IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

Biodegradation Efficiency of Fungi for Lignocellulosic Biomass of Water Hyacinth (*Eichhornia crassipes*)

Nur Azmira Zainuddin[1], Mohd Fadhil Md Din[2], Mohanadosss Ponraj[3*], Mimi Haryani Hassim[4], Shazwin Mat Taib[2], Eli Hendrik Sanjaya[5]
School of Civil Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Skudai, Malaysia[1]
Centre for Environmental Sustainability and Water Security (IPASA),
Research Institute of Sustainable Environment (RISE), School of Civil Engineering, Faculty of Engineering,
Universiti Teknologi Malaysia, Skudai, Malaysia[2]
Department of Biological Sciences, School of Mathematics and Natural Sciences,
The Copperbelt University, Riverside, Jambo Drive, P. O. Box: 21692, Kitwe, Zambia[3]
School of Chemical and Energy Engineering, Faculty of Engineering
Universiti Teknologi Malaysia, Skudai, Malaysia[4]
Department of Chemistry, State University of Malang (Universitas Negeri Malang), Indonesia[5]
***Corresponding Author:** Mohanadoss Ponraj**, Email:** goldking1977@gmail.com

Abstract: *Nowadays, renewable energy has become alternative energy to reduce consumption of fossil fuels. Lignocellulosic materials such as crop residues, grass, and aquatic macrophytes that does not compete with food supply have become potential renewable sources of bioethanol. In this study, water hyacinth was selected as potential resource because it is abundant in nature and can be easily propagated and cultivated to be used as substrate. This study is emphasized on the biodegradation of water hyacinth using white rot fungi collected from decayed wood and soil. Trichoderma citrinoviride M3, Schizophyllum commune M8 and Pestalotiopsis sp M12 were selected in degrading water hyacinth substrate. These fungal are further used for degradation of lignocellulosic materials contained in the water hyacinth. Based on the result, all fungi caused the deliginification on water hyacinth. Selectivity value (SV) was used as an indicator for the selective lignin-degrading fungal as SV more excellent than 1.0 was considered a function of delignification efficiency. Trichoderma citrinoviride M3 had the highest degrading ability of cellulose, hemicellulose, and lignin, followed by Schizophyllum commune M8 and Pestalotiopsis sp. M12. Hence, Trichoderma citrinoviride M3 had the highest value of SV, 1.01 (>1.0), and recognizes as a lignin-degrading fungus for water hyacinth biomass.*

Keywords: Lignocellulosic biomass; Water hyacinth; Biodegradation; Delignification; Fungi; Bioethanol

I. INTRODUCTION

Higher demand for energy and concern for the environment are the crucial factors leading to renewable energy sources. Energy demand in Malaysia is constantly increasing due to the advancements in the industrial and transportation sectors (Tye et al., 2011). The global depletion of energy resources due to energy consumption and environmental degradation caused by the use of fossil fuels has prompted research into alternative renewable energy sources. As a result, the demand for petroleum-derived fuels is not decreasing, but has increased sharply in recent decades (Shamsuddin, 2012). However, the challenges in finding a long-term solution for a reliable and infinite source of clean energy supply in the future are

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

enormous. 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 with the intention of promoting the prosperity and sustainable development of the country. The Sustainable Energy Development Authority (SEDA) proposed National Renewable Energy Policy has set an ambitious target by aiming for 20% of the capacity mix to be renewable energy sources in order to reduce greenhouse gas emissions and create a more conducive environment (Abdullah et al., 2019). Therefore, renewable bioethanol becomes a potential energy source for Malaysia's future renewable energy development prospects for the country.

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 carbonaceous feedstocks such as sugarcane, corn and starchy materials. Recently, however, there has been interest in the low-cost, readily available, and abundant lignocellulosic biomass that could serve as a potential feedstock for bioethanol production (Wyman et al., 2019). Lignocellulosic biomass, which includes agricultural and forestry residues and wastes, has the advantage of providing a more excellent choice of potential feedstocks that do not conflict with land use for food production and is cheaper than conventional bioethanol sources. Many researchers around the world are working hard to convert lignocellulosic biomass such as straw and other plant wastes into bioethanol production. Although the biological conversion of lignocellulosic biomass into monomeric sugars is more complicated than the conversion of starch 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).

