LIGNOCELLULOSICS BIOMASS BIODEGRADATION OF WATER HYACINTH AND WATER LETTUCE BY WHITE ROT FUNGI FOR BIOETHANOL PRODUCTION

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DEDICATION

This thesis is dedicated to all my beloved family and friends.

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ABSTRACT

Nowadays, renewable energy has become alternative energy to reduce the consumption of fossil fuels. Therefore, lignocellulosic materials such as crop residues, grass and wood, and aquatic plants that are inedible has become potential sources for bioethanol production. In this study, water hyacinth (WH) and water lettuce (WL) were selected as potential resources of their abundance in nature and can be easily propagated and cultivated. Although these floating aquatic plants are considered as the most problematic plants due to their uncontrollable growth in water bodies worldwide, their ability to remove pollutants from wastewater has created a sustainable approach for their use in phytoremediation and further use as biomass substrates for bioethanol production. The use of phytoremediation by implementing invasive floating aquatic plants can support the sustainable management of wastewater treatment in the future. This study aims to determine the potential of WH and WL as bioindicators for phytoremediation and at same time to produce a high amount of sugar consumption for bioethanol production. In addition, this study emphasizes the biodegradation of WH and WL by white-rot fungi collected from decayed wood and soil. White-rot fungi have the ability to degrade lignin, hydrolyze cellulose, and hemicellulose, and ferment alcohols for bioethanol production. *Trichoderma citrinoviride* M3, *Schizophyllum commune* M8, and *Pestalotiopsis* sp. M12 were selected from twelve fungal species on the basis of rapid growth rate after five days of incubation and further use for degradation of lignocellulosic materials from water hyacinth and water lettuce and for bioethanol production. These fungal species were identified by morphological characterization and 18S rRNA sequence analysis. To date, the use of biological pretreatment using *T. citrinoviride* M3, *S. commune* M8, and *Pestalotiopsis* sp. M12 with regard to water hyacinth and water lettuce substrates as well as its further use for the fermentation process to produce bioethanol has not been explored before. The parameters involved are sugar content by the dinitrosalicylic acid (DNS) Method, the determination of lignin by the Klason Method, the determination of cellulose and hemicellulose by the Chesson Method, and the determination of bioethanol by Gas Chromatography (GC). The results showed that both WH and WL indicated the same correlation trend between biomass growth and sugar content, as the sugar content increased when the plants reached the highest growth. However, WH has more extractable sugar than WL, which is more significant because fermentable sugar is needed for the fermentation process to produce bioethanol. The results also showed that *T. citrinoviride* M3 has the highest rate of degradation of lignocellulosic materials compared to *S. commune* M8 and *Pestalotiopsis* sp. M12. Therefore, *T. citrinoviride* M3 was selected for further investigation to evaluate the simultaneous saccharification and fermentation process for bioethanol production. The results showed that WH and WL produced 8.6 g/L and 7.4 g/L yield of ethanol, respectively, proportional to the fermentation time, with an increasing time of up to 96 hours. Overall, it can be concluded that WH is a promising biomass when the simultaneous co-cultivation of *T. citrinoviride* M3 with *S. cerevisiae* is used for bioethanol production and the fermentation process is fully optimized. The findings of this study would be beneficial for future investigation, especially in exploring the potential production of bioethanol from phytoremediation technology systems.

