A NEW APPROACH OF APPLICATION OF SUPERHYDROPHOBIC COATING ON CULTIVATION OF GIANT DUCKWEED (*SPIRODELLA POLYRHIZA*)

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by

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LIST OF SYMBOLS

Greek letter

θ	Contact angle of water droplet	-

LIST OF ABBREVIATION

Symbol	Description
A_{649}	Absorbance value at wavelengths 649 nm
A_{664}	Absorbance value at wavelengths 664 nm
ANOVA	Analysis of Variance
BCA	Bicinchoninic Acid
BSA	Bovine Serum Albumin
CA	Contact angle
Chl a	Chlorophyll a
Chl b	Chlorophyll b
DCA	Dynamic contact angle
M_1	Initial weight of the plant (g)
M_2	Final weight of the plant (g)
RGR	Relative Growth Rate
SDG	Sustainable Development Goals
SHP	Superhydrophobic coating
SP	Superhydrophobic coated pool without metal mesh
SPM	Superhydrophobic coated pool with metal mesh
t_1	Time where M_1 measured (days)
t_2	Time where M_2 measured (days)
V	95% ethanol volume (ml)
W	Dried weight of the sample (g)
W_f	Final weight of the vial (g)
W_i	Initial weight of the empty vial (g)
W_s	Dried weight of sample (g)

PENDEKATAN BARU APLIKASI LAPISAN SUPERHYDROPHOBIC PADA PENANAMAN GIANT DUCKWEED (*SPIRODELLA POLYRHIZA*)

ABSTRAK

Dalam kajian ini, telah dikaji mengenai pendekatan baru menerapkan lapisan superhidrofobik untuk penanaman *Spirodella polyrhiza* (*S. polyrhiza*). Pendekatan baru ini dilakukan untuk mengkaji peningkatan pertumbuhan spesies ini dengan meningkatkan luas permukaan penanaman dan kecekapan penggunaan air melalui penggunaan lapisan superhidrofobik. Hal ini demikian kerana terdapat suatu kelemahan tidak dapat dielak dalam cara penanaman konvensional iaitu keluasan permukaan untuk penanaman spesies. Kandungan biokimia spesies juga dianalisa untuk membandingkan kecekapan pendekatan baru ini berbanding kaedah penanaman konvensional. Lapisan superhidrofobik disediakan menggunakan lilin lebah kerana ianya mesra alam dan boleh diperbaharui. Lapisan yang disediakan dianalisa melalui kaedah Sessile drop di mana sudut hubungan lebih daripada 150^{o} menunjukkan bahawa suspensi lilin yang disediakan sangat superhidrofobik seperti mana yang diperlukan. Suspensi beeswax yang disediakan (lapisan superhidrofobik) kemudian disalut melalui semburan di sisi perspex bersaiz A4 untuk digunakan sebagai kultur kolam. *Spirodella polyrhiza* (*S. polyrhiza*) ditanam di kolam yang dilapisi dan pada masa yang sama sebagai kultur kawalan, *S. polyrhiza* juga ditanam di dalam balang kaca yang dipanggil sebagai cara kultur yang konvensional. Kedua-dua kultur tersebut dilaksanakan dalam keadaan pertumbuhan yang serupa dan dituai selepas 7 hari. Daripada keputusan yang diperolehi telah diperhatikan bahawa kadar pertumbuhan kultur kolam (0.1002 ± 0.012 day^{-1} and 0.0912 ± 0.011 day^{-1}) 2 kali lebih besar daripada kultur kawalan (0.0467 ± 0.009 hari^{-1}). Hasil yang didapati ini kompromasi dengan objektif yang dicadangkan dari penyelidikan ini iaitu untuk

meningkatkan luas permukaan dan kecekapan penggunaan air untuk penanaman *S. polyrhiza* serta memaksimumkan kadar pertumbuhan melalui lapisan superhidrofobik daripada cara penanaman konvensional. Sampel yang dituai kemudian terlibat dengan analisis kandungan biokimia di mana jumlah klorofil, lipid dan protein dianalisa. Jumlah kandungan klorofil spesies yang dituai dari kultur kolam bersalut superhidrofobik (1.02 ± 0.08 mg/g and 1.01 ± 0.02 mg/g) lebih tinggi daripada kultur kawalan (0.85 ± 0.07 mg/g). Semasa membandingkan antara kolam 2, kandungan klorofil sampel dari kolam dengan mesh logam (SPM) lebih tinggi daripada kolam tanpa mesh logam (SP). Begitu juga untuk lipid, kandungan lipid spesies kultur kolam (5.50 ± 0.49 % dan 6.21 ± 0.75 %) lebih tinggi daripada spesies kultur kawalan (4.79 ± 0.49 %). Semasa membandingkan antara kolam, SPM memberikan kandungan lipid yang lebih tinggi daripada sampel dari SP. Bagi protein juga nilai untuk spesies kultur kolam 11.75 ± 0.77 % dan 14.59 ± 0.58 % sementara untuk spesies kultur kawalan kandungan protein 10.83 ± 0.80 %. Keputusan serupa diperhatikan dalam membandingkan 2 kumpulan di mana sampel dari SPM menunjukkan kandungan protein yang lebih tinggi daripada yang lain. Hasil dari penyelidikan ini menunjukkan bahawa penggunaan lapisan superhidrofobik untuk penanaman *S. polyrhiza* bukan sahaja memaksimumkan kadar pertumbuhan tetapi juga meningkatkan kandungan biokimia spesies berbanding kaedah konvensional penanaman.

