OPTIMISATION OF *Arthrospira platensis* HARVESTING USING EDIBLE FUNGAL BIOFLOCCULANT

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ABSTRACT

Arthrospira sp. is considered a sustainable and completely natural microalgae-based food supplement to solve nutritional diseases, specifically malnutrition. However, both cultivation and harvesting methods for this microalgae takes up to 40% of energy consumption. Therefore, this study is aimed at maximizing *Arthospira platensis* biomass productivity under outdoor cultivation as well as propose safe and efficient harvesting method using edible fungi. Results from data obtained by comparing three types of photobioreactor (PBR) configurations conducted under indoor conditions demonstrated that macrobubble column (MA-CP) showed the highest dry cell weight yield compared to microbubble column (MI-CP) and airlift loop column (ALCP), with 0.536 ± 0.044 g/L, 0.477 ± 0.034 g/L and 0.274 ± 0.014 g/L, respectively. Thus, based on this result, MA-CP was carried out during the outdoor cultivation studies. Covered MA-CP showed a comparable but steady growth compared to non-covered MA-CP due to limited exposure of the microalgae to solar radiation. Whereas outdoor MA-CP PBRs resulted in significantly higher growth compared to indoor MA-CP due to the influence of temperature and light intensity. The result suggested that by taking advantage of Malaysia's weather conditions, integration of solar panel systems for outdoor cultivation of *Arthrospira* sp. in covered MA-CP is a viable and sustainable option. Meanwhile, a promising harvesting technique via bioflocculation is recommended as an alternative to conventional flocculation because of its simplicity and efficiency. In this study, *Rhizopus microsporus* was locally isolated and demonstrated the highest harvesting efficiency compared to other fungi. One-factor-at-time (OFAT) technique was used for the preliminary screening of different factors including mycelia concentration, pH of mycelia and temperature. The results were then applied in response surface methodology (RSM) modelling for optimization through central composite design (CCD). The harvesting efficiency (HE, %) for bioflocculation of *A. platensis* using *R. microsporus* was maximized (65.89 ± 2.795%) when 3.85% mycelial concentration (w/v) with initial pH of 2.5 at 38.8°C were used as the harvesting parameter conditions. Overall, the results showed that the overall process is viable and economical when the outdoor cultivation setup was integrated with solar panels as the system produced 2-fold biomass compared to the indoor cultivation coupled with harvesting microalgae step via locally isolated fungi as bioflocculant.

ABSTRAK

Arthrospira sp. dianggap sebagai makanan tambahan berasaskan mikroalga yang lestari untuk menyelesaikan penyakit pemakanan, khususnya malnutrisi. Namun begitu, kaedah penumbuhan dan penuaian mikroalga ini memerlukan sehingga 40% penggunaan tenaga. Oleh itu, tujuan kajian adalah untuk memaksimumkan produktiviti biojisim *Arthrospira* sp. di bawah penumbuhan luar serta mencadangkan kaedah penuaian yang selamat dan cekap dengan menggunakan kulat yang boleh dimakan. Hasil kajian dari data yang diperolehi dengan berbandingkan tiga jenis konfigurasi fotobioreaktor (PBR) di bawah keadaan tertutup telah menunjukkan bahawa reaktor buih-makro (MA-CP) menghasilkan berat sel kering yang tertinggi berbanding dengan reaktor buih-mikro (MI-CP) dan reaktor gelung pengangkutan udara (ALCP), dengan 0.536 ± 0.044 g/L, 0.477 ± 0.034 g/L dan 0.274 ± 0.014 g/L, masing-masing. Oleh itu, reaktor MA-CP telah dijalankan semasa kajian penumbuhan luar berdasarkan keputusan ini. MA-CP yang dilindungi menunjukkan pertumbuhan yang setanding tetapi stabil, berbanding dengan MA-CP yang tidak dilindungi kerana pendedahan terhad mikroalga kepada penyinaran suria. Manakala PBR MA-CP luar menghasilkan pertumbuhan yang lebih tinggi berbanding dengan MA-CP tertutup kerana pengaruh suhu dan kekuatan cahaya. Hasil kajian telah mencadangkan bahawa integrasi sistem panel suria dalam MA-CP tertutup untuk penanaman luar *Arthrospira* sp. adalah sebuah pilihan yang berdaya maju dan mampan dengan mengambil kesempatan keadaan cuaca Malaysia. Sementara itu, teknik penuaian melalui bioflokulasi disarankan sebagai alternatif kepada penggumpalan konvensional kerana kemudahan dan kecekapannya. Dalam kajian ini, *Rhizopus microsporus* telah diasingkan daripada tempeh dan menunjukkan kecekapan penuaian tertinggi semasa saringan. Teknik satu faktor pada suatu masa (OFAT) digunakan untuk pemeriksaan awal faktor-faktor yang berbeza termasuk kepekatan miselium, pH miselium dan suhu. Hasilnya kemudian digunakan dalam pemodelan kaedah rangsangan permukaan (RSM) untuk pengoptimuman melalui reka bentuk komposit tengah (CCD). Kecekapan penuaian (HE, %) untuk bioflokulasi *A. platensis* menggunakan *R. microsporus* telah dimaksimumkan (65.89 ± 2.795%) apabila kepekatan miselium (b/i) 3.85% dengan pH awal 2.5 awal pada suhu 38.8°C digunakan sebagai syarat faktor penuaian. Secara keseluruhan, hasil kajian menunjukkan bahwa kesuluruhan proses adalah berdaya maju dan menjimatkan apabila penanaman luar disepadukan dengan panel suria kerana system ini menghasilkan biojisim 2 kali ganda berbanding dengan penumbuhan tertutup, dan digandingkan dengan langkah penuaian mikroalga melalui kulat tempatan sebagai bioflokulan.

