culture mixing.

Light, together with CO_2 are very important growth parameters for all oxygenic photoautotrophs, essentially converting the inorganic carbon into organic compounds essential for growth and deposited as storage products, once growth reaches stationary phase or slows (Carvalho et al., 2011). A vast body of literature has investigated the effect of light on the growth of *Arthrospira*, investigated in detail in terms of intensity and quality (Ravelonandro et al., 2008) (Shi et al., 2016). The tubular Wuhan Friendship New Tech CO. LTD. photobioreactor and the bubble column inoculator used in this study were equipped by LEDs as a source of light set at one side of the system. The starting light intensity in the bubble column inoculator and the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor

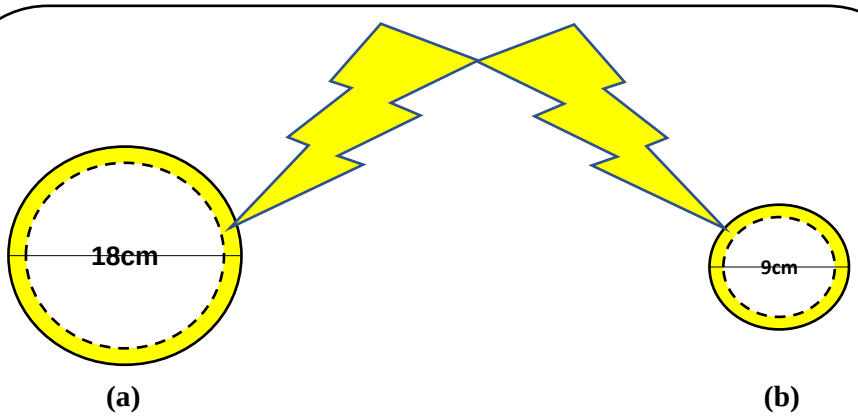


Figure 4.1. Schematic of light penetration and diameter size difference between the bubble column inoculator (a) and the tubular Wuhan Friendship New Tech CO. LTD. reactor (b).

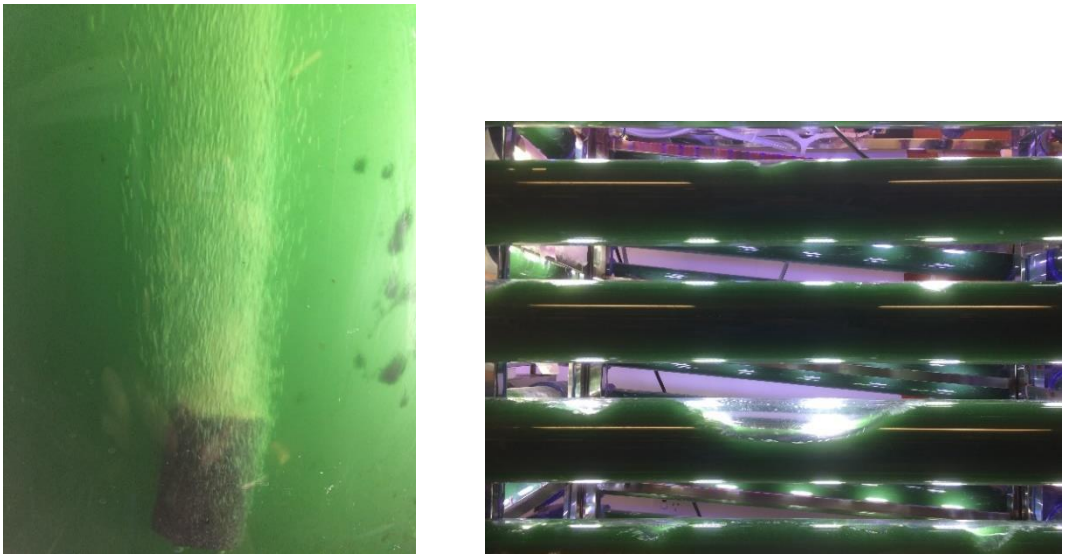


Figure 4.2. Difference in culture mixing between the two growth systems. (a) air bubbles from a sparger aiding culture suspension in the bubble column inoculator, (b) waves and large air pockets created in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor.

were 347 and 254 μmol photons m^{-2} s^{-1}, respectively. If we compare the structure of both systems, the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor consists of tubes with an outer diameter of 9 cm. In contrast, the bubble column inoculator is a cylindrical tank with an outer diameter of 18 cm (**Fig. 4.1**). Based on diameter differences between the systems, it was more likely that the culture in the bubble column inoculator would be light-limited due to an increased dark zone. The dark zone is the zone where the cell growth rate is negative due to the lack of light required for photosynthesis (Merchuk and Wu, 2003, Bitog et al., 2011).

An additional difference between the two growth systems is the mixing (suspension and gas exchange) process, which has an impact on light and nutrient availability (Borowitzka, 1999, Vadiveloo and Moheimani, 2018). Mixing also prevents cell clumping, cell sedimentation and cell attachment to the reactor's walls (Anjos et al., 2013, Carvalho et al., 2006). Hence, the mixing process might have also contributed to the observed differences in growth performance of *Arthrospira* spp. In the bubble column inoculator, bubbling air from the sparger was used for culture suspension and CO_2 mass transfer (**Fig. 4.2a**). In contrast, culture circulation was driving by negative pressure, creating waves and large air pockets in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor (**Fig. 4.2b**). Culture mixing process in the bubble column inoculator seemed to be more effective than in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor, as the air bubbles created provided faster culture circulation, shuttling the cells between the dark zone and the illuminated zone. Cycling between the dark zone and illumination zone allows efficient photosynthetic electron transport in photosystems I and II,

which is widely thought to produce more ATP and reducing equivalents ($NADPH+H^+$) essential for effective CO_2 fixation (Xue et al., 2013, Grobbelaar, 1991). Culture circulation and light/dark cycling also occurs in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor, it is, however, not as efficient due to possibly lower gas exchange rates. The efficiency of mixing can be determined based on the time it takes a system for homogenous suspension of the biomass following inoculation. This took ~24 h for the novel bioreactor, whereas the bubble column inoculator homogeneously suspended the inoculate within minutes (personal observation). Lower gas flow rate have been shown to decrease photosynthetic efficiency and poorer growth performance (Janssen et al., 2003).