A NEW APPROACH OF APPLICATION OF SUPERHYDROPHOBIC COATING ON CULTIVATION OF GIANT DUCKWEED (SPIRODELLA POLYRHIZA)

ABSTRACT

In this thesis, it has been studied on new approach of applying superhydrophobic coating for the cultivation of giant duckweed (*Spirodella polyrhiza*). This new approach is postulated to be able to maximize the growth rate of this plant by increasing the surface area of cultivation and increased water usage efficiency through application of superhydrophobic coating. This is because there is a major drawback with the conventional cultivation method which is the surface area of the growing environment. Biochemical content of the species was also analyzed to compare the efficiency of this new approach over the conventional cultivation method. Superhydrophobic coating was prepared using beeswax since it is environmentally friendly and renewable. The coating was analyzed through sessile drop method where the contact angle obtained was more than 150^{o} showing that the prepared wax suspension was superhydrophobic. The prepared wax suspension (superhydrophobic coating) spray coated on the sides of an A4 sized Perspex to be used as a pool culture. *Spirodella polyrhiza* (*S. polyrhiza*) cultivated in the pools which were coated while as a control, the *S. polyrhiza* also cultivated in glass jars as a conventional way. Both the cultures were carried out in similar growing conditions and harvested after 7 days. It is observed that the growth rate of the pool culture (0.1002 ± 0.012 day^{-1} and 0.0912 ± 0.011 day^{-1}) is 2 times greater than the control culture (0.0467 ± 0.009 day^{-1}). This results compromises with the proposed objectives of this research which is to increase the surface area and water usage efficiency for *S. polyrhiza* cultivation as well as maximizing the growth rate through

superhydrophobic coating over the conventional way of cultivation. Comparing the different pools where one with metal mesh while the other without metal mesh, pool with metal mesh (SPM) (0.1002 ± 0.012 day^{-1}) provided a higher growth rate than the one without metal mesh (SP) (0.0912 ± 0.011 day^{-1}). Harvested samples were then subjected to biochemical content analysis where total chlorophyll, crude lipid and crude protein were analyzed. Total chlorophyll content of the species harvested from superhydrophobic coated pools culture (1.02 ± 0.08 mg/g and 1.01 ± 0.02 mg/g) much greater than the control culture (0.85 ± 0.07 mg/g). While comparing between the 2 pools, chlorophyll content of the samples from SPM were quite higher than the SP. Similarly, for crude lipid, the lipid content of pool cultured species (5.50 ± 0.49 % and 6.21 ± 0.75 %) was higher than control cultured species (4.79 ± 0.49 %). While comparing the pools, SPM gives higher lipid content than the samples from SP. As for the protein, the values for pool cultured species were also higher (11.75 ± 0.77 % and 14.59 ± 0.58 %) than control cultured species (10.83 ± 0.80 %). Similar trends were observed in comparing the 2 pools where the sample from SPM exhibits higher protein content than the other. These outcomes from this research show that the application of superhydrophobic coating for *S. polyrhiza* cultivation not only maximizes the growth rate but also enhances the biochemical content of the species over the conventional method of cultivation.

CHAPTER 1

INTRODUCTION

Chapter 1 introduces the overview of this research and significance of new approach of applying superhydrophobic coating for *S. polyrhiza* cultivation for maximisation of the productivity of the species. In general, this chapter summarizes the research background of *Spirodella polyrhiza (S. polyrhiza)*, the problem statement and the objectives of this final year project.

1.1 *Spirodella polyrhiza (S. polyrhiza)* Research Background

Duckweed species are the smallest rapidly growing free-floating aquatic plant with a fast growth rate which are highly productive under favorable conditions. Due to its floating capabilities, the harvesting process is relatively simple. Duckweeds are widely applied in wastewater treatment and environmental water purification due to their high nutrient removal efficiency (Toyama et al., 2022). Duckweed becomes a better feedstock for industrial protein, animal feed and biofuels due to its high protein and starch content (Toyama et al., 2022). There are five main genera of duckweed such as *Lemna, , Wolffia, Wolffiella, and Landoltia* (Mun et al., 2020; Ali et al., 2016).

Spirodella polyrhiza (S. polyrhiza) also commonly known as Giant Duckweed falls under the duckweeds (Lemnaceae) family. As a duckweed *species, S. polyrhiza* also exhibits higher growth rates, higher removal of nutrients with a nutrient removal percentage of 60% NH_3 –N, 30% NO_3 –N and 72% $PO_4{}^{3-}$ from the synthetic wastewater (Mun et al., 2020) which allows this species to be promising choice for phytoremediation. This species lacks true leaves and stems which consists of an oval leaf-like body called a thallus. The underside of the thallus is typically reddish-purple with a cluster of 4-16 roots hanging in the water. Due to its colonial habit, it usually looks like a mat of green pumpkin seeds floating on the surface of still or slow-moving waters which grows extremely rapid and can form extensive floating mats, especially in nutrient enriched waters. *S. polyrhiza* reproduces by asexual budding, seeds, and overwinters as dark-green or brown buds on the sediments (Washington State Department of Ecology, 2022). Figure 1.1 shows the lifecycle of the *S. polyrhiza* on how this species grows vegetatively.

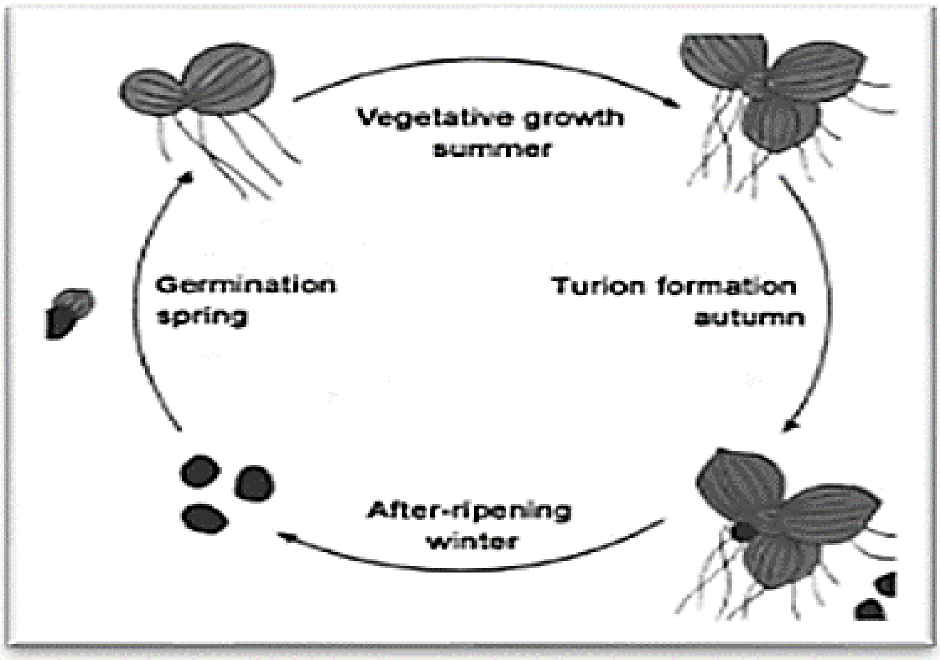

Figure 1.1 : Life cycle of S. polyrhiza on how the species grows vegetatively (Washington State Department of Ecology, 2022)