ABSTRAK

Pada masa kini, tenaga boleh diperbaharui telah menjadi tenaga alternatif untuk mengurangkan penggunaan bahan bakar fosil. Oleh itu, bahan lignoselulosa seperti sisa tanaman, rumput dan kayu, dan tumbuhan akuatik yang tidak boleh dimakan telah menjadi sumber yang berpotensi untuk pengeluaran bioetanol. Dalam kajian ini, keladi bunting (WH) dan pokok kiambang (WL) dipilih sebagai sumber daya yang berpotensi kerana sifatnya mudah ditanam dan boleh membiak dengan banyaknya. Walaupun tumbuhan akuatik terapung ini dianggap sebagai tumbuhan yang paling bermasalah kerana pertumbuhannya yang tidak terkawal di atas air sisa, namun keupayaan mereka untuk membuang bahan pencemar daripada air sisa telah mewujudkan pendekatan yang mampan untuk kegunaannya dalam fitoremediasi dan penggunaan selanjutnya sebagai bahan untuk pengeluaran bioetanol. Oleh itu, penggunaan pokok akuatik terapung ini pastinya dapat menyokong pengurusan rawatan air sisa yang mampan pada masa hadapan. Kajian ini bertujuan untuk menentukan potensi WH dan WL sebagai bioindikator untuk fitoremediasi dan pada masa yang sama menghasilkan jumlah penggunaan gula yang tinggi untuk pengeluaran bioetanol. Di samping itu, kajian ini menekankan pada biodegradasi WH dan WL oleh kulat yang dikumpulkan dari kayu dan tanah yang reput. Kulat ini mempunyai kemampuan untuk menurunkan kandungan lignin, menghidrolisis selulosa dan hemiselulosa, dan fermentasi alkohol untuk pengeluaran bioetanol. *Trichoderma citrinoviride* M3, *Schizophyllum commune* M8 dan *Pestalotiopsis* sp M12 dipilih dari dua belas spesies kulat berdasarkan kadar pertumbuhan pesat setelah lima hari inkubasi. Spesies kulat dikenal pasti dengan ciri morfologi dan analisis urutan rRNA 18S. Sehingga kini, penggunaan prarawatan biologi menggunakan *T. citrinoviride* M3, *S. commune* M8, dan *Pestalotiopsis* sp. M12 menggunakan substrat WH dan WL serta penggunaan selanjutnya untuk proses penapaian untuk menghasilkan bioetanol belum pernah diterokai sebelum ini. Tiga spesies ini seterusnya digunakan untuk mendegradasi bahan lignoselulosa dari WH dan WL dan parameter yang terlibat adalah kandungan gula dengan Kaedah asid dinitrosalicylic (DNS), penentuan lignin dengan Kaedah Klason, penentuan selulosa dan hemiselulosa dengan Kaedah Chesson dan penentuan bioetanol oleh Kromatografi Gas (GC). Hasil kajian menunjukkan bahawa kedua-dua pokok menunjukkan korelasi trend yang sama antara kadar pertumbuhan dan kandungan gula, ketika kandungan gula meningkat maka tanaman akuatik juga mencapai peningkatan pertumbuhan tertinggi. Namun, WH mempunyai lebih banyak gula yang dapat diekstrak berbanding dengan WL, kerana gula fermentasi diperlukan untuk proses fermentasi untuk menghasilkan bioetanol. Hasil kajian menunjukkan bahawa *T. citrinoviride* M3 mempunyai kadar degradasi tertinggi bahan lignoselulosa berbanding dengan *S. commune* M8 dan *Pestalotiopsis* sp M12. Oleh itu, *T. citrinoviride* M3 dipilih untuk penyelidikan seterusnya untuk menilai proses sakarifikasi dan fermentasi serentak untuk pengeluaran bioetanol. Selain itu, keputusan menunjukkan bahawa WH dan WL menghasilkan 8.6 g/L dan 7.4 g/L hasil bioetanol, masing-masing, berkadar dengan masa fermentasi, dengan masa yang meningkat sehingga 96 jam. Ini dapat disimpulkan bahawa WH adalah bahan biomas yang berpotensi untuk digunakan apabila digabungkan *T. citrinoviride* M3 dengan *S. cerevisiae* untuk pengeluaran bioetanol dan proses fermentasi dioptimumkan sepenuhnya. Hasil kajian ini akan bermanfaat untuk kajian masa hadapan, terutamanya dalam menerokai potensi penghasilan bioetanol daripada sistem teknologi fitoremediasi.