Bioethanol production is seen as an alternative fuel for the future as fossil fuel reserves have been depleted. Ethanol production requires the use of lignocellulosic biomass as feedstock, which has many advantages in terms of energy and environmental concerns (Aswathy et al., 2009; Singh et al., 2012; Menon and Rao, 2012). However, the main problem in converting biomass to fuel is substrate cost and conflict of interest in agricultural products. For example, growing sugarcane for bioethanol can increase the price of sugar production and tighten food supplies. Hence, it is important to realize the potential of floating aquatic plants, which are rich in fermentable carbohydrates, as they are abundant and cheaper than others in Malaysia. Therefore, this study focused on the abundant floating lignocellulosic macrophyte of water hyacinth, which consist of significant components, namely lignin, cellulose and hemicellulose. The presence of high percentage of cellulose and hemicellulose in this aquatic plants is the main advantage for their use in bioethanol production (Maurya et al., 2015). The use of this floating aquatic plants as feedstock for bioethanol production can remove various pollutants and grow rapidly even under extreme conditions without requiring land area (Akinbile et al., 2012; Akinbile et al., 2016).

Water hyacinth (*Eichhornia crassipes*) is fast-growing invasive plants that can cover the entire water surface of a variety of habitats (Rezania et al., 2015; Arefin et al., 2021). In fact, water hyacinth is known for its reproductive potential and can double its reproduction within only 6-12 days. However, this floating aquatic macrophytes can disturb the underlying flora and fauna, ecosystem and aquatic activities and cause problems for navigation. However, the potential of utilizing this macrophyte for bioethanol production is feasible due to its high hemicellulose content and low lignin content (Arefin et al., 2021). Therefore, attention has been emphasized in exploring water hyacinth as substrates for bioethanol production in present and future works.

Another major problem in the production of bioethanol from lignocellulosic material is the low solubilization of the cellulosic and hemicellulosic components during the hydrolysis process. During this process, the sugar is converted for bioethanol production. Therefore, pretreatment is essential to break down the lignocellulosic components and improve the cost-effective hydrolysis process (Eshtigahi et al., 2012). In the conversion of lignocellulosic material into bioethanol, the use of chemicals in the pretreatment method is one of the major problems in terms of cost and hazardous effects on the environment. Due to environmental concerns, a biological pretreatment using particular white rot fungi was adopted in this study to produce bioethanol. Biological pretreatment is considered a cost-effective method compared

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

to other pretreatment methods such as acidic or alkaline pretreatment. However, large-scale use leads to high operating costs as the pretreatment is carried out under sterile conditions, which increases the processing costs. Therefore, the process of biological pretreatment is too slow and is not recommended for industrial purposes (Chaturvedi and Verma, 2013).

Since the cost of lignocellulosic substrates plays a key role in determining the economics of the delignification process, previous research has focused on the use of low-cost substrates. In contrast, this study investigated the potential of floating aquatic plant which is water hyacinth as an abundant plant. Thie aquatic plants are composed of cellulose, hemicellulose and lignin linked by covalent cross-links and non-covalent forces. The high content of cellulose and hemicellulose is the main advantage for bioethanol production. However, resistance to enzymatic hydrolysis is a major limitation in the conversion of lignocellulosic biomass into fermentable sugars. 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 a lot of energy, and produce inhibitors that interfere with enzymatic hydrolysis and fermentation (Abo et al., 2019). Therefore, biological pretreatment using metabolites of a microorganism in nature for ethanol production from biomass is a promising technology due to its numerous advantages, such as an environmentally friendly and economically viable strategy to increase the enzymatic saccharification rate. Several studies indicated that fungal pretreatment could improve hydrolysis efficiency, resulting in energy consumption limitation. This study investigates the suitability of naturally occurring fungal species for the biodegradation process of lignocellulosic plants of water hyacinth as biomass substrate. Since no chemicals are used in this process, no recycling of chemicals is required and no toxic compounds are released into the environment. Therefore, biological pretreatment with certain white-rot fungi has been used, which seems to be promising as an environmentally friendly process. In contrast, no inhibitors are generated during the pretreatment process. Therefore, in this study, *Trichoderma citrinoviride* M3, *Schizophyllum commune* M8 and *Pestalotiopsis* sp M12 were introduced to degrade the lignocellulosic biomass of water hyacinth subtrate. It is also crucial to replace polluting fossil fuels with more environmentally friendly lignocellulosic biomass. Thus, bioethanol can reduce the emissions that are released when it is burned as a fuel. Therefore, sustainable energy efficiency plays an essential role in addressing the high energy demand for future low-carbon strategies supported by renewable resources.