S. polyrhiza was found to be a hyperaccumulator of cadmium and chromium in research investigating phytoremediation capability of *S. polyrhiza* (Chauduri *et al*., 2014; Singh *et al*., 2019) which makes this species to be a prospective contender for wastewater treatment. In recent days, *S. polyrhiza* not only has gained interest for its phytoremediation capability but also for biohydrogen production where it contains promising substrate for this application. Moreover, *S. polyrhiza* also as a feed material for fish, poultry and pigs due to its less fibre content which makes it to be an easily digestible compound (Sharma et al., 2019). Due to increasing demand and application of *S. polyrhiza*, maximizing the growth of this species becomes a crucial challenge for current research.

1.2 Problem Statement

The major drawback with the conventional cultivation method is the surface area of the growing environment. It is reported that surface area is one of the major factors that influences the growth rate of the giant duckweeds (Sharma et al., 2019). In conventional method, the flasks and the bottles were used for the cultivation where this type of cultivation method provides smaller surface area for the growth. This will cause an occurrence of layering of plants which eventually reduces the light penetration to the bottom layer of plants. Besides surface area, light intensity is also an important criterion for cultivation. Thus, a bigger surface area is essential to ensure the plant gets optimum light to enhance the plant's growth rate. Besides that, current research involves high water usage for cultivation when considering increasing the surface area of the cultivating environment especially for pond culture (Sharma et al., 2019; Guo et al., 2020). This huge amount of water consumption for the cultivation reduces the water usage efficiency in previous research.

In this paper, a new approach of applying superhydrophobic coating for cultivation of *S. polyrhiza* is being proposed to increase the growth rate of this plant. This proposed approach shall increase the surface area utilized for the cultivation and eventually increase water usage efficiency by minimizing the excessive amount of water/growth medium requirement for the cultivation. This is because when the superhydrophobic coating maximizes the surface area for the cultivation, the growth medium would spread wide all over the area of the cultivation which allows the species to obtain maximum accessibility to the growth medium. This eventually will prevent higher depth of medium/water required for the cultivation to maintain the growth rate which will reduces excessive water wastage. Moreover, application of superhydrophobic coating for the cultivation would allow the growth medium/water being retained well in the pool. Through this approach, the productivity of the *S. polyrhiza* would also increase. Characterization of superhydrophobic coating that is being prepared and biomass relative growth rate and biochemical content of shall also be investigated in this work.

1.3 Research objectives

The objectives of this research:

i. To characterize the superhydrophobic properties of wax coating

ii. To increase the surface area of the cultivation, water usage efficiency and productivity of *S. polyrhiza* by applying superhydrophobic coating

iii. To analyze the effect of different cultivation methods on biomass relative growth rate and biochemical content

CHAPTER 2

LITERATURE REVIEW

Chapter 2 represents the previous and current discoveries and reviews available from credible scientific records and research that are related to this final year project topic. This chapter covers the overview of *Spirodella polyrhiza*, conventional lab scale and pilot scale cultivation of *S. polyrhiza*, factors affecting the productivity of *S. polyrhiza*, overview of superhydrophobic coating, raw materials and preparation methods applied so far for superhydrophobic coating. Besides that, this chapter also presents importance of applying beeswax for the preparation of superhydrophobic coating.

2.1 *Spirodella polyrhiza* Overview

S. polyrhiza is an aquatic plant which highly involves phytoremediation applications in wastewater industries due to high capability of absorbing the nutrients especially the heavy metals. Phytoremediation approach involving *S. polyrhiza* is a cost effective and environmentally friendly biological treatment in current wastewater treatment due to several upsides such as fast accumulation, wide range of heavy metal removal capability and most importantly chemical free. *S. polyrhiza* capable of removing heavy metals such as cadmium, nickel, ammoniacal nitrogen, phosphorus and etc (Mun et al., 2020; Chausuri et al., 2011). The removal efficiencies of all these components in wastewater treatment is highly researched and still in research to improvise the phytoremediation capabilities of the *S. polyrhiza*.

2.1.1 Biochemical content of *S. polyrhiza*

S. polyrhiza has high potential of being a food source for humans and aquatic animals due to its high crude protein content. According Appenroth et al., 2022 and Bhantumnavin et al., 1971, duckweed has been utilized in Laos, Thailand and Myanmar as food for poor people since many generations. Not only protein, *S. polyrhiza* also contains other nutritional contents such as starch, lipid, fibers and water (90% of the weight of the plant in common). Appenroth et al., 2022 has reported that *S. polyrhiza* contains 20-30 % of protein, 4-6% of lipid and 6-8% of starch content. In another study, it has been suggested that the crude protein in *S. polyrhiza* to be 30.52% (Ansal et al., 2007). In a recent study conducted in 2021, it has been reported that the protein, lipid and fiber content of *S. polyrhiza* to be 32.13 ± 1.1, 12.93 ± 3.8 and 28.26 ± 0.8 respectively on percentage of dry weight basis. Besides these nutritional content (Patel et al., 2021), *S. polyrhiza* is also rich in its chlorophyll content which ranges up to 1.20 mg/g. According to Su et al., 2010, chlorophyll content obtained through 95% ethanol is 0.745 ± 0.018 mg/g. While Seth et al., 2007, proves that the chlorophyll content for the *S. polyrhiza* control eventually 0.86 mg/g. In another study by Suzuki et al., 2014, the chlorophyll content of the duckweed species is about 1.031 mg/g.