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LIST OF ABBREVIATIONS

DNS	-	Dinitrosalicylic acid
GC	-	Gas Chromatography
PDA	-	Potato Dextrose Agar
RBBR	-	Brilliant Blue R
SEM	-	Scanning Electron Microscopy
SSF	-	Simultaneous Saccharification and Fermentation
UTM	-	Universiti Teknologi Malaysia
WH	-	Water Hyacinth
WL	-	Water Lettuce

LIST OF SYMBOLS

h	-	hour
g	-	gram
g/L	-	gram per litre
g/g	-	gram per gram
mg/g	-	milligram per gram
^{o}C	-	degree Celsius
μm	-	micrometer
PO_4^{3-}	-	phosphate
NO_3^-	-	nitrate
NO^-_2	-	nitrite

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Higher demand for energy and concern for the environment is the crucial factor leading to renewable energy sources. Energy demand in Malaysia is constantly increasing due to advancements in the industrial and transportation sectors (Salleh et al., 2020; Ayodele et al., 2021), and the global depletion of energy resources due to energy consumption and environmental degradation caused by fossil fuels has prompted research on alternative renewable energy sources. As a result, the demand for petroleum-derived fuels has not decreased but increased sharply in recent decades (Shamsuddin, 2012; Li et al., 2021). In contrast, the challenges in finding a long-term solution for a reliable and infinite source of clean energy supply in the future are enormous. The Five-Fuel Diversification Policy Malaysia has consistently promoted renewable energy (RE) over non-renewable energy sources in its energy policy, along with fossil fuels and hydropower (Chin and H'ng, 2013). Malaysia has formulated the National Biofuel Policy to promote the prosperity and sustainable development of the country, and the Sustainable Energy Development Authority (SEDA) has proposed the National Renewable Energy Policy, setting an ambitious target by aiming for 20% of the capacity mix constituting renewable energy sources to reduce greenhouse gas emissions and creating a more conducive environment (Abdullah et al., 2019). Therefore, renewable bioethanol becomes a potential energy source for Malaysia's future renewable energy development prospects.

Bioethanol production deals with biotechnological production from various feedstock sources. The selection of the most suitable feedstock for bioethanol production depends on local conditions. Conventional bioethanol production has been reported from various feedstocks such as sugarcane, corn, and starchy materials; however, there has been a rising interest in low-cost, readily available, and abundant lignocellulosic biomass that could serve as a potential feedstock for bioethanol production (Wyman et al., 2019; González-Gloria et al., 2022). Lignocellulosic biomass, which includes agricultural and forestry residues and wastes, provides a more excellent choice of potential feedstocks that do not conflict with land use for food production and is also cheaper than conventional bioethanol sources. Lignocellulosic biomass typically consists of carbohydrates that can be derived from monosaccharides and disaccharides (Singh et al., 2019).

Conversely, carbohydrates comprise cellulose and hemicellulose that are tightly wrapped by lignin. Many researchers worldwide are working hard to convert lignocellulosic biomass, such as straws and other plant wastes, into bioethanol production (Arefin et al., 2021). Although the biological conversion of lignocellulosic biomass into monomeric sugars is more complicated than the conversion of starch that is currently used for bioethanol production, many countries (Sweden, Australia, Canada, and Japan) are making efforts to utilize this lignocellulosic biomass into ethanol (Singh and Satapathy, 2018; Singh et al., 2022).