In addition, due to a larger surface to volume ratio, the tiny bubbles in the bubble column inoculator can be predicted to provide higher concentrations of dissolved CO_2 compared to the large air pockets created in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor. Concentrations of dissolved CO_2 are an important parameter governing together with light the efficient conversion of CO_2 into biomass (Posten, 2009, Fernandez et al., 2013). As microalgae cannot utilize gaseous CO_2, but can access dissolved CO_2 (Huang et al., 2017), insufficient provision of dissolved CO_2 would result in carbon-limitation of the culture. Carbon dioxide does not easily dissolve in water. The dissolution process of CO_2 in a liquid involves the transfer of carbon dioxide gas ($CO_{2\,(g)}$) into carbon dioxide in aqueous solution ($CO_{2\,(aq)}$). In this process, carbon dioxide molecules in the gas mixture must pass the air barrier first, and then pass through the water barrier to dissolve in water (Batema, 2018). Bubbling the gas mixture in tiny air bubbles would achieve faster gas-to-liquid mass transfer (Holdt et al., 2014), compared to the large air

pocket/water interface in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor. This together with observed light differences and suspension efficiency would also result in improved light and nutrient supply (Barbosa et al., 2003, Merchuk and Wu, 2003), explaining the better growth performance of *Arthrospira* spp. in the bubble column inoculator.

Documentation of culture pH showed that gradual pH increase was comparable in both growth systems during the cultivation period. Increase in pH is a consequence of reduction of H^+ concentration in the culture medium through assimilation of HCO_3^-, accordingly to the equilibrium changes through conversion of H_2CO_3 ===> HCO_3^- ===> $CO_2 + H_2O$ (Chi et al., 2011). The change of the culture pH in the bubble column inoculator was slightly faster than in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor. CO_2 and nitrate uptake results in an increased of medium pH as demonstrated by Scherholz and Curtis (2013). Although culture internal light intensities could not be measured, and nitrate consumption was not monitored, the almost identical development of culture pH between the systems suggests that faster growth of *Arthrospira* spp. in the bubble column inoculator was driven by mixing-determined better light and nutrient availability.

Based on final culture pH, both cultures in growth systems could have been slightly carbon-limited, but according to Pandey et al. (2010) the optimum pH for growth of *Arthrospira* is 9.0-9.5. In another study by Richmond and Grobbelaar (1986), the optimum pH for the cultivation of *Arthrospira* was documented as 10.5, with growth decreasing at a pH close to 11.0. The final pH reached in both systems was 9-9.5, thus not exceeding the optimal pH range *Arthrospira platensis* (Ismaiel et al., 2016).

4.2. Biomass yields in batch and semi-continuous harvesting regimes

In this study, the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor was run as batch- and semi-continuously harvested system. Each harvesting regime was replicated in triplicate for a period of 7 days each. Under batch harvest operation, the culture medium was added once at the start of the cultivation and *Arthrospira* spp. was harvested at the end of cultivation period. In contrast, under semi-continuous harvest operation, a portion of culture suspension was removed periodically and replaced with fresh medium to the original volume (Livansky, 1979). The amount of culture removed was calculated so that the optical density (OD) of the remaining culture was maintained at 1. In other words, we did not set a certain rate for the daily renewal of the culture volume. It was expected that semi-continuous harvesting would yield more biomass than batch harvesting, as the culture is not harvested completely, and nutrients are resupplied periodically at the time of harvest.

As expected, total *Arthrospira* spp. biomass yields were 10% higher in semi-continuous harvesting regime than for the batch-harvest operated system. Similarly, semi-continuous harvest of the cyanobacterium *Synechococcus* sp. resulted in 20% daily harvests (Rosales Loaiza et al., 2004). This result obtained here for *Arthrospira* spp. is in general accord with semi-continuously harvested *S. platensis* but 2-4-times higher biomass yields were achieved compared to batch-operated PBRs (Reichert et al., 2006). The lower biomass yield achieved in this study is probably due to the difference in cultivation period (7 days this study vs. 90 days). This suggests that, for biomass yields, semi-continuous harvesting regimes are more beneficial than batch cultivation.

In general, it has been shown that semi-continuous harvesting seems to be a most feasible cultivation strategy for microalgae, due to higher growth rates, maximum cell densities obtained and biomass productivities (Fuentes-Grunewald et al., 2015). According to Fabregas et al. (1996), semi-continuous harvesting also results in operational benefits over batch harvesting, such as maintaining the inoculum in a constant amount and maintaining cultured microorganisms at high specific growth rate. However, one of the drawbacks of semi-continuous harvesting is increased risk of contamination and the introduction of competitors or predators, which result in the build-up of metabolites, interfering with culture growth eventually (Otero et al., 1998).

4.3. Biochemical profiles of *Arthrospira* spp.

4.3.1 Phycocyanin content

Phycocyanin (PC) is a water-soluble blue pigment, belonging to a group of light-harvesting proteins called phycobiliproteins (Gantt, 1981). Phycocyanin, along with other phycobiliproteins such as phycoerythrin (PE) and allophycocyanin (APC), plays an important role in photosynthetic light harvesting with maximum absorption at 620 nm (MacColl, 1998). Another biological role of phycobiliproteins is as intracellular nitrogen store, which are degraded selectively when the cells experience nitrogen starvation (Allen and Smith, 1969, Sloth et al., 2006, Boussiba and Richmond, 1980, Yamanaka and Glazer, 1980, Lewitus and Caron, 1990).

PC content is determined by biomass productivity and biomass productivity is determined by light supply and the efficiency of light utilization to make biomass (Eriksen, 2008). In a growth system where culture circulations between light and dark zone is rapid and the exposure times of the cells to the high surface light

intensities are short, photoinhibition is decreased and the photosynthetic efficiency of the culture increased (Gitelson et al., 1996). Higher PC content (95.5 mg g^{-1} DW) of *Arthrospira* spp. biomass cultivated in the bubble column inoculator can be explained as the impact of effective culture mixing which leads to better light/dark cycling and faster culture growth. As a result, culture in the bubble column inoculator became denser more quickly, resulting in light limitation. *Arthrospira* cells respond to light limitation by providing more and larger antenna complexes in order to capture sufficient light for photosynthesis (Olaizola and Duerr, 1990). Phycocyanin is a part of the antenna complexes, hence, the more or larger antenna complexes, the higher the PC content in the cell. Moreover, as PC also serves as nitrogen store (Liotenberg et al., 1996), higher PC content indicated that *Arthrospira* spp. culture in the bubble column inoculator was nitrogen sufficient.

4.3.2 Total Lipid

Total lipid contents of *Arthrospira* spp. biomass cultured in the bubble column and in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor were 17% and 14.16% (w/w DW), respectively. Total lipid content was much higher than reported contents for both *S. maxima* and *S. platensis* (5.6-7% of dry weight (Bujard et al., 1970, Challem et al., 1981, Santillan, 1974)) and more similar to 11% of total lipids reported for *Arthrospira* by Hudson and Karis (1974).