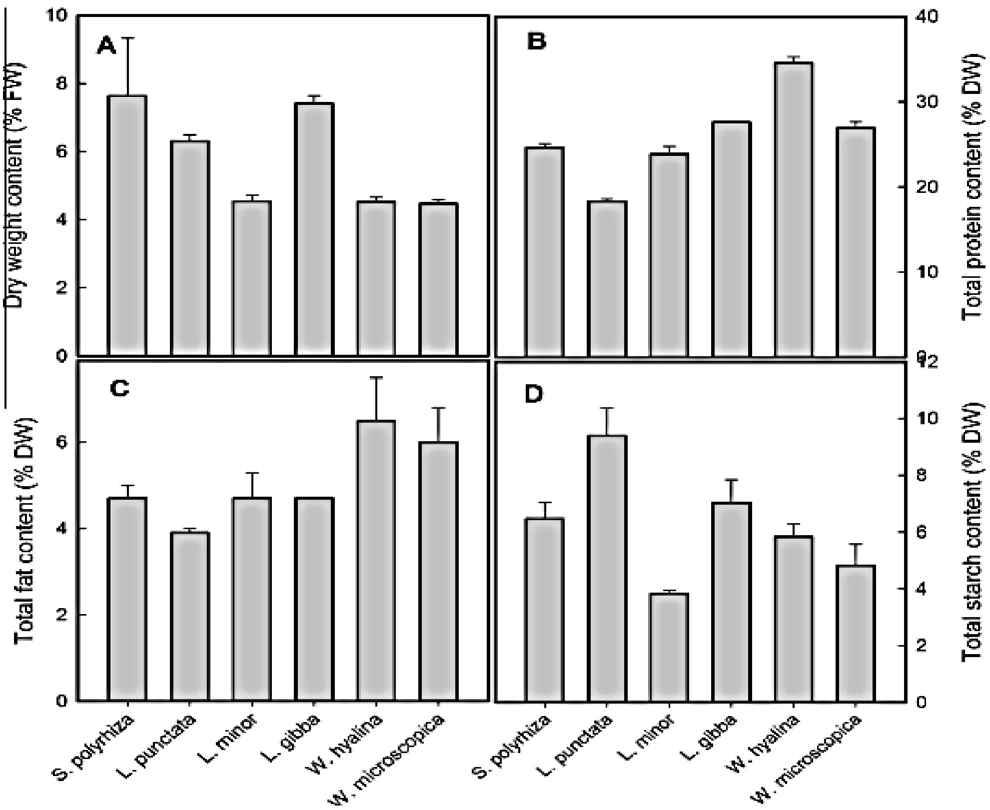

Figure 2.1 : Nutritional content of the duckweeds. A refers to Dry weight content (%FW) of all the duckweed species. B refers to total protein content (%DW). C refers to Total lipid content (%DW) and D refers to Total starch content (%DW) (Appenroth et al., 2022)

2.1.2 Current emerging technology/application of *S. polyrhiza*

Currently, *S. polyrhiza* is also found to be a promising substrate for biofuel production due to its faster growth rates. In a study conducted in 2011, it has been proved that duckweed-to-ethanol conversion is a promising technology compared to maize-to-ethanol conversion since duckweed provides higher yield of ethanol. During this, duckweed not only performed as a biomass to produce ethanol but also the plant reduced significant amount of ammonia and phosphorus from the pond used in the cultivation (Xu et al., 2011). This shows that *S. polyrhiza* as a biomass for biofuel production becomes a renewable resource and sustainable approach towards the environment which compromising with Goal 7 in Sustainable Development Goals (SDG) that emphasizing on '*Affordable and Clean Energy*' (United Nation, 2022). This shows that *S. polyrhiza* is gaining high demand in many fields such as phytoremediation, biofuel production and food production, which are very much essential for everything. Thus, cultivating and maximizing the production of this species also gaining more industry by researchers.

2.2 Conventional lab scale cultivation of *S. polyrhiza*

Due to the increasing demand of *S. polyrhiza* for its wide range of applications for phytoremediation, cultivation methods / modes of this species currently becoming emerging research by the researchers to increase the growth rate of the plant. In conventional method for cultivation *of S. polyrhiza* in lab scale, the plant is cultured in batch mode in beakers, glass jars, Erlenmeyer flask or 2L Duran Scott bottle containing Hoagland medium consisting of respective nutrients according to their composition (Nesan et al., 2020; Guo et al., 2020). Hoagland medium is the culture medium which will be prepared with certain compositions of several components resembling the wastewater concentration to acclimatize the species to attain a maximum growth in lab scale culture. Typically, this species is cultured in Hoagland medium under controlled conditions at a temperature of 25 ± 2 ^{o}C with a light intensity of 1600 lux for 16 hours light and 8 hours dark respectively (Nesan et al., 2020). Figures 2.2 and 2.3 show the lab scale cultivation of the species in glass jar and 2L Duran Schott bottle respectively.

Figure 2.2 : S. polyrhiza cultivated in glass jar

Figure 2.3 : S. polyrhiza cultivation in 2L Duran Schott bottle in lab

Unfortunately, this lab scale cultivation mode in glass jars will not provide higher growth rates of the species due to some restrictions in this mode. First, surface area for the cultivation is very small which would cause layering to occur in the culture. Because of this layering the light being passed will not be sufficient enough to reach the plant in the bottom layer. Thus, this eventually affects the growth rate of the species.

2.3 Large scale cultivation of *S. polyrhiza*

To overcome these constraints, the species were started to cultivate in bigger surface areas which are commonly in ponds and batch tanks. Commonly, the species is first acclimatized under laboratory conditions and then transferred to the pond/tank with a bigger surface area for cultivation (Leblebici et al., 2010; Basilico et al., 2013).