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LIST OF ABBREVIATIONS

CCD	-	Composite Central Design
pH	-	pH value of liquid
rpm	-	Rotation per minute
UTM	-	Universiti Teknologi Malaysia
HE	-	Harvesting efficiency
ANOVA	-	Analysis of Variance
OFAT	-	One factor at A Time
PDA	-	Potato Dextrose Agar
PDB	-	Potato Dextrose Broth
DCW	-	Dry cell weight
ITS	-	Internal Transcribed Spacer
OD	-	Optical density
EPS	-	Exopolysaccharide
RSM	-	Response Surface Methodology
ZM	-	Zarrouk's Media
PBR	-	Photobioreactor
MA-CP	-	Macrobubble column photobioreactor
MI-CP	-	Microbubble column photobioreactor
ALCP	-	Airlift column photobioreactor
PPFD	-	Photosynthetic Photon Flux Density
UV-Vis	-	Ultraviolet-visible
SEM	-	Scanning electron microscopy
CV	-	Coefficient of variation
AP	-	Adequate precision
R^2	-	Regression coefficient
PI	-	Prediction interval

LIST OF SYMBOLS

°C	-	Degree celsius
mL	-	Millilitre
μL	-	Microlitre
v/v	-	Volume per volume
w/v	-	Weight per volume
Lux	-	Luminous flux per unit area
g/L	-	Gram/litre
t	-	Amount of time that has passed
T_c	-	Cultivation time
$x_m - x_i$	-	Cell concentration variation
P_x	-	Cell productivity
X_m	-	Maximum biomass
vvm	-	Volume of air per unit of medium per unit of time
μmol/m^2/s	-	Micromole per second and square meter
mV	-	millivolts

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CHAPTER 1

INTRODUCTION

1.1 Background of study

In recent years, photoautotrophic microalgae are widely recognised due to their diverse yet significant natural values, particularly in healthcare, including pharmaceuticals, cosmetics, and feedstock industries (Almomani, 2020; Kanchanatip et al., 2016; Kumar et al., 2020). In this respect, the filamentous blue-green microalgae called *Arthrospira* sp. has been addressed as a potent superfood and become one of the most exploited microalgal species for nutraceutical purposes (Liestianty et al., 2019; Saha & Murray, 2018; Soni et al., 2017). Since *Arthrospira* sp. or commonly known as *Spirulina* contains an extraordinary proportion of protein components, this microalgae has been proposed to alleviate malnutrition globally (Abed et al., 2016; Matondo et al., 2016). In 2019, about 203 million children below five years old were affected by undernutrition. In extreme cases, the deaths of 45% of children under 5 years old were associated with undernutrition (FAO et al., 2020; WHO, 2020). In Malaysia, the indigenous community are the most affected ethnic group with severe energy and protein undernutrition due to inaccessibility to expensive nutritious food (Kamaruzaman et al., 2020; Khor & Shariff, 2019; Murtaza et al., 2019). *Arthrospira* sp. is rich in nutrients, high digestibility, and presenting lesser difficulties in the production compared to other food sources such as soy bean and beef (Masuda & Chitundu, 2019; Mathur, 2018; Soni et al., 2017). Therefore, *Arthrospira* sp. is one of the best food candidates in combatting against nutritional deficiencies which lead to health problems including marasmus and marasmus-kwashiorkor (Kamaruzaman et al., 2020; Khor & Shariff, 2019). Hence, from a research perspective, the cultivation process should be optimised to produce low-cost *Arthrospira* sp. as a complete supplement to meet the global market of malnourished people.

Nevertheless, a low cost yet high productivity of microalgal biomass rarely can be achieved, mainly due to the cultivating and harvesting system (Okoro et al., 2019). Under outdoor cultivation, the growth and biomass productivity of *Arthrospira* sp. can be quite concerning as the microalgal growth rate depends on the geographical locations (de Jesus et al., 2018). On the other hand, the microalgal harvesting process could incur 3-15% of the total production cost and may achieve 40% of the total energy consumption for the entire process (Kanchanatip et al., 2016; Fasaei et al., 2018; Liber et al., 2020). To solve this problem, bioflocculation as the harvesting technique has been proposed as a more efficient (>90% efficiency) and feasible method due to cost-effectiveness and sustainability compared to other flocculant and harvesting methods (Matter et al., 2019). However, fungi as flocculant in large-scale flocculation is not fully elucidated yet and may cause contamination to the harvested biomass, making it unsuitable for consumption as it poses detrimental health risks (Nazari et al., 2020; Yin et al., 2020). Economical cultivation and safe harvesting of *Arthrospira* sp. should be studied in the local environment to ensure it could help local producers maximise production and help the indigenous people process the supplement with a feasible system to prevent malnutrition.