Floating aquatic plants are potential lignocellulosic biomass when it comes to bioethanol production. Currently, the extensive focus has been placed on developing methods to produce bioethanol from aquatic plants with high cellulose-hemicellulose contents. Water hyacinth (WH) and water lettuce (WL) are potential feedstocks since they constitute abundant floating aquatic plants and are quick to propagate for bioethanol production. Moreover, water hyacinth and water lettuce also act as bioindicators for phytoremediation technology to remediate contaminated domestic wastewater. Phytoremediation refers to a new plant-based technology that can be used as an alternative option to purify contaminated water. This technology focuses on using floating aquatic plants to eliminate pollutants from wastewater (Rezania et al., 2015). The selection of aquatic plants is essential as it is the main tool for removing contaminants (De Stefani et al., 2011; Ansari et al., 2020). Thus, the selection is based on the high contaminant uptake as well as fast and easy growth (Roongtanakiat et al., 2007; Mustafa and Hayder, 2021). Microorganisms attached to plant roots can oxidize biodegradable materials in contaminated wastewater (Nayanthara and Bindu, 2017).

Water hyacinth (WH), which is also known as Eichhornia crassipes, is a fast-growing invasive plant that can double its reproduction in less than 13 days (Rezania et al., 2015; Arefin et al., 2021). As a result, this plant can disturb aquatic ecosystems and activities, thus causing problems for navigation. However, due to their high hemicellulose and low lignin content, WH is feasible for bioethanol production (Arefin et al., 2021).

Water lettuce (WL), also known as Pistia stratiotes L., has been widely used to mitigate pollutants from contaminated water (Gupta et al., 2012; Karmakar et al., 2018; Amalia et al., 2019) and belongs to the Araceae family, which can reproduce rapidly and vegetatively on the water surface (Hussnera et al., 2014; Walsh and Maestro, 2014; Lien et al., 2019). WL floats on the water surface and its roots submerse beneath water bodies. The size of WL leaves can reach up to 13 cm long (Dipu et al., 2011). WL also has a soft body that can increase biochemical responsiveness and low labor harvesting due to its small body size (Mishima et al., 2008). Therefore, an emphasis has been placed on exploring WH and WL as bioindicators of phytoremediation technology and as substrates for bioethanol production in future works.

1.2 Problem Statement

The excess concentration of nutrients released from domestic wastewater has become of concern because it has triggered eutrophication, harmful algal blooms, and fish kills in water bodies. Eutrophication typically causes excessive growth of algae on the water surface and subsequently leads to the deterioration of water quality (Sabeena et al., 2018; Feng et al., 2023). Due to the accumulation of organic and nutrients, such as nitrogen and phosphorus, which come from the nearby wastewater domestic ponds, this condition is relatively similar to the selected location of Desa Bakti wastewater. As a result, discharge from the drainage system, encompassing residential and gardening areas as well as nonpoint surface runoff on rainy days, had an impact on it. Therefore, it is essential to remove the wastewater's nutrients to achieve environmental sustainability (Mulling et al., 2014; Qv et al., 2023).

Nowadays, several treatment processes, including physical, chemical, and biological processes, have been used to remove nutrients from wastewater (Nizam et al., 2020; Nidheesh et al., 2022). However, these treatment methods require high operational and maintenance costs (Ali et al., 2020). Among all the treatment methods, phytoremediation is a promising technique for removing nutrients from wastewater owing to the eco-friendly, cost-effective, and efficient process it offers. This technology focuses on the use of floating aquatic macrophytes as a tool to eliminate pollutants from wastewater (Rezania et al. 2015; Ansari et al., 2020). To date, comprehensive information on how to choose plants that will maximize the contribution of each phytoremediation mechanism and how to improve phytoremediation technology performance are still lacking (Qin et al., 2020). Several studies have described the effectiveness of aquatic plants, such as water hyacinth (E. crassipes), water lettuce (P. stratiotes), duckweed (L. minor), and giant salvinia (S. molesta) to remove various pollutants from wastewater (Rezania et al., 2015; Qin et al. 2016; Ting et al., 2018; Ansari et al., 2020; Mustafa & Hayder, 2021). Nevertheless, very few studies have been performed to determine the effectiveness of aquatic plants for removing nutrients from wastewater (Qin et al. 2016; Nayanthara & Bindu, 2017). However, the use of WH and WL is proven superior to other plants as a bio-indicator for phytoremediation in terms of nutrient removal efficiency and sugar recovery for bioethanol production (Dixit et al., 2011; Akinbile and Yusoff, 2012; Nivetha et al., 2016; Qin et al., 2016). Hence, it is needed to evaluate the correlation between sugar content for bioethanol production, biomass growth rate, and nutrients recovered from WH and WL from wastewater.