In a study conducted by Basílico et.al,2013, the cultivation was carried out in batch tanks of 433 m^2 of free surface area. The tanks were filled with 1 L of wastewater from Pampean stream to analyse the removal of nutrients from the wastewater by the species. This culture is done for 6 days. About 20.1 g of fresh weight was added to each of the 15 tanks and about > 90% of the free surface was covered and also the sides of the tanks were wrapped with an opaque plastic membrane to prevent algal growth. The containers were illuminated with fluorescent light in a 16:8 h (light:dark) photoperiod (Basilico et al., 2013). The productivity of the species was determined to be 8 g/m^2 per day on a fresh weight basis.

In Punjab (India), there is a pilot duckweed project initiated for bio- remediation of village pond in 2001 by the State Government in Sanghol and Chanarthal kalan villages in Fatehgarh Sahib district. The village ponds were divided into 2 different ponds where one is a duckweed pond, and the other is a fish culture pond. Bio-remediated water from the duckweed pond is used for culture of carps in the fish culture pond while the harvested duckweed biomass is used to feed the fish. This project is still in application to date due to its encouraging results. This way of cultivation provides an ecological and economical approach for rural development which provides cost effective village pond maintenance through *S. polyrhiza* cultivation in the polluted ponds. Harvested species were used for fish ponds as a feed for the fish growth. This eventually increases the economy of the villages in aquaculture industry. Duckweed productivity from the duckweed ponds reported from 10 to 50 ton (dry weight)/ ha/ yr (Ansal et al., 2010).

In other study (Chen et al., 2018), *S. polyrhiza* along with other duckweed species cultivated in pilot scale reactor made up of steel plate with a dimension of 24m x 0.5m x 0.5m at a surface area of 12 m^2. Domestic wastewater fed as the growth medium which contains following nutrients such as NH_4^+–N = 5.98 ± 0.09 mg L^{-1}, NO_x^-–N = 0.03 ± 0.00 mg L^{-1}, TN = 6.00 ± 0.09 mg L^{-1}, PO_4–P = 0.41 ± 0.02 mg L^{-1}, TP = 0.57 ± 0.02 mg L^{-1}, COD = 29.88 ± 6.36 mg L^{-1} . The species were grown at pH f 8.31 and turbidity of 12.21. The samples were collected from the reactors at 4 days interval to analyze the growth rate of the biomass. *S. polyrhiza* attained a growth rate of 9.5 to 12.5 g/m^2/day on a dry weight basis. This productivity of the species obtained relatively larger compared with lab scale cultivation in other studies (Nesan et al., 2020).

In large scale cultivation, there are several factors affecting the productivity of the *S. polyrhiza* such as surface area of the cultivation, growth conditions and nutrient availability. Most dominantly, surface area of the cultivation and nutrient availability in the growth medium becomes the important factors widely researched to maximize the growth rate of *S. polyrhiza*.

2.4 Factors affecting productivity of *S. polyrhiza*

As mentioned, there are several factors that will be affecting the productivity of the species, especially the surface area of the cultivation and nutrient availability in the growth medium in large scale cultivation. Even though the cultivation is scaled up to large scale from lab scale for betterment in the species growth but still these factors play an important role in affecting the growth rate of the species. Moreover, much research has been done by carrying out different approaches to overcome these constraints to increase the growth rate of *S. polyrhiza*.

2.4.1 Surface area of cultivation

In a recent paper (Sharma et al., 2019), *S. polyrhiza* was first cultured in 3 tanks in batch mode where each containing 3 different manures of different nutrient compositions. Three replicates were performed for each manure where 15g fresh weight of *S. polyrhiza* introduced into each tank (1.2 m × 0.35 m × 0.30 m). The duckweeds were harvested after 118 days of culture where 50% of total duckweeds were harvested during each harvest. Based on the growth rate of the *S. polyrhiza* in tank culture, manure that exhibits the highest relative growth rate were used for the pond culture. Manure 1 consisting of cattle manure, poultry droppings and mustard oil cake was the medium provides the highest growth rate in the tank culture, thus it was used for the pond culture. In pond culture (200 m^2 {20 m × 10 m} with water level maintained as 50 cm) the plants were harvested three times in 10 days intervals. In that case, a higher relative growth rate obtained for the pond culture than the tank culture which concluded that higher surface area of cultivation provides higher growth rate.

In a study to utilize *S. polyrhiza* for bioethanol production, the plants were cultivated in a pilot scale duckweed culture pond 300 m^2 and 0.6 m in depth (Xu et al., 2011). As a nutrient medium for growth pig effluent which was treated in an anaerobic digester were pumped on a maintained flow from a lagoon into the culture pond. The treated pig effluent medium consists of irons such as NH_4-N (290 mg/L), NO_3-N (5.47

mg/L), NO_2-N (4.70 mg/L), TP (12.1 mg/L), Potassium (926 mg/L), sodium (384 mg/L), calcium (22.2 mg/L), magnesium (41.4 mg/L), zinc (0.28 mg/L) and copper (0.09 mg/L). The temperature and light intensity of the culture pond were maintained at 20-30 ^oC and 2.89 mmol/m^2s respectively. The fresh biomass was harvested three times in a week while keeping the pond covered by approximately two layers of duckweed fronds. In total, 1008 kg of fresh duckweed were harvested from this cultivation. Again, this proves, bigger surface area for cultivation provides higher production of duckweed.