Variations in total lipid obtained could be affected by extraction methods used. In the present study, we applied a single-step extraction method by using chloroform:methanol (2:1) as solvent. Extraction method using this solvent mixture seemed to be more effective and result in more lipids being extracted compared to other solvents such as n-hexane (Ambrozova et al., 2014). A higher lipid content

may indicate the presence of other lipid-soluble compounds, especially carotenoid pigments (von Alvensleben et al., 2013, Lim et al., 2012).

Overall, fatty acids content in *A. platensis* has been reported to be higher than in *S. maxima* (Pascaud, 1993), which is at odds with results obtained here for *Arthrospira* spp., which represented a two species *Arthrospira* culture (*A. platensis* and *A. maxima*), dominated by *A. maxima* in both systems (personal observations). As such, the observed higher total lipid content in the bubble column inoculator is unlikely due to a dominance shift between *S. platensis* and *S. maxima* in the two cultivation systems. Indeed, total lipid and fatty acid composition varies in different microalgal strains including *Arthrospira*. Changes in lipid accumulation in microalgae are usually influenced by culture conditions. Various stress sources such as nutrient depletion, salinity, temperature, light, etc. have been reported as the factors that affect the composition of lipid and fatty acids in *Arthrospira* (Vonshak et al., 1996, Rafiqul et al., 2003, Isik et al., 2006, Ayachi et al., 2007). Previous studies showed an increase in lipid and fatty acid content of microalgae following nutrient-depletion (Converti et al., 2009, Dean et al., 2010, von Alvensleben et al., 2016). Higher lipid content of *Arthrospira* spp. in the bubble column inoculator was more likely due to nutrient-limitation, indicated conclusion supported by the faster increase in culture pH.

4.3.3 Total Protein

Total protein obtained may vary depending on the analytical method used for determination. According to Maehre et al. (2018), most of analytical methods used for protein quantification are inaccurate, due to the application of indirect measures such as nitrogen content and subsequent conversion. In this study, total protein was

determined through amino acids analysis, which is considered an accurate method because the results are not affected by interfering substances (Wilson and Walker, 2010).

Total protein of *Arthrospira* spp., determined as sum of amino acids (AA), were not significantly different in the two growth systems (64 vs 61% in the bubble column inoculator vs. the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor, respectively). This result is comparable to the high protein content (60-70% of its dry weight) reported for *Arthrospira* (Gutierrez-Salmean et al., 2015). In fact, the major proteins present in *Arthrospira* is C-phycocyanin, a type of phycobiliprotein, representing ~20% of *Arthrospira* dry weight (Khan et al., 2005). Factors such as harvesting regime and timing in regard to light/dark cycle may affect total protein content in the obtained biomass. It has been reported that highest protein content in *Arthrospira* was achieved when harvested at early daylight (Falquet, 2006).

Arthrospira is considered to provide a complete protein diet as it contains all essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) and nonessential amino acids (aspartic acid, arginine, serine, tyrosine, glutamic acid, glycine, alanine, cysteine and proline) (Belay et al., 1996, Becker, 1986). In the analysed samples, glutamic acid had the highest proportion among all amino acids, followed by asparagine, leucine and alanine, while cysteine accounted for the smallest proportion, and all other amino acids present in adequate concentrations. Glutamic acid is a non-essential amino acid, believed to have important roles in the human brain and spinal cord as a major excitable neurotransmitter. Glutamic acid is also recognized for its ability to

detoxify muscle cells and help shuttle potassium between the blood-brain barrier and spinal fluid (NCBI, 2018). In contrast, leucine is an essential amino acid, which plays important role in protein synthesis, regulating blood-sugar levels, promoting the growth of muscle and bone, and producing somatotropin (growth hormone) (Garlick, 2005). High concentrations of both glutamic acid and leucine obtained in the *Arthrospira* spp. biomass cultivated here validate its use as an important nutritional supplement (Gutierrez-Salmean et al., 2015).

4.3.4. Total Carbohydrate

Calculation on carbohydrate content revealed that total carbohydrate content of *Arthrospira* spp. grown in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor was 7% higher compared to those grown in the bubble column inoculator. These results may suggest light-, CO_2- and/or nutrient-limitation in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor due to less effective culture mixing which leads to less gas exchange. As a result, culture growth in the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor was limited. However, at the same time, photosynthesis and CO_2 fixation was still running and produces organic carbon (sugars). Because of the limited growth, organic carbon was not utilised for growth, but it was stored as carbohydrate.

The availability of CO_2 affects the carbohydrate content of microalgae. As pointed by Thyssen et al. (2001), decrease in CO_2 availability resulted in an increase in microalgal carbohydrate accumulation. Izumo et al. (2007) also demonstrated an increase in carbohydrate content in microalgal biomass by 2.5-fold when CO_2 concentration was decreased from 3 to 0.04%. Increasing in carbohydrate accumulation cannot only be induced by depletion of CO_2, but also by limiting the

amounts of other nutrients such as nitrogen, phosphorus, sulphur and iron (Markou et al., 2012). That is why nutrient starvation could be a good strategy for increasing carbohydrate content in microalgal biomass. Nitrate-assimilation is a light-dependent process (Malerba et al., 2015). Therefore, light-limitation together with a poor circulation of nutrients within the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor could have resulted in increased diversion of photosynthetically fixed carbon from growth to storage (von Alvensleben et al., 2016, Malerba et al., 2015).

Chapter 5. Conclusion and future research directions

5.1. Conclusion

Conclusions that can be drawn from the results obtained in this research project are as follows:

1. Based on publish scientific principle on impact of growth systems on performance, this study validated the expected positive effects of positive pressure air bubbling on growth performance.
2. Higher phycocyanin content of *Arthrospira* spp. in the bubble column inoculator generated biomass leads to the recommendation to use this system specifically for phycocyanin production.
3. Semi-continuous harvesting is recommended for future operation of the tubular Wuhan Friendship New Tech CO. LTD. photobioreactor as it resulted in higher *Arthrospira* spp. biomass yields compared to the batch harvesting.
4. Overall, the performance of this novel system was great and can be recommended for fresh *Arthrospira* production.

5.2. Limitations and future research directions

The only issue found during investigating this cultivation system was biofouling which leads to long downtime for cleaning. Chemical cleaning is the only way to clean the tubular compartment of this cultivation system, and the cleaning process took a long time (almost one week for cleaning).