Thus, the utilization of WH and WL substrates for bioethanol production is explored in this study. Moreover, since the use of WH and WL as substrates for bioethanol production can remove various pollutants in the wastewater and proliferate even under extreme conditions without requiring the land area, several studies have described the potential of WH and WL as substrates for bioethanol production, which consist of significant components such as lignin, cellulose, and hemicellulose. The high percentage of cellulose and hemicellulose in these aquatic plants is the main advantage of their use in bioethanol production (Arefin et al., 2021). Therefore, extensive interest has been emphasized in exploring water hyacinth and water lettuce as a substrate for bioethanol production in the present study and in future studies.

Previously, conventional bioethanol production was reported from various carbonaceous feedstocks such as sugarcane, corn, and starchy (Kassim et al., 2022). However, the main problem in converting carbonaceous feedstocks to bioethanol is the substrate cost and conflict of interest in agricultural products. For example, growing sugarcane for bioethanol production can increase the price of sugar production and tighten food supplies.

Consequently, there has been an emphasis on utilizing lignocellulosic biomass as a source of fermentable sugars for bioethanol production. Since lignocellulosic biomass is readily available, low-cost, and abundant, it could serve as a potential feedstock for bioethanol production (Bilal et al., 2020). Lignocellulosic biomass, which includes agricultural and forestry residues and wastes, also provides a more excellent choice of potential feedstocks that do not conflict with land use for food production and is, in fact, cheaper than conventional bioethanol feedstocks (Krishnan et al., 2020). Hence, in the present study, it is crucial to realize the potential of lignocellulosic biomass of WH and WL, which are rich in fermentable carbohydrates, as they are abundant in nature and cheaper than others in Malaysia.

Generally, bioethanol production relies on the following processes: pretreatment, fermentation, and distillation. However, the major limitation in converting substrates to sugar is the resistance to enzymatic hydrolysis (Li et al., 2018). The low solubilization of the cellulose and hemicellulose components during hydrolysis is another problem in bioethanol production (Arefin et al., 2021). During this process, the sugar is converted for bioethanol production. Therefore, pretreatment is essential for breaking down the lignocellulosic components and improving the cost-effective hydrolysis process (Eshtigahi et al., 2012; Krishnan et al., 2020). To convert lignocellulosic material into bioethanol, using chemicals in the pretreatment method is one of the significant problems in terms of cost and its hazardous effects on the environment. Previous studies have shown that most physical and chemical pretreatments using acid, alkali, microwave, steam explosion, ionizing radiation, or combined processes require specialized instrumentation, consume too much energy, and produce inhibitors that interfere with enzymatic hydrolysis and fermentation (Abo et al., 2019; Krishnan et al., 2020). Hence, biological pretreatment using the metabolites of a microorganism in nature for ethanol production from biomass is a promising technology due to its numerous advantages, such as its environmentally friendly benefit and economically viable strategy for increasing the enzymatic saccharification rate (Zabed et al., 2019). Several studies have indicated that fungal pretreatment could improve hydrolysis efficiency, resulting in energy consumption limitations (Zabed et al., 2019; Krishnan et al., 2020). Hence, this study explores the suitability of naturally occurring fungal species for the biodegradation process of lignocellulosic plants of water hyacinth and water lettuce as biomass substrates. Since no chemicals are used in this process, no recycling of chemicals is required and, thus, no toxic compounds are released into the environment.