In another study (Tonon et al., 2017), the species were cultivated in a pilot scale pond culture at a dimension of 4.2m x 2.4m x 1m (surface area of $10m^2$) with a 0.42m depth of growing medium. The ponds were constructed of fiber glass while the growth medium was a municipal wastewater from residential condominium in South Brazil. The growth medium was fed to the ponds continuously at a flowrate of 200 L/day. Municipal wastewater contains COD of 25.1 kg/ha/day and NH_4^+-N of 10.5 kg/has/day. The species samples were collected every week and the biomass growth rate were measured in terms of g/m^2/day. Relative growth rate of the biomass was relatively higher for the pond culture which is about 3.27 to 5.72 g/m^2/day. This study again concluded that new cultivation method by maximizing the surface area for cultivation eventually increases the growth rate of the species over the conventional lab scale cultivation

All these current conventional cultivation methods of *S. polyrhiza* so far discussed on improvising the growth rate of the species through manipulating the nutrient contents of the culture medium and influence of surface area of the cultivation to maximize the growth rate. So far, the approach to increase the production of *S. polyrhiza* is only carried out through maximizing the pond size, high / continuous growth medium usage and manipulating the growth medium's nutrient composition. But, the new / innovative approach for the *S. polyrhiza* cultivation is still in the development stage.

2.5 Superhydrophobic coating (SHP) Overview

Superhydrophobic coatings are types of engineered coatings that are specifically designed to excel at repelling water. This superhydrophobic coating attracted wide attention in the current world due to its broad applications in many industries such as oil and water separation, drag reduction, metal coatings and etc (Zhao et al., 2018; Vazirinasab et al., 2018). Currently, superhydrophobic coating is applied mainly in textile industry, coating industry, constructions, automobile, energy and aerospace industry (Nguyen et al., 2019). Superhydrophobic coatings are excellent candidates to eliminate liquid waste which minimizes the water usage in an application (Liu et al. 2019). Most of the superhydrophobic coatings are prepared through construction of micro/nanostructures on the surface of substrates followed by decreasing the surface energy of the micro/nanostructures (Zhao et al., 2018). Characterization of these coatings described based on several properties such as surface wetting, surface roughness and surface tension and surface energy (Nguyen et al., 2019).

The SHP coating is commonly characterized by surface wetting characterization which is one of its properties (Huhtamaki et al., 2018; Nguyen et al., 2019). Surface wetting characterization is usually determined based on the contact angle of the liquid on the surface. The coating is known to be superhydrophobic when it is characterized by water contact angle (CA) of more than 150^o and low sliding angles (SA) of less than 10^o (Zhao et al., 2018). Figure 2.4 shows the shape of a water drop and the contact angle on different surface types. There are several methods available for the water contact angle measurement, such as Sessile drop, Needle method, Meniscus method, Tilting method and Wilhelmy method (Biolin Scientific, 2020). The sessile drop method is the most widely applied method for contact angle measurement. In nature, the best example of superhydrophobicity is lotus leaf. This is the reason why the superhydrophobicity also called the lotus effect (Bayer, 2020).

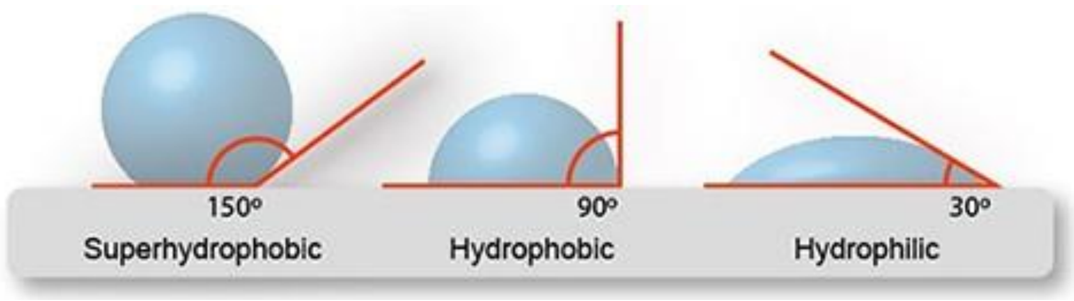

Figure 2.4 : Shape of a water drop and the water contact angle

2.6 Raw materials for SHP coating preparation

Superhydrophobic coating is prepared from different types of raw materials. Commonly, fluorinated compounds are widely used as raw materials for superhydrophobic coating preparation due to its high efficiency in providing coatings that are highly superhydrophobic (CA>150°). Currently, there is much research emerging on applying naturally available materials as raw ingredient for the SHP coating preparation considering the sustainability factors.

2.6.1 Fluorinated compounds

Traditionally, superhydrophobic coatings are prepared from fluorinated compounds and their derivatives (Liu et al. 2019; Wang et al., 2019). In a study by Favia et al., superhydrophobic coating was deposited into modulated RF glow discharges that are fed with tetrafluoroethylene. This coating was characterized by high fluorination degree, ribbon-like randomly distributed surface microstructures and crystallinity. This combined high fluorination and surface roughness provides the superhydrophobicity where the contact angles obtained to be >150^{o}. In another study conducted later in 2015, fluorinated carbon at gram scale were synthesized by reacting fluorinated alcohol with the sodium in Teflon reactor at elevated temperature in order to increase the efficiency of the carbon material in term of its electronic and magnetic properties by inducing the superhydrophobic characteristics (S.Lyth et al., 2015). Again, in this study, fluorinated alcohol provides the better superhydrophobic behaviour to the particles.

Other than the fluorinated compounds, there are also other chemicals such as resin and toluene used to prepare superhydrophobic coating. In a study conducted by Yu et al., 2018, durable superhydrophobic coating was prepared through a facile one pot route. In this facile one pot route, superhydrophobic P25 (nano TiO_2)/ATP (attapulgite)/ER (epoxy resin)/PDMS (polydimethylsiloxane) coating with good durability and self-cleaning property were obtained. The coating also exhibits a contact angle of more than 150^{o} during its characterization through sessile drop method (Yu et al., 2018). Epoxy resin and polyamide curing agent were in a toluene at a ratio of 2:1 and continuously stirred. Then, the P25 and ATP are added to the solution and then mixed for 2.5 hours followed by mixing of H-PDMS. This mixture then vigorously stirred until off-white superhydrophobic dispersion which is then used to coat on different substrates such as glass slides, filter paper and aluminium sheet. The coating is then analyzed for its characterization which finally provides promising results.