Therefore, for the future research direction, we need to work on system optimization to shorten the downtime, either by improving cleaning method (i.e. improving fouled biomass removal) or by reducing biofilm formation (i.e. coating on tubes). In addition, because the final goal of this project is to produce fresh-live

Arthrospira products, it is important to characterize the bacteria which are associated with this *Arthrospira* culture, to determine whether the bacteria are 'good' or 'bad'. It is recommended to eliminate the present of harmful bacteria to meet the food grade requirement. From a human fresh food application perspective, it is also interesting to investigate the growth performance of an axenic culture of *Arthrospira* in this cultivation system, to see whether the axenic cultures behave differently to the non-axenic culture.

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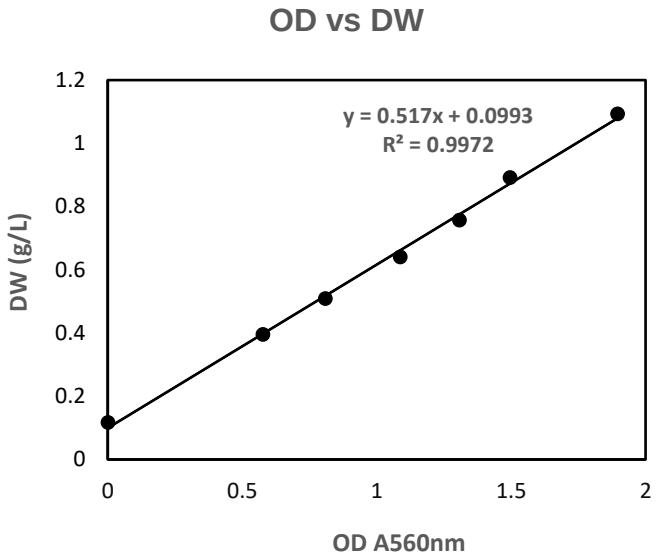
Appendices

1. Calibration curve showing relationship between the absorbance of *Arthrospira* spp. culture at 560nm and the dry weight (DW).



2. Required components to make 0.1M phosphate buffer

Component	Mass	Molar
Sodium phosphate dibasic (mw: 268 g/mol)	20.209 g	0.0754 M
Sodium phosphate dibasic (mw: 138 g/mol)	3.394 g	0.0246 M

3. Summary of total amino acids (AA) content (ng/mg)

Amino Acids	Bubble column			Tubular Wuhan Friendship New Tech CO. LTD.		
	r1	r2	r3	r1	r2	r3
Asp	62473.63	63247.21	62831.04	53524.87	67263.97	59929.13
Thr	32870.19	33556.07	32496.58	27516.13	35020.28	30993.12
Ser	33111.11	34140.56	32196.22	27770.65	35484.66	31137.8
Glu	90557.4	87424.43	93841.32	77284.75	98584.14	84941.99
Gly	32768.9	33378.48	33352.92	28207.69	35758.1	31552.07
Ala	50772.05	52757.42	50301.81	43733.78	55248.07	47891.95
Val	40166.75	41423.89	40407.58	35053.09	44200.95	39299.09

Cys	4602.53	5084.68	5146.5	4341.01	5309.36	4328.27
Met	14829.96	16224.44	14806.16	12402.43	16313.33	14355.77
Ile	36390.89	38006.98	36714.62	31462.57	40288.42	35559.02
Leu	60662.49	62783.08	60696.64	52293.7	66579.72	58896.2
Tyr	31543.04	33455.12	31423.69	27050.85	34846.59	30197.74
Phe	30165.87	31425.46	30429.86	25946.94	33017.99	28859.15
Lys	31343.25	32306.17	31148.32	25651.19	33840.23	30427.51
His	10307.35	10741.23	10618.52	8916.88	11437.88	10323.39
Arg	45024.34	46261.85	44977.41	36866.47	48841.27	43928.45
Pro	26191.67	24331.55	23587.78	20475.13	27046.36	22595.03
Total	**633781.42**	**646548.62**	**634976.97**	**538498.13**	**689081.32**	**605215.68**

Growth system	**Total Amino acids (ng/mg)**			**Mean**	**Standard deviation**	**Standard error**
	r1	r2	r3			
Bubble Column	633781.42	646548.62	634976.97	638435.67	7051.404344	4071.130196
Tubular Wuhan Friendship New Tech CO. LTD.	538498.13	689081.32	605215.68	610931.71	75454.15182	43563.47486

Growth system	**Protein content (%)**			**Mean**	**Standard deviation**	**Standard error**
	r1	r2	r3			
Bubble Column	63.37	65.65	63.49	64.17	1.283121195	0.740810367
Tubular Wuhan Friendship New Tech CO. LTD.	53.85	68.91	60.52	61.09333	7.54635232	4.356888543

4. Hitachi Amino Acid Analyzer Reports of 6 *Arthrospira* spp. powder's samples from Shanghai Jiaotong University

- **Sample 1 (Bubble column r1)**

Hitachi Amino Acid Analyzer Report

Analyzed: 2018/8/16 18:22:45 Reported: 2018/8/18 13:34:10
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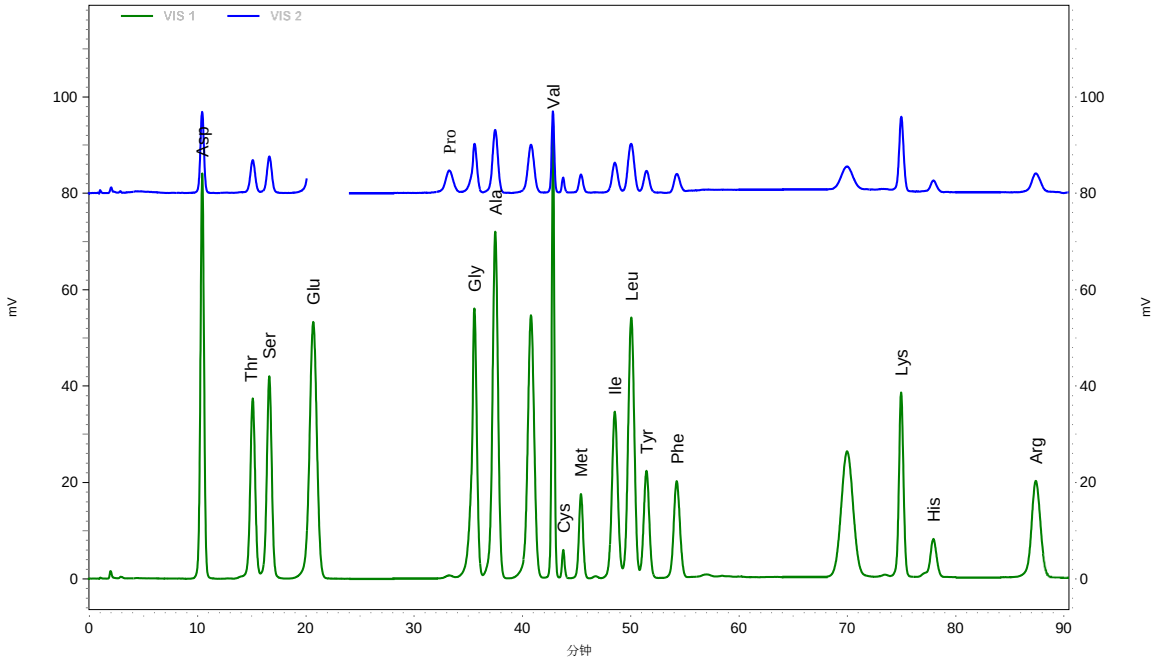
Vial Number: 171 Inj. Volume(uL): 10



