OPTIMISATION OF PHYCOCYANIN PRODUCTION FROM MICROALGA *Arthrospira (Spirulina) maxima*

By

MUHAMMAD SYAWALUDDIN HILMI BIN YAHYA

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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Chairman : Mohd Shamzi bin Mohamed, PhD
Faculty : Biotechnology and Biomolecular Sciences

Phycocyanin is one of the valuable pigments produced by *Arthrospira* spp. that possess nutritional and colouring properties. It has been widely used in food, nutraceutical and biotechnology applications. Presently, *Arthrospira platensis* is very much established for producing phycocyanin commercially. Given how abundant and widely understood it is, this leaves room for less utilised yet promising strains like *Arthrospira maxima* to be studied for pigment production. The study aims to optimise the phycocyanin production from the *Arthrospira maxima* strain by first considering which of these media components are vital factors toward phycocyanin secretion by the microalgal cells, i.e., sodium nitrate, sodium bicarbonate, dipotassium phosphate and sodium chloride and precursor. Upon identifying the most significant factors, their composition in the production medium would be manipulated using response surface methodology (RSM). Subsequently, the environmental effect of cultivating *A. maxima* inside the column photobioreactor was investigated. The preliminary result indicates that the Nallayam Research Centre Media (NRCM) was the best option, as it has produced slightly higher phycocyanin production (163.12 mg/L) and dry cell weight (1.25 g/L) than the Zarrouk Media (ZM) and Central Food Technological Research Institute Media (CFTRIM). The preliminary screening using the Plackett-Burman design showed two macronutrients and a precursor significantly affecting the target response ($\rho > 0.05$): sodium nitrate, dipotassium phosphate and glutamic acid. The three factors were further refined using the Box-Behnken design (BBD). BBD's highest phycocyanin concentration was 224.86 mg/L, achieved at a recipe comprising of 0.0125 M sodium nitrate, 0.375 mM dipotassium phosphate and 0.625 mM L-glutamic acid. This resulted in a 37.85% improvement over the unoptimised NRC medium. BBD's validating recipe comprising 0.0125 M sodium nitrate, 0.375 mM dipotassium phosphate and 0.625 mM L-glutamic acid produces 235.98 mg/L of phycocyanin. It has a 44.67% improvement in term phycocyanin concentration compared with an unoptimised NRC medium. In the column photobioreactor setup, an aeration rate of 0.5 L/min yielded the highest phycocyanin concentration (560.30 mg/L) and biomass (4.4 g/L), surpassing rates of 0.25 L/min, 0.75 L/min, and 1.0 L/min, all maintained under a consistent light intensity of 40.5 ± 4.73 µmol/m^2/s. This configuration resulted in a 137.44% increase in phycocyanin concentration and a remarkable 214.29% increase in biomass compared to the validation model. Alternatively, with aeration remained fixed at 0.5 L/min, the same reference light intensity of 40.5 ± 4.73 µmol/m^2/s still registered the highest phycocyanin concentration (560.30 mg/L). In contrast, a higher light intensity of 101.25 ± 6.75 µmol/m^2/s was eventually optimal to produce the highest *A. maxima* biomass density (6.2 g/L). In conclusion, optimizing the composition of medium could enhance phycocyanin production by *Arthrospira maxima*, and culturing *Arthrospira maxima* in a bubble column photobioreactor with optimal light intensity and aeration may increase phycocyanin concentration.

Keywords: *Arthrospira maxima*, Box-Behnken Design, Phycocyanin, Plackett-Burman Design, Medium Formulation

SDG: GOAL 12: Responsible Consumption and Production and GOAL 14: Life under Water

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN PENGELUARAN FIKOSIANIN DARIPADA MICROALGA *Arthrospira (Spirulina) maxima*