2.6.2 Natural and biodegradable compounds

Major disadvantage of using fluorinated compounds here is that the fluorinated compounds and the other chemicals such as epoxy resins and toluene are potentially harmful to the environment. These materials that are being used for the SHP coating preparation are not environmentally friendly or follow green fabrication processes. Moreover, these materials are bio accumulative, and potentially toxic to humans and aquatic wildlife (Liu et al. 2019; Wang et al., 2019). In order to comply with sustainability goals, currently it is encouraged on SHP coating preparation through natural, biodegradable, nontoxic and food/medical grade materials using ecofriendly fabrication techniques (Bayer, 2020). According to Ilker S. Bayer in 2020, superhydrophobic coatings can be prepared through natural waxes, proteins, fatty acids, cellulose (and its derivatives) and biomass and agricultural wastes. Each of these raw materials has its own advantages and disadvantages depending on the mode of its application.

In a study conducted by Zhao, X., Hu, T. and Zhang, J. (2018), the superhydrophobic coating was prepared using beeswax, paraffin wax, carnauba wax and microcrystalline wax (Zhao et al., 2018). The method used to prepare these coatings is also much easier. Each wax dissolved in 95% ethanol at 80 ^{o}C water bath. Then, once all wax suspensions dissolved completely in the solvent, the solution cooled to room temperature followed by spray coating the prepared suspension onto the glass slides. All the prepared wax suspension analyzed through contact angle analysis. As for the results, it is reported that the contact angle obtained for the beeswax-based SHP suspension is greater than the other waxes (162^{o}) which at the end concluded that the beeswax provides the promising results than the other waxes. In another study, similar preparation of SHP coating applied (Liu et al. 2019). In this study, candelilla wax and rice bran wax were used to prepare the SHP coating. These waxes are mixed in ethanol at 85 ^{o}C water bath under continuous stirring for 3 minutes. Then the prepared suspension spray coated on the food packaging material since this coating prepared for the purpose of food packaging. Both the waxes provide a contact angle greater than 150^{o}. Other than wax, the SHP coating is also produced from biomass such as bagasse and epoxidized soybean oil. According to research conducted in 2021, bagasse which is a high yield agricultural waste used as a raw material to produce superhydrophobic coating since bagasse is cheap, renewable and biodegradable (Qin et al., 2021). This biobased SHP coating is prepared by esterifying mechanically treated bagasse with stearoyl chloride. The prepared suspension is then spray coated onto the substrates which is a glass slide. And successfully this prepared coating provides the SHP characteristics in terms of its contact angle and sliding angle.

2.7 Superhydrophobic coating preparation methods

There are many different superhydrophobic coating preparation methods that have been established in many papers such as sol-gel method, cold spray method, chemical vapor deposition, physical vapor deposition, thermal spray method, in-situ polymerization, dip-coating and electrodeposition (Nguyen et al., 2019). A comparison of these preparation methods is provided in Table 2.1. The Sol gel method has been used for superhydrophobic coating preparation while another research team produced superhydrophobic films by coating a substrate with polyurethane and fluorinated silica particles that repels most type of liquids (Liu et al. 2019; Wei et al., 2016; Vahabi et al., 2016).

Table 2.1 : Comparison of the preparation methods of SHP coating

Superhydrophobic coating preparation method	Advantages	Disadvantages
Sol-gel	High quality films	Thickness limit, mechanical stress, high energy consumption and cost
Spin coating	Quick dry, thickness controllable	Suits only lab scale, small surface coating only allowed
Chemical vapor deposition	Applied for complex surfaces and high-quality coating	Involves high temperature and high cost
Cold spray	Provides low defect, only low temperature required	High energy consumption and used for selective substrates
Physical vapor deposition	Provides high quality coating and ecofriendly technique	High pressure required and high cost too
Dip coating	Suitable for complex structure and used for industrial scale	Used only for soluble polymers
Electrodeposition	Low cost and simple manufacturing process	Time consuming and gives non uniform coatings

2.8 Advantages of beeswax-based SHP coating

As mentioned above, while preparing the superhydrophobic coating, it is important to consider sustainability factors since materials used for the coating preparation can be bio accumulative, toxic to environment and also can pollute ground water and aquatic wildlife. Since in this paper, superhydrophobic coating will be applied in water environment, it is important to ensure the coating is sustainably compliable to the environment it is being exposed to. Based on the reviews from the articles, in this paper beeswax is the material that has been used for the superhydrophobic coating preparation as this preparation is easier and edible to be handled in lab. Moreover, beeswax is also an environmentally friendly material that will not leave any footprint to the environment and is also a renewable resource unlike other waxes (Zhao et al., 2018). Other than its environmentally friendly nature, wax also exhibits more advantages such as (Bayer, 2020):

i. Easy to be emulsified with water due to their low melting points.
ii. Can be compounded in other hydrophobic polymers for encapsulation and stabilization.
iii. Polymer compounded waxes tend to migrate to the surface improving coating hydrophobicity.
iv. Can be dispersed in alcohols.
v. Crystallization of wax can be tuned to form roughness features similar to lotus leaf.
vi. Easily can impregnate into rough or porous surfaces

CHAPTER 3

METHODOLOGY

This chapter discloses the information on the methods applied in this final year project. It includes the general research flow diagram, experimental procedure along with statistical analysis.

3.1 Research flow

Figure 3.1 shows the flow diagram of the research project for cultivation of *S. polyrhiza* on superhydrophobic (SHP) coated Perspex. The species will be subcultured while the SHP wax suspension will be prepared in the lab. After the characterization of the SHP coating is done, cultivation of the species will carried out on the prepared coating. Then, the growth rate analyzed comparing it with conventional cultivation method along with the biochemical content of the species cultured. Report writing done after all of the data required for this research is collected.