VIS 1 Results

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
5	10.480	Asp	7832873	11.52	469.37	62473.63
6	15.153	Thr	4853127	6.43	275.99	32870.19
7	16.680	Ser	5444972	1.81	315.04	33111.11
8	20.733	Glu	10147932	3.86	615.62	90557.40
10	35.627	Gly	7626598	2.30	436.51	32768.90
11	37.540	Ala	10344572	2.21	569.90	50772.05
13	42.880	Val	6436988	2.62	343.01	40166.75
14	43.827	Cys	399467	2.10	19.15	4602.53
15	45.467	Met	1884551	2.82	99.40	14829.96
17	48.593	Ile	5262242	1.99	277.37	36390.89
18	50.113	Leu	8621264	1.47	462.37	60662.49
19	51.513	Tyr	3184211	1.38	174.08	31543.04
20	54.293	Phe	3174671	2.75	182.60	30165.87
26	75.033	Lys	4488396	1.70	214.39	31343.25
28	78.007	His	1262809	0.00	66.41	10307.35
29	87.460	Arg	4636830	7.25	258.46	45024.34
Total			85601503		4779.67	607589.75

VIS 2

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
6	33.287	Pro	941045	3.06	227.56	26191.67
Total			941045		227.56	26191.67

- **Sample 2 (Bubble column r2)**

Hitachi Amino Acid Analyzer Report

Analyzed: 2018/8/16 22:07:33 Reported: 2018/8/18 13:37:43
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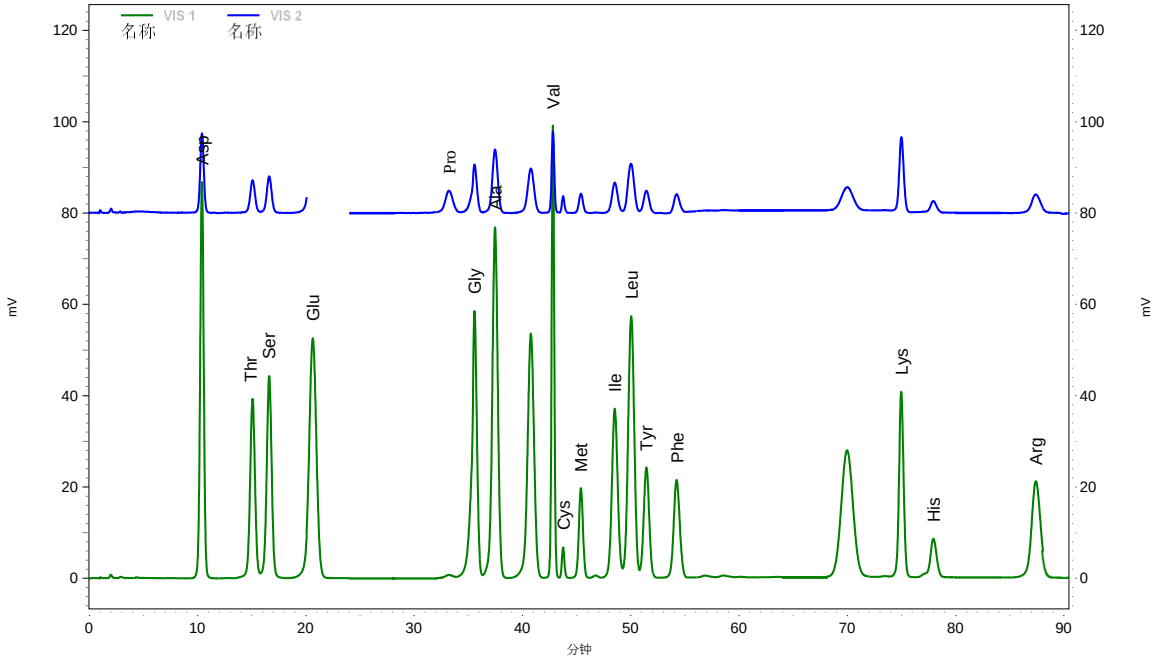
Vial Number: 172 Inj. Volume(uL): 10



VIS 1 Results

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
5	10.467	Asp	8119423	12.22	475.19	63247.21
7	15.133	Thr	5072826	5.60	281.75	33556.07
8	16.667	Ser	5748466	1.82	324.84	34140.56
9	20.707	Glu	10031037	3.84	594.32	87424.43
11	35.620	Gly	7954170	2.25	444.63	33378.48
12	37.527	Ala	11006033	2.19	592.18	52757.42
14	42.880	Val	6797141	2.63	353.75	41423.89
15	43.827	Cys	451863	2.10	21.16	5084.68
16	45.460	Met	2111043	2.81	108.74	16224.44
18	48.580	Ile	5627310	1.96	289.69	38006.98
19	50.100	Leu	9135929	1.47	478.53	62783.08
20	51.500	Tyr	3457962	1.38	184.63	33455.12
21	54.287	Phe	3386288	2.77	190.23	31425.46
25	75.040	Lys	4736875	3.51	220.97	32306.17
27	78.007	His	1347424	0.00	69.21	10741.23
28	87.453	Arg	4878162	7.22	265.57	46261.85
Total			89861952		4895.38	622217.07

VIS 2

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
6	33.287	Pro	895110	9.91	211.39	24331.55
Total			895110		211.39	24331.55