Oleh

MUHAMMAD SYAWALUDDIN HILMI BIN YAHYA

APRIL 2024

Pengerusi : Mohd Shamzi bin Mohamed, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Fikosianin merupakan salah satu pigmen yang berharga yang dihasilkan oleh *Arthrospira* spp. yang mempunyai sifat pewarna dan mempunyai pelbagai manfaat kesihatan. Ia telah digunakan secara meluas dalam pelbagai bidang seperti makanan, nutraseutikal dan bioteknologi. Pada masa sekarang, *Arthrospira platensis* merupakan strain yang digunakan secara besar-besaran dalam penghasilan fikosianin secara komersial kerana ianya banyak dan difahami secara terperinci. Oleh sebab ini, terdapat ruang yang boleh digunakan untuk menyelidik potensi strain lain terutamanya *Arthrospira maxima* sebagai pengeluar fikosianin. Di dalam kajian ini, tujuan utama ialah mengoptimumkan pengeluaran fikosianin dari *A. maxima* dengan mempertimbangkan komponen media yang boleh mempengaruhi pengeluaran fikosianin seperti natrium nitrat, natrium bikarbonat, dipotasium fosfat dan natrium klorida serta prekursor. Selepas mengenalpasti faktor penting, komposisi faktor tersebut dalam medium dimanipulasi mengunakan teknik kaedah sambutan permukaan (RSM). Akhir sekali, kesan persekitaran terhadap kultur *A. maxima* dalam fotobioreaktor turut dikaji. Dari kajian awal didapati media Nallayam Research Centre

(NRCM) adalah opsyen terbaik mendorong kepekatan fikosainin (163.12 mg/L) dan biojisim (1.25 g/L) diperolehi sedikit tinggi berbanding dengan media Zarrouk (ZM) dan media Central Food Technological Research Institute (CFTRIM). Penyaringan awal mengunakan kaedah reka bentuk Plackett-Burman menunjukkan dua makronutrien dan satu prekursor yang mempengaruhi pemboleh ubah sasaran (natrium nitrat, dipotassium fosfat dan L-glutamat asid). Tiga faktor tersebut diperhalusi lagi dengan mengunakan reka bentuk Box-Behnken (BBD). Kepekatan fikosainin paling tinggi dalam BBD adalah 224.86 mg/L yang dicapai berdasarkan resepi berikut: 0.0125 M natrium nitrat, 0.375mM dipotassium fosfat dan 0.625mM L-glutamat asid. Ia menunjukan peningkatan 37.85% berbanding dengan medium NRC. Resipi yang dihasilkan oleh BBD secara teori yang kemudiannya disahkan melalui eksperimen mengandungi 0.0125 M natrium nitrat, 0.375 mM dipotassium fosfat dan 0.625 mM L-glutamat asid menghasilkan 235.98 mg/L kepekatan fikosainin. Ia menunjukkan peningkatan sebanyak 44.67% dalam pengumpulan fikosianin berbanding medium NRC yang tidak dioptimumkan. Dalam fotobioreaktor turus, kadar pengudaraan sebanyak 0.5 L/min didapati memberikan kepekatan fikosianin tertinggi (560.30 mg/L) dan biojisim (4.4 g/L), melebihi kadar 0.25 L/min, 0.75 L/min, dan 1.0 L/min, semuanya ini diselaraskan di bawah keamatan cahaya yang konsisten pada 40.5 ± 4.73 µmol/m^2/s. Konfigurasi ini menghasilkan peningkatan sebanyak 137.44% dalam kepekatan fikosianin dan peningkatan luar biasa sebanyak 214.29% dalam biojisim berbanding dengan keputusan model validasi. Sementara itu, pada kadar pengudaraan dikawal 0.5 L/min, keamatan cahaya pada kadar rujukan sebanyak 40.5 ± 4.73 µmol/m^2/s masih memberikan kepekatan fikosianin tertinggi (560.30 mg/L), sementara keamatan cahaya sebanyak 101.25 ± 6.75 µmol/m^2/s adalah dikira optimum untuk penghasilan biojisim tertinggi *A. maxima* (6.2 g/L). Sebagai kesimpulan, mengoptimumkan komposisi media dapat meningkat pengeluaran fikosianin oleh *Arthrospira maxima* dan mengkultur *Arthrospira maxima* dalam fotobioreaktor turus gelembung dengan intensiti cahaya dan pengudaraan optimum meningkatkan kepekatan fikosianin.