Therefore, biological pretreatment with certain isolated white-rot fungi has been used, which seems promising as an environmentally friendly process. In contrast, no inhibitors are generated during the pretreatment process (Kumar et al., 2020. In this study, *Trichoderma citrinoviride* M3, *Schizophyllum commune* M8, and *Pestalotiopsis* sp. M12 were adopted as an indicator of the biological pretreatment of WH and WL and to be used for the fermentation process to produce bioethanol. The current study focuses on the utilization of biological pretreatment using *Trichoderma citrinoviride*, *Schizophyllum commune*, and *Pestalotiopsis* sp. on WH and WL substrates for bioethanol production, which has not been explored before. However, large-scale use leads to high operating costs as the pretreatment is carried out under sterile conditions, thus increasing the processing costs. Therefore, biological pretreatment is too slow and not recommended for industrial purposes (Chaturvedi and Verma, 2013).

Another bottleneck is the feedback inhibition of cellobiose in the fermentation process after hydrolysis during bioethanol production (Cheng et al., 2015). The most effective method to solve the feedback inhibition problem is simultaneous saccharification and fermentation (SSF) where an enzymatic process hydrolyzes lignocelluloses to sugars and ferments to bioethanol simultaneously, which has been used in many lignocelluloses fermentation systems (Huang et al., 2013; Soares and Gouveia, 2013). To date, the SSF process using *Trichoderma citrinoviride*, *Schizophyllum commune*, and *Pestalotiopsis* sp. with yeast (Saccharomyces cerevisiae) on WH and WL substrates for bioethanol production has yet to be discovered.

1.3 Research Objectives

The objectives of this study are as follow:

a) To determine the critical harvesting time of aquatic plants (water hyacinth and water lettuce) based on the correlations among biomass growth, nutrient removal rate, and sugar content from domestic wastewater in the batch system.

b) To identify the isolated white-rot fungi collected from nature that can degrade the lignocellulosic biomass of water hyacinth and water lettuce.

c) To determine the efficiency of the lignocellulosic degradation rate of biological pretreatment of aquatic plants (water hyacinth and water lettuce) using isolated white-rot fungi.

d) To evaluate the efficiency of recombinant isolated white-rot fungi and yeast (*Saccharomyces cerevisiae*) using simultaneous saccharification and fermentation (SSF) modes from aquatic plants (water hyacinth and water lettuce) to produce bioethanol.

1.4 Scope of Study

The scope of this study entails evaluating the ability of WH and WL to remove nutrient pollutants from Desa Bakti domestic wastewater that lead to the production of a high amount of sugar, which is a crucial parameter for the simultaneous saccharification and fermentation process to produce bioethanol. The two floating aquatic plants (WH and WL) were placed in a tank of a batch system and one tank without plants to serve as a control. The plants were grown in a tank using a batch flow system. The wastewater samples were collected from the tank, and the plants were harvested from the tank with 3 days of interval till the plants became matured and reached the highest growth of plants. The efficiency of biomass growth and nutrient removal were carried out based on of phosphate (PO_4^{3-}), nitrate (NO_3^-), and nitrite (NO^-_2) and were compared to determine the best harvested time of WH and WL to produce the highest sugar content for the phase.

12 types of fungi were collected from UTM tropical rainforest. Fungi samples were cultivated and maintained on potato dextrose agar (PDA) and observed at the time interval of 7 days by measuring the diameter of fungal colonies. The screening method was used to test fungi based on speed to choose the preferable fungi for the degradation of aquatic plants. White-rot fungi were identified using 18S rRNA sequence and microscopic observation. The best preferred white-rot fungi were used further for biological pretreatment to degrade lignocellulosic biomass of WH and WL in order to determine the efficiency of isolated white-rot fungi based on the highest sugar content for bioethanol production. The lignocellulosic components and the sugar content of WH and WL were estimated using the Klason Method and Dinitrosalicyclic (DNS) Method, respectively.