- **Sample 3 (Bubble column r3)**

Hitachi Amino Acid Analyzer Report

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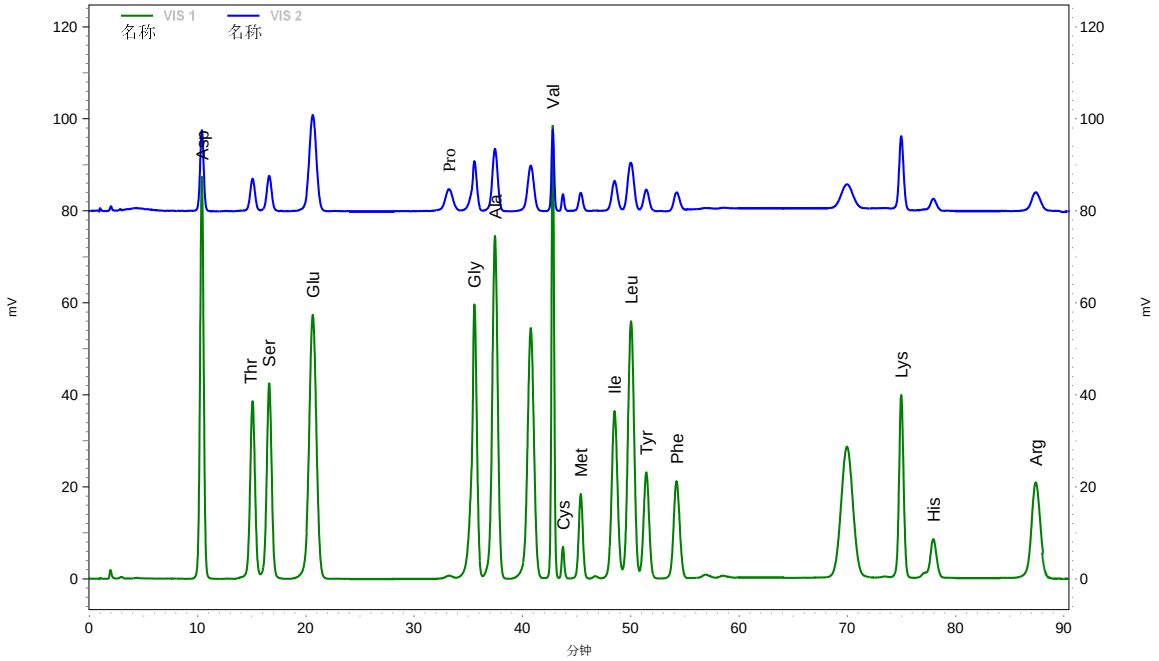

VIS 1 Results

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
5	10.460	Asp	8191537	11.23	472.06	62831.04
6	15.140	Thr	4989120	6.41	272.85	32496.58
7	16.667	Ser	5505460	1.81	306.34	32196.22
8	20.707	Glu	10934893	3.84	637.94	93841.32
10	35.613	Gly	8071786	2.34	444.29	33352.92
11	37.520	Ala	10657080	2.21	564.62	50301.81
13	42.867	Val	6733574	2.62	345.07	40407.58
14	43.807	Cys	464475	2.08	21.42	5146.50
15	45.447	Met	1956488	2.82	99.24	14806.16
17	48.560	Ile	5520570	1.97	279.84	36714.62
18	50.087	Leu	8969788	1.47	462.63	60696.64
19	51.493	Tyr	3298544	1.39	173.42	31423.69
20	54.280	Phe	3330041	2.77	184.20	30429.86
25	75.033	Lys	4638190	1.77	213.05	31148.32
27	78.000	His	1352763	0.00	68.42	10618.52
28	87.447	Arg	4816538	7.22	258.19	44977.41
Total			89430847		4803.57	611389.19

VIS 2

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
6	33.253	Pro	881254	9.93	204.93	23587.78
Total			881254		204.93	23587.78

- **Sample 4 (Tubular Wuhan Friendship New Tech CO. LTD. r1)**

Hitachi Amino Acid Analyzer Report

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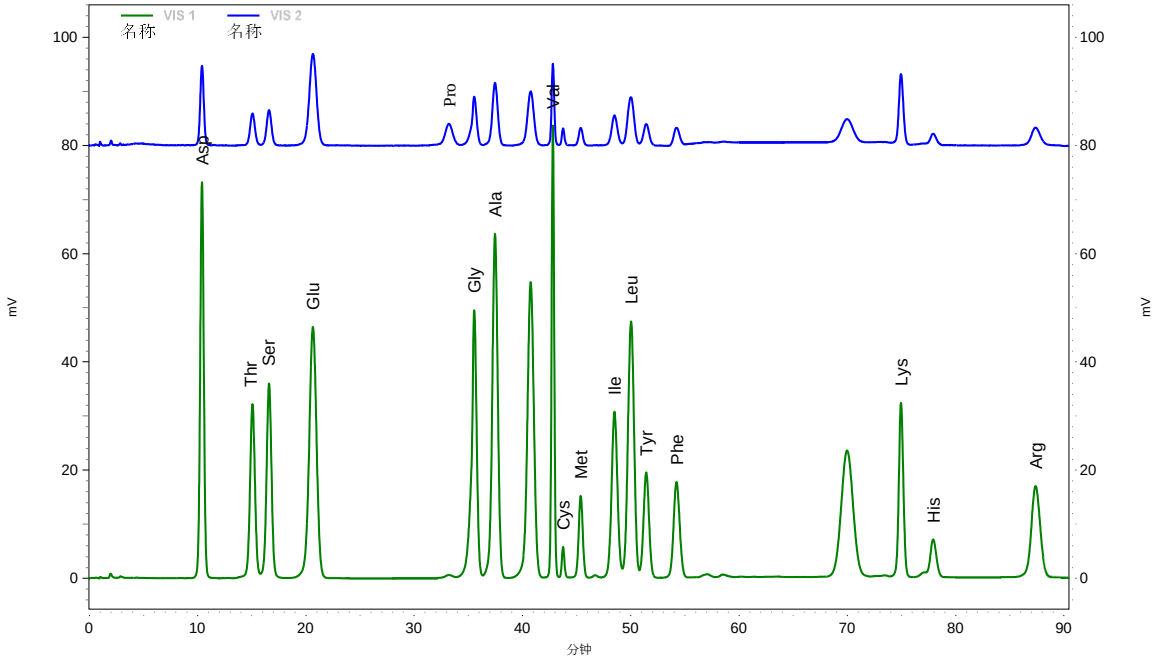
Sample ID: {Sample ID}
Vial Number: 174 Inj. Volume(uL): 10



VIS 1 Results

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
5	10.460	Asp	6844571	11.52	402.14	53524.87
6	15.127	Thr	4143555	6.40	231.03	27516.13
7	16.653	Ser	4657729	1.81	264.23	27770.65
8	20.700	Glu	8833111	3.85	525.39	77284.75
10	35.613	Gly	6695803	2.36	375.75	28207.69
11	37.520	Ala	9088058	2.20	490.89	43733.78
13	42.867	Val	5729393	2.63	299.34	35053.09
14	43.807	Cys	384274	2.08	18.06	4341.01
15	45.440	Met	1607463	2.81	83.13	12402.43
17	48.560	Ile	4640219	2.05	239.81	31462.57
18	50.093	Leu	7579950	1.48	398.58	52293.70
19	51.493	Tyr	2785130	1.38	149.29	27050.85
20	54.280	Phe	2785064	2.77	157.06	25946.94
25	75.020	Lys	3746458	2.30	175.45	25651.19
27	77.993	His	1114218	0.00	57.45	8916.88
28	87.440	Arg	3872323	7.25	211.63	36866.47
Total			74507319		4079.25	518023