Keywords: *Arthrospira maxima*, Kaedah Reka Bentuk Box-Behnken, Fikosianin, Kaedah Reka Bentuk Plackett-Burma, Rumusan Media

SDG: MATLAMAT 12: Penggunaan dan Pengeluaran Bertanggungjawab and MATLAMAT 14: Kehidupan dalam Air

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Mohd Shamzi bin Mohamed, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecule Sciences
Universiti Putra Malaysia
(Chairman)

Murni binti Halim , PhD
Associate Professor
Faculty of Biotechnology and Biomolecule Sciences
Universiti Putra Malaysia
(Member)

Fadzlie wong bin Faizal Wong, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecule Sciences
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia
Date: 7 November 2024

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LIST OF ABBREVIATIONS

%SEP	Standard Error Prediction
µm	Micrometre
A_f	Accuracy Factors
ANOVA	Analysis of Variance
BBD	Box-Behnken Design
B_f	Bias Factor
BRM	Baranyi-Robert Model
CFTRI Media	Central Food Technological Research Institute Media
CFTRIM	CFTRI Medium
K_2HPO_4	dipotassium Phosphate
KNO_3	Potassium Nitrate
L-Glu	L-glutamic acid
LLM	Logistic With Lag Model
meq/L	Miliequvalent per litre
MGM	Modified Gompertz Model
mM	Milimolar
$NaN0_3$	Sodium Nitrate
NH_4Cl	Ammonium Chloride
NRC Media	Nallayam Research Centre Media
Obs	Observed
PBD	Plackett-Burman Design
Pred	Predicted
R^2	R-square
RMSE	Root Mean Squared Error
U	Urea
ZM	Zarrouk's Medium
σ_{est}	Standard Error Estimate

CHAPTER 1

INTRODUCTION

1.1 Background Study

Microalgae have been an integral part of human life since ancient times. Microalgae are often used by humans as a source of food or supplement. Furthermore, microalgae have been studied extensively for their commercial application as a renewable, economical and sustainable source of feed, food for humans, pharmaceuticals and cosmetics (Camacho et al., 2019; Khan et al., 2018; Yarkent et al., 2020). Although numerous microalgal species exist, *Arthrospira* sp. and *Chlorella* sp. are among the most commonly used in various industries. *Arthrospira sp.* is a filamentous, multicellular cyanobacterium known for its extremophilic characteristics, thriving in environments with high alkalinity and pH (Karemore et al., 2020). It particularly flourishes in temperatures around 35ºC. Although the *Arthrospira* genus has 23 species identified and accepted into their genus, *Arthrospira platensis* and *Arthrospira maxima* are the most well known (Nowicka-Krawczyk et al., 2019). *Arthrospira* sp. comtains a unique pigment group called phycobiliprotein.

Phycobiliprotein is a colour protein pigment that captures light energy in phycobilisomes, only found in certain algae. Among the phycobiliproteins derived from microalgae, phycoerythrin (PE) and allophycocyanin (AP) can be found in small amounts, but the most abundant is phycocyanin (PC), a brilliant blue pigment that shows great potential for different important applications (Cuellar-Bermudez et al., 2015; Pagels et al., 2019). Phycocyanin is a blue fluorescent protein pigment with many applications in industry, such as colourant, medicine and fluorescent marker.

The single visible absorption for this strongly fluorescent pigment is between 615 and 620 nm, while the maximum fluorescence emission is around 650 nm. Besides having high absorption and visible wavelengths, phycocyanin has a large Stokes shift and high quantum efficiency. It is well-known as the ingredient used by Dainippon Ink & Chemicals (Sakura, Japan) to develop a product called “Lina blue” (PC extract from *Spirulina platensis*) which is used as a colourant in chewing gum, ice sherbets, popsicles, candies, soft drinks, dairy products and wasabi (Roda-serrat et al., 2018). Phycocyanin had a global market value of almost USD 112.3 million in 2018 and is expected to reach USD 409.8 million in 2030 (Thevarajah et al., 2022). Phycocyanin is often produced by *Arthrospira platensis* culture in open pond or raceway in autotroph conditions (Eriksen, 2008). This gives room to explore using other species under the *Arthrospira* genus, especially *Arthrospira maxima* which can provide alternative and different perspectives regarding phycocyanin production from less known *Arthrospira* species. Additionally, the main setback in commercialising phycocyanin was low biomass and pigment accumulation in cells (Eriksen, 2008; Hsieh-Lo et al., 2019). Thus, the culture condition must be optimised to yield optimum phycocyanin production.