The determination of bioethanol production from WH and WL as carried out using the simultaneous saccharification and fermentation (SSF) method. This method conducted the enzymatic hydrolysis and fermentation process simultaneously. The significant advantage of SSF is the released sugar will be instantly consumed by the fermenting agent (yeast), hence indirectly reducing the inhibition of the enzyme due to the low sugar concentration for the bioethanol production inhibition. This study evaluated the feasibility of using monoculture and co-cultures of isolated white-rot fungi and yeast (S. cerevisiae) on WH and WL biomass for bioethanol production. The bioethanol production was determined using Gas Chromatography (GC).

1.5 Significance of the Study

The significance of this study entails the ability of abundant WH and WL as substrates for bioethanol production. Simultaneously, they were used as bioindicators for the phytoremediation process, offering an alternative way to replace the high-cost treatment by utilizing the white-rot fungi in nature to degrade lignocellulosic biomass for bioethanol production. Moreover, in the current study, reducing waste wealth is significant to substitute fossil fuels with more environmentally friendly lignocellulosic biomass. The findings would be beneficial for future investigation in exploring the potential production of bioethanol from phytoremediation technology systems. As such, this study is significant in several ways:

(a) The aquatic plants that possess the potential for phytoremediation and bioethanol production can ideally be used to redeem wastewater pollutants. After the phytoremediation process, the aquatic plants' biomass can be utilized as a raw material for bioethanol production.

(b) The ability of fungi to degrade lignocellulose biomass of WH and WL due to their highly effective enzymatic system.

(c) Biological pretreatment using white-rot fungi in nature for bioethanol production from biomass is a promising technology due to its numerous advantages, such as its environmentally friendly benefits and economically viable strategy for enhancing the enzymatic saccharification rate.

(d) Studies on the fungal degradation of lignocellulosic material together with yeast as fermenters could yield promising candidate strains that could subsequently be used for bioethanol production.

(e) Bioethanol can reduce the emissions released when it is burned as a fuel. Therefore, sustainable energy efficiency is essential in addressing the high demand for future low-carbon strategies supported by renewable resources.

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LIST OF PUBLICATIONS

Journal

1. **Zainuddin, N.A**., Md Din, M.F., Ponraj, M., Hassim, M.H., Mat Taib, S., Sanjaya, E.H. (2022). Biodegradation Efficiency of Fungi for Lignocellulosic Biomass of Water Hyacinth (Eicchornia crassipes). *International Journal of Advanced Research in Science, Communication and Technology*. 2(8): 46-56.
2. **Zainuddin, N.A**., Md Din, M.F., Nuid, M., Abdul Halim, K., Salim, N.A.A., Elias, S.H., and Mat Lazim, Z. (2022). The Phytoremediation using Water Hyacinth and Water Lettuce: Correlation between Sugar Content, Biomass Growth Rate, Nutrients. *Jurnal Kejuruteraan*. 34(5): 915-924.
3. Krishnan, S., Ahmad, M.F., **Zainuddin, N.A.,** Md Din, M.F., Rezania, S., Chelliapan, S., Mat Taib, S., Nasrullah, M. And Wahid, Z.A. (2020). Bioethanol production from lignocellulosic biomass (water hyacinth): a biofuel alternative. *Bioreactors: Sustainable Design and Industrial Applications in Mitigation of GHG Emissions*. 123-143.

Book Chapter

1. **Zainuddin, N.A.,** Md Din, M.F. and Aminudin, E. (2020). Water hyacinth (eichhornia crassipes) and water lettuce (pistia stratiotes L.) as nutrient uptake from domestic wastewater. *Issues and Technologies in Water Contaminants*. UTM Press. Universiti Teknologi Malaysia.
2. **Zainuddin, N.A**., Md Din, M.F., Hassim, M.H. and Mat Taib, S. (2022). Application of floating aquatic macrophytes for Phytoremediation of wastewater. *Water and Wastewater Treatment Technology*. UTM Press. Universiti Teknologi Malaysia.