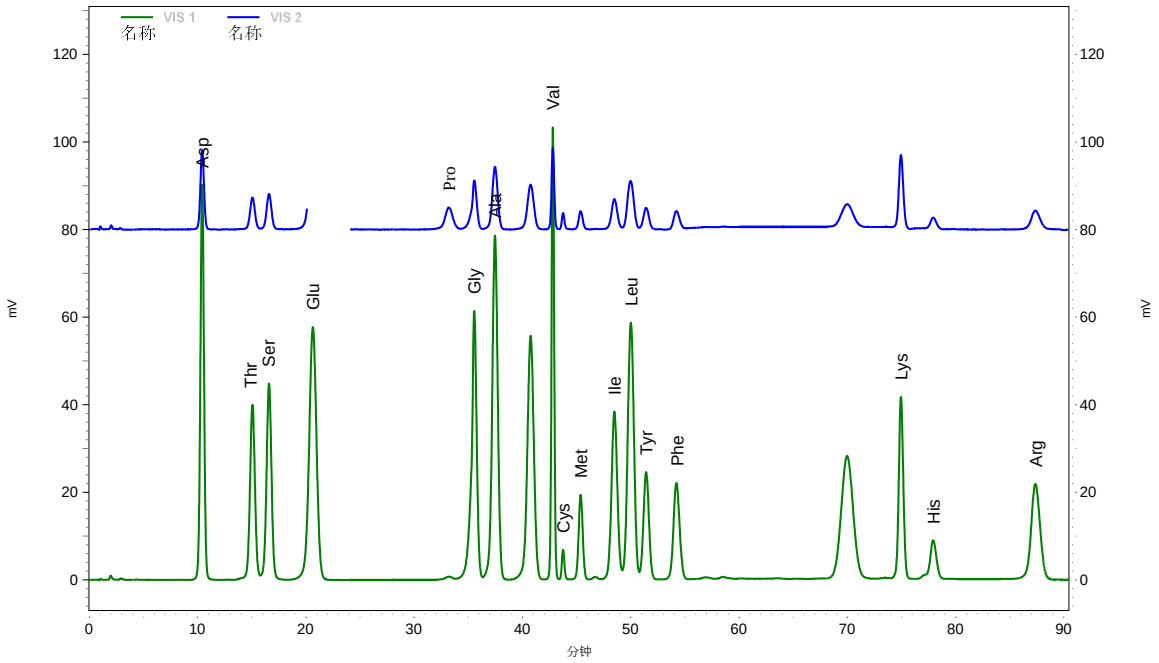
VIS 2

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
5	33.287	Pro	750309	9.95	177.89	20475.13
Total			750309		177.89	20475.13

- **Sample 5 (Tubular Wuhan Friendship New Tech CO. LTD. r2)**

Hitachi Amino Acid Analyzer Report

Analyzed: 2018/8/17 3:44:39 Reported: 2018/8/18 13:48:07
Data File: E:\Data\20180813 PF hjl\PF-luoxuanzao-TAA-B2-10ul-2018-8-17 3-42-53.dat
Method: E:\Method\analysis\PF-20180705-110min-analysis-PH.met
Sample ID: {Sample ID}
Vial Number: 175 Inj. Volume(uL): 10



VIS 1 Results

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
5	10.473	Asp	8399881	11.50	505.36	67263.97
6	15.127	Thr	5149978	6.38	294.04	35020.28
7	16.653	Ser	5812043	1.80	337.63	35484.66
8	20.693	Glu	11003402	3.83	670.18	98584.14
10	35.613	Gly	8289144	2.33	476.33	35758.10
11	37.513	Ala	11211694	2.20	620.14	55248.07
13	42.860	Val	7055274	2.62	377.46	44200.95
14	43.807	Cys	458979	2.10	22.09	5309.36
15	45.433	Met	2064794	2.80	109.34	16313.33
17	48.553	Ile	5802627	1.93	307.08	40288.42
18	50.073	Leu	9424515	1.46	507.47	66579.72
19	51.480	Tyr	3503683	1.38	192.31	34846.59
20	54.267	Phe	3460985	2.77	199.87	33017.99
25	75.020	Lys	4826659	1.78	231.47	33840.23
27	77.987	His	1395734	0.00	73.70	11437.88
28	87.427	Arg	5009877	7.24	280.37	48841.27
Total			92869269		5204.84	662034.96

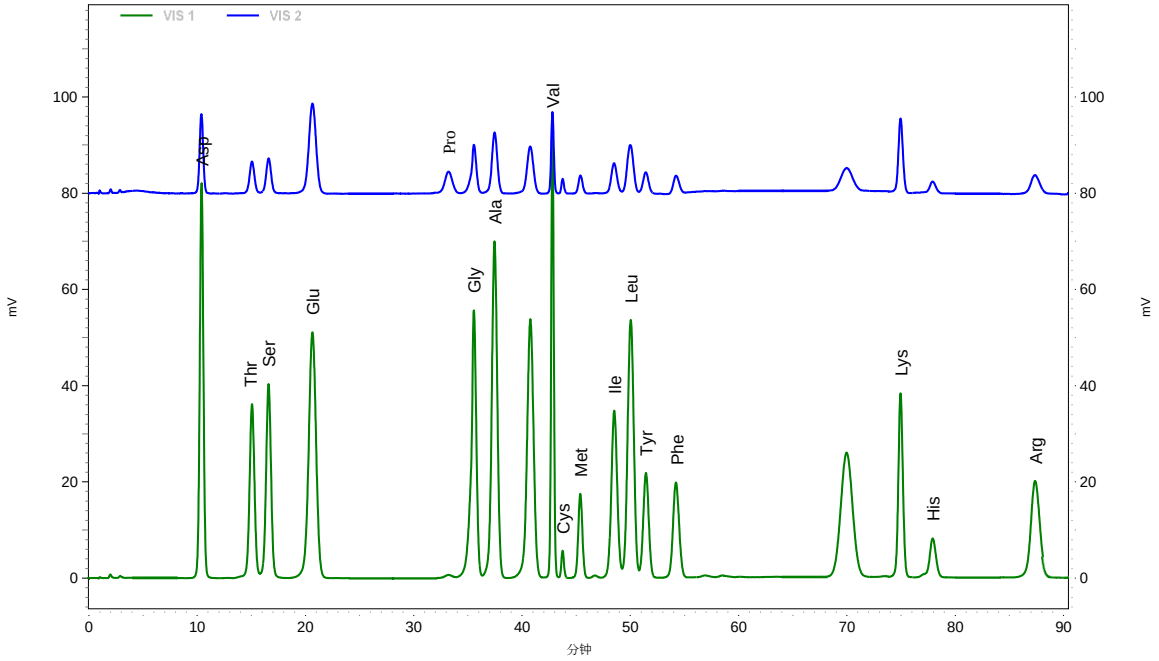
VIS 2

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
5	33.260	Pro	967882	9.83	234.98	27046.36
Total			967882		234.98	27046.36