The culturing conditions, especially medium composition, can decisively influence the growth phases of microalgae, causing changes in its composition and increasing or decreasing the proportion of phycobiliproteins, including phycocyanin. For example, using different nitrogen sources and concentrations experimenters may affects phycocyanin accumulation differently (Fagiri et al., 2013; Mirhosseini et al., 2021; Shanthi et al., 2018). Besides that, other macronutrients (carbon and phosphorous) usually affect growth and cell accumulation, influencing phycocyanin production (Hao et al., 2019; Magwell et al., 2023). Metabolic stressors as part of the medium components have shown promise in optimising phycocyanin production (Manirafasha et al., 2018; Musifa et al., 2023). Therefore, the focus here is on determining the ideal medium composition to maximise phycocyanin production without hampering growth and biomass accumulation. This could reduce costs and increase the commercial value of phycocyanin in various industries.

Besides that, environmental factors such as light intensity and aeration play important in cultivating microalgae. Light intensity is another important parameter whereby it is suggested that having the right amount of light will boost production. At the same time, too much or too little will have the opposite effect (Chaiklahan et al., 2007; Khatoon et al., 2018). Besides that, aeration and mixing are also integral to the optimum production of biomass and proteins. Studies show that *Arthrospira* aerated with air or agitated tend to have better growth performance (Pandey & Tiwari, 2010; Ronda et al., 2012; Soni et al., 2019). Therefore, empirical investigations into how environmental factors, including light intensity and aeration rates, influence phycocyanin production are essential to harness the full potential of these microalgae.

1.2 Problem statement

Phycocyanin is a valuable pigment primarily sourced from *Arthrospira* sp. It has a wide range of potential uses, including serving as a colouring agent and providing health benefits such as antioxidant, anti-inflammatory, and immune-boosting properties. Despite its potential, phycocyanin production faces several challenges, particularly in optimizing media formulation and environmental conditions for maximizing yield using *Arthrospira* sp. Although *Arthrospira platensis* is well-established for phycocyanin production, exploring other species within the Arthrospira genus remains limited. Variations in phycocyanin yield and quality among different species could present new opportunities for enhancing production efficiency. This study focuses on *Arthrospira maxima* to enhance its phycocyanin production.

Current research on phycocyanin production from *Arthrospira* sp. mainly emphasizes extraction methods rather than optimizing media formulation. Studies on media formulation for Arthrospira sp. often focus on growth and other bioproducts, such as lipids, chlorophyll α, and polyhydroxybutyrate, rather than on phycocyanin specifically. Additionally, there is a lack of comprehensive studies on how environmental factors such as light intensity and aeration affect phycocyanin production in *Arthrospira* sp. Therefore, this study aims to address these gaps by investigating the optimal media formulation and environmental conditions for maximizing phycocyanin production in *Arthrospira maxima*.

1.3 Scope of the Study

This study determined the best production media for phycocyanin concentration and growth for *Arthrospira maxima*. The assessment of media production is based on phycocyanin concentration, carbon consumption, and a growth model. The most promising medium from this assessment would serve as the basal medium for investigating the effects of various nitrogen sources and concentrations. Subsequently, statistical methods were employed to fine-tune the medium composition and precursors. Nine factors were screened for significance, guided by insights from a comprehensive literature review using Plackett-Burman Design. The significant factors were optimised using the steepest ascent and then further optimised in Box-Behnken Design (BBD). Lastly, two operating parameters of the column photobioreactor, namely, aeration rate and light intensity, were studied on a 500 mL bubble column photobioreactor using the BBD optimised media.

1.4 Objectives of the Stud

1. To investigate suitable medium constituents affecting the production of phycocyanin pigment from *Arthrospira maxima*.

2. To optimise medium component and environmental factors affecting phycocyanin formation through statistical technique of Response Surface Methodology.

3. To assess the impact of operating parameters such as aeration and light intensity in a photobioreactor affecting phycocyanin pigment production from *Arthrospira maxima*

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