1.2 Problem statement

Since the last decade, *Arthrospira* sp. has been considered a sustainable and completely natural supplement to nutritional diseases (Abed et al., 2016; Sinha et al., 2018). However, the mass production of the nutritious compounds are highly depends on the cultivation conditions, harvesting and drying methods (Aouir et al., 2017). Although few studies demonstrate the cultivation under outdoor Malaysia tropical climate, none of the articles has yet to economically cultivate and utilize the local weather by integrating into a complete and cheap solar system. Consequently, the *Arthrospira* sp. growth performances using this system to the surrounding local physical environments have not been well understood.

Apart from the cultivation process, the efficiency of a harvesting method is also affected by several factors, including the microalgal morphology (Cuellar-Bermudez et al., 2020). Among the methods established, a cost-effective and energy-efficient method is flocculation. Nevertheless, the literature reports on using efficient yet biodegradable and environmentally safe flocculant are limited (Luo et al., 2019). The common flocculant is chemical as high efficiency can be achieved quickly despite the toxicity and harmful effects on the culture (Singh & Patidar, 2018). As an alternative, edible fungi is a safer flocculant for harvesting high-value microalgae without affecting the end product (Kinyoki et al., 2020). However, there has yet enough literature that report the optimized harvesting conditions to the local edible fungi. Thus, the relation of the local cultivation of fungi based on the variable conditions to the effectiveness of harvesting efficiency remains unclear due to the activity of harvesting using mycelium fungi is highly dependable and correlated to their cultivation process (Bhattacharya et al., 2017)

Besides, the optimization flocculation method assisted by fungi using response surface methodology (RSM) is still lacking, thus hindering the evaluation of the significance of interaction between a series of controllable experimental variables and the experimental findings (Bai et al., 2015; Jaafari & Yaghmaeian, 2019). In this regard, there is a need to investigate several parameters influencing the fungal bioflocculation efficiency, simultaneously through RSM. By doing so, the highly reliable data generated from this method would allow the interactions between the variables and the response to be comprehended more precisely. In short, this study aims to develop a cheap mass-cultivation system and harvesting via safe, efficient edible fungi bioflocculation.

1.3 Objectives

The objectives of the research are :

(a) To investigate the growth performances under indoor and outdoor conditions of *Arthrospira* sp. cultivated in several 2 L photobioreactor including the integration of solar panel system with different configurations and bubble sizes

(b) To isolate, screen and identify potential local edible fungi for microalgal harvesting

(c) To examine the optimized bioflocculation harvesting parameters of the identified edible fungi on *Arthrospira platensis* using response surface methodology (RSM)

1.4 Scope of study

This study covers the study of optimization in microalgal cultivation step to study the growth performances of *Arthrospira* sp. using different 2 L photobioreactor (PBR) systems including indoor and outdoor condition for biomass production. Particularly, there were three different PBRs configurations and bubble sizes studied indoor including macrobubble column (MA-CP), microbubble column (MI-CP) and airlift loop column (ALCP). The best PBR contributing to the highest biomass was then further carried out to be conducted under outdoor condition. There were two different PBRs have been integrated with solar panel systems which were covered MA-CP and non-covered MA-CP. Soon after, local edible fungi were isolated, screened and identified with the best fungal strain as bioflocculant was selected for further investigation. Lastly, the efficiency of the parameters studied for bioflocculation harvesting process were screened using one-factor-at-time (OFAT) prior to the optimization using a statistical method, response surface methodology (RSM). In this study, fungal mycelium concentration (%, w/v), initial pH of fungal mycelium and temperature were the parameters that have been subjected for both OFAT and RSM flocculation optimization.

1.5 Significant of study

The aim of this study was to discover the optimized conditions for biomass cultivation of *Arthrospira* sp. integrated with the solar system under outdoor conditions while considering the environmental factors that would positively impact the production of a high-quality microalgae culture. The potential of using this solar system is an alternative in sustainability than the lab-scaled systems as it may economically lower the cost and more feasible to be applied. Apart from that, by identifying edible fungi in Malaysia that can harvest *Arthrospira* sp., it would certainly reduce the cost, time and conserve the byproduct of *Arthrospira* sp. during its manufacturing stage. Edible fungi as a flocculant would be safe for environment and human consumption which is the opposite effect of using chemical, toxic and harmful flocculant. Furthermore, as the fungi itself may provide additional added value to the final product hence, the consumer would acquire extra nutrients.

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