- **Sample 6 (Tubular Wuhan Friendship New Tech CO. LTD. r3)**

Hitachi Amino Acid Analyzer Report

Analyzed: 2018/8/17 5:37:00 Reported: 2018/8/18 13:50:35
Data File: E:\Data\20180813 PF hjl\PF-luoxuanzao-TAA-B3-10ul-2018-8-17 5-35-08.dat
Method: E:\Method\analysis\PF-20180705-110min-analysis-PH.met
Sample ID: {Sample ID}
Vial Number: 176 Inj. Volume(uL): 10



VIS 1 Results

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
5	10.460	Asp	7693461	13.39	450.26	59929.13
6	15.133	Thr	4685373	6.38	260.23	30993.12
7	16.660	Ser	5242872	1.80	296.27	31137.80
8	20.700	Glu	9746203	3.83	577.44	84941.99
10	35.613	Gly	7518934	2.32	420.30	31552.07
11	37.520	Ala	9991019	2.20	537.57	47891.95
13	42.867	Val	6448488	2.62	335.60	39299.09
14	43.807	Cys	384643	2.07	18.01	4328.27
15	45.440	Met	1867901	2.80	96.22	14355.77
17	48.567	Ile	5264865	1.95	271.03	35559.02
18	50.087	Leu	8570327	1.46	448.90	58896.20
19	51.487	Tyr	3121275	1.38	166.65	30197.74
20	54.273	Phe	3109752	2.76	174.69	28859.15
25	75.020	Lys	4461418	1.89	208.12	30427.51
27	77.980	His	1295008	0.00	66.52	10323.39
28	87.427	Arg	4632112	7.23	252.17	43928.45
Total			84033651		4579.99	582620.65

VIS 2

Peak #	Retention Time	Name	Area	Resolution	Mol concentration (nmol/mg)	CalcMol (ng/mg)
5	33.260	Pro	831227	9.91	196.31	22595.03
Total			831227		196.31	22595.03

5. Independent-Samples T-Test Results

5.1. Specific Growth Rate (SGR)

Group Statistics

	System	N	Mean	Std. Deviation	Std. Error Mean
SGR	Air-lift tank	3	.2533	.02517	.01453
	PBR	3	.1467	.01528	.00882

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SGR	Equal variances assumed	.643	.468	6.276	4	.003	.10667	.01700	.05948	.15386
	Equal variances not assumed			6.276	3.298	.006	.10667	.01700	.05523	.15810

5.2. Productivity (P)

Group Statistics

	System	N	Mean	Std. Deviation	Std. Error Mean
Productivity	Air-lift tank	3	89.5400	14.36897	8.29593
	PBR	3	46.5033	2.02040	1.16648

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Productivity	Equal variances assumed	8.102	.047	5.137	4	.007	43.03667	8.37754	19.77690	66.29644
	Equal variances not assumed			5.137	2.079	.033	43.03667	8.37754	8.27311	77.80022

5.3. Phycocyanin Concentration (PC)

Group Statistics

	System	N	Mean	Std. Deviation	Std. Error Mean
Phycocyanin	Air-lift tank	3	3.8167	.35907	.20731
	PBR	3	.8933	.10214	.05897

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Phycocyanin	Equal variances assumed	5.595	.077	13.563	4	.000	2.92333	.21554	2.32491	3.52176
	Equal variances not assumed			13.563	2.322	.003	2.92333	.21554	2.10881	3.73786

5.4. Lipid Content

Group Statistics

	System	N	Mean	Std. Deviation	Std. Error Mean
Lipid	Air-lift tank	3	17.0000	.50000	.28868
	PBR	3	14.1667	.28868	.16667

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Lipid	Equal variances assumed	.400	.561	8.500	4	.164	2.83333	.33333	1.90785	3.75882
	Equal variances not assumed			8.500	3.200	.227	2.83333	.33333	1.80906	3.85761

5.5. Ash Content

Group Statistics

	System	N	Mean	Std. Deviation	Std. Error Mean
Ash	air-lift tank	3	6.4000	1.51327	.87369
	PBR	3	5.3333	.90738	.52387

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Ash	Equal variances assumed	1.272	.322	1.047	4	.354	1.06667	1.01871	-1.76174	3.89507
	Equal variances not assumed			1.047	3.274	.366	1.06667	1.01871	-2.02735	4.16068

5.6. Protein Content

Group Statistics

	System	N	Mean	Std. Deviation	Std. Error Mean
Protein	Bubble column	3	64.1700	1.28312	.74081
	PBR	3	61.0933	7.54635	4.35689

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Protein	Equal variances assumed	3.264	.145	.696	4	.525	3.07667	4.41942	-9.19361	15.34695
	Equal variances not assumed			.696	2.116	.555	3.07667	4.41942	-14.97718	21.13051

5.7. Carbohydrate Content

Group Statistics

	System	N	Mean	Std. Deviation	Std. Error Mean
Carbohydrate	Bubble column	3	12.4300	.82486	.47624
	PBR	3	19.4067	7.04162	4.06548

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Carbohydrate	Equal variances assumed	4.841	.093	-1.704	4	.164	-6.97667	4.09328	-18.34144	4.38810
	Equal variances not assumed			-1.704	2.055	.227	-6.97667	4.09328	-24.14549	10.19216

5.7. Total Biomass

Group Statistics

	System	N	Mean	Std. Deviation	Std. Error Mean
Biomass	Batch	3	48.5667	14.82644	8.56005
	Semi-continuous	3	58.6333	4.40606	2.54384

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Biomass	Equal variances assumed	3.910	.119	-1.127	4	.323	-10.06667	8.93004	-34.86042	14.72709
	Equal variances not assumed			-1.127	2.351	.361	-10.06667	8.93004	-43.48881	23.35547