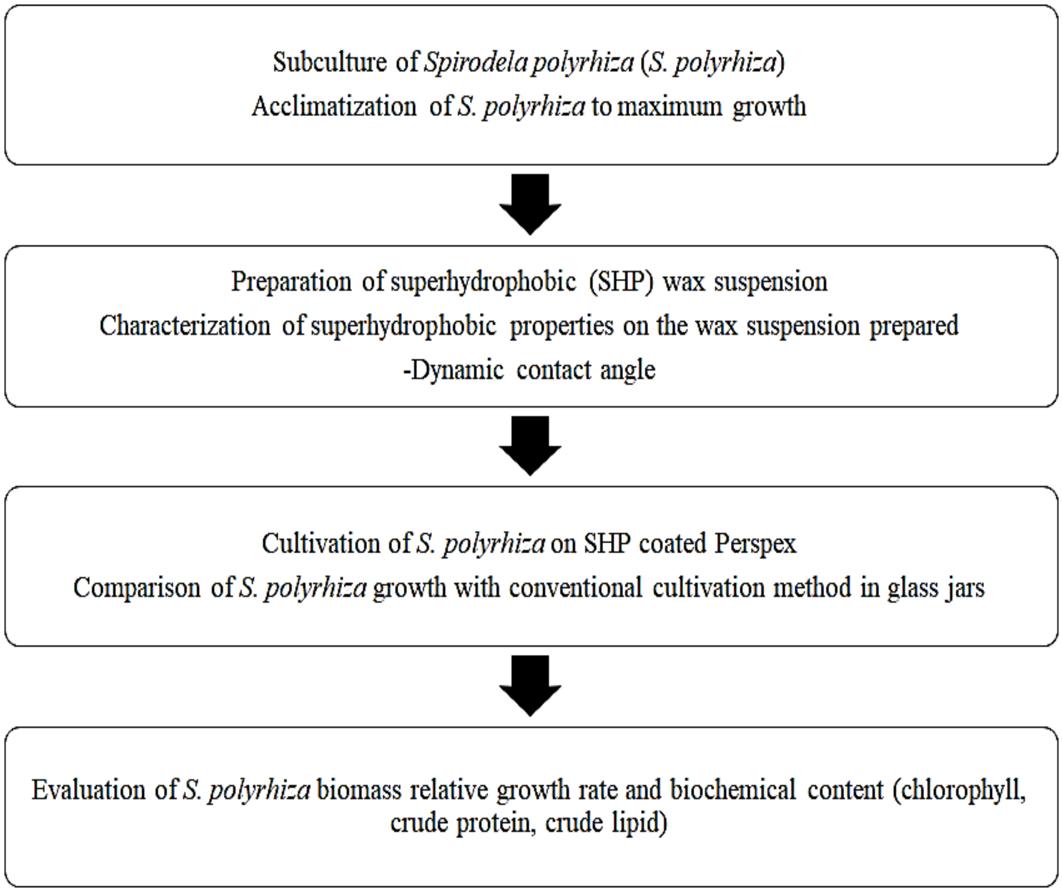

Figure 3.1 : Flow diagram of the research project for cultivation of S. polyrhiza on superhydrophobic (SHP) coated Perspex

3.2 Subculture and acclimatization of *S. polyrhiza* to maximum growth

S. polyrhiza samples for this study subcultured from plantlets subcultures initially collected out from local waterway in Nibong Tebal. The Hoagland medium was prepared in glass jars according to the composition as shown in Table 3.1 with addition of 1.5% sucrose. Then, the medium sterilized by autoclaving it at 121°C for 15 minutes after adjusting the medium to a pH of 5.6 by NaOH solution. Once the Hoagland medium was autoclaved, the *S. polyrhiza* samples then cultured in 100 ml of sterile medium in glass jar where the initial biomass covers 50% of the surface under a temperature of 25 ± 2 °C and light intensity of 1600 lux with 16h light : 8h dark photoperiod. This subculturing of the samples carried out for 2 weeks before starting to cultivate the *S. polyrhiza* on SHP coated Perspex (Nesan et al., 2020; Ng et al., 2017).

Table 3.1 : Composition of Hoagland medium (growth medium)

Components	**Concentration (mg/L)**
$(NH_4)_2SO_4$	94.34
$MgSO_4.7H_2O$	246.4
KH_2PO_4	27.22
$Ca(NO_3)_2.4H_2O$	153.49
K_2SO_4	243.96
$CaSO_4.2H_2O$	232.36
$MnCl_2.2H_2O$	0.74
H_3BO_3	1.43
$ZnSO_4.7H_2O$	0.11
$CuSO_4.5H_2O$	0.04
$Na_2MoO_4.2H_2O$	0.013
$FeSO_4.7H_2O$	2.51
$Na_2.EDTA.2H_2O$	3.37

3.3 Superhydrophobic wax suspension preparation and characterization of the superhydrophobic properties

1.2 g of beeswax mixed with 100 mL of 95% ethanol in a conical flask at 65 ℃ water bath. By stirring continuously, beeswax dissolved gradually in ethanol in 5 minutes. Next, the flask taken out from the water bath and let to cool at room temperature for 20 minutes until the beeswax completely precipitate. Glass slides were used as substrates.

They then washed with ethanol and distilled water followed by drying under nitrogen (N_2) flow. Then, 10 ml of the beeswax suspension was spray-coated (2 layers of coating) on the whole surface (75mm x 25mm) of the vertically placed glass slide using a spray gun with 0.2 MPa N_2 (Zhao et al., 2018; Wang et al., 2019).

The prepared SHP wax coating was characterized by dynamic contact angle where the water contact angle on the prepared SHP coating analyzed using Sessile Drop mode (Huhtamaki et al., 2018). One droplet of water (10 μL) was placed on the wax coated slide using a 0.5 ml syringe and an image of the drop recorded (Liu et al., 2019). The static contact angle was then defined by fitting Young-Laplace equation around the droplet (Biolin Scientific, 2020; Tiab et al., 2016). The contact angle determined by averaging all the values that were measured at 10 different points on the wax coating sample.

After the further verification of superhydrophobic property of the wax suspension that has been prepared, 60 ml of the suspension was spray-coated (2 layers of coating) on an A4 sized Perspex (29.7 cm x 21 cm) forming a frame (Figure 3.2).