II. METHODOLOGY

2.1 Experimental Design

A pilot fabrication tank system was constructed near the contaminated oxidation pond in Skudai, Johor, Malaysia as shown in Fig. 1. The fabricated pilot tank system consist of five tanks with dimension of 48 x 90 x 90 cm, holding capacity of 380 liters interconnected through PVC pipes and equipped with a pump (Fig. 2).

Figure 1 Contaminated Oxidation Pond

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

The pump is switched on until the water in each tank is overflown. Then, the valve between each tank is closed. The water hyacinth was placed in all the tanks except in the control tank.

Figure 2 Pilot Plant Fabrication Tank System

2.2 Preparation of Biomass

Fresh water hyacinth was harvested and washed thoroughly with tap water to remove adhering dirt and other particles. The plant was split into leaves, roots, and rhizomes and further chopped into small pieces (approximately 1~2 cm) and dried in a hot air oven at 105°C until it reached a constant weight. Next, the dried biomass was blended using a home-mixer and passed through 850 µm sieve. Then, the dried biomass was kept in airtight container at room temperature until further use. The preparation process is shown in Fig.3.

Figure 3 The preparation of water hyacinth A: Harvesting of water hyacinthplants; B: Separation of leaves and rhizome; C: Dried biomass

2.3 Sampling of Fungi

Three fungi species were collected from soil and decayed wood at the recreation forest of Skudai, Johor. The fungi were labelled in sealed plastic bags as M3, M8 and M12 and stored in the freezer to preserve their growth before use. Hence, the apparatus was soaked with 10.0% nitric acid overnight to remove all the contaminants that adhere to glassware surfaces, rinsed with distilled water, and dried in the oven at 80°C.

2.3 Cultivation of Fungi on Agar Medium

The fungal strains of M3, M8 and M12 were isolated and cultured in potato dextrose agar (PDA) (39 g/L) medium. The agar medium was sterilized in autoclave at 121°C for 15 min. After that, 20 mL of PDA medium was moved into petri dish. The fungal tissue was cut and cultured in agar medium in petri dish and incubated for 5 to 7 days to have a similar radial growth rate (Hadibarata and Tachibana, 2009).

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

2.4 Fungi Strain Identification

The fungi species on the PDA medium were rapidly growing within seven days of incubation time. The morphology of mycelia of fungi species observes using a microscope (Biolux-12; Kyowa). The one plug of actively growing culture of mycelia fungus was transferred onto a microscope slide, and the mycelia observation was captured. The same procedure applies to all the best three species of fungi. The identification of fungi species was made via a PCR analysis (submitted to a PCR analysis). First, the DNA of the fungal tissue was extracted using Wizard® 47 Genomic DNA Purification Kit (Promega, USA, Cat No: A1120). PCR process involving Taq polymerase, dNTPs mix, and DNA template. Then, several steps had to be carried out: thermal cycling, which consists of cycles of heating and cooling. The process reaction was performed as follow: 1 cycle at 94°C for 30s, 50°C for 30 s, and 72°C for 2 min and ended with 1 cycle at 72°C for 10 min. The sequence was further compared with the known gene sequence, 18S rDNA from the Basic Local Alignment Search Tool (Blast) on National Center for Biotechnology (NCBI) Genbank database (Hadibarata et al., 2012). The phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis 5 (MEGA5) based on the neighbor-joining method.

2.5 Biodegradation of Lignocellulosic Biomass

Biodegradation of lignocellulosic biomass of water hyacinth was conducted for a period of 30 days interval time. 5 g of dried biomass samples and 20 mL of distilled water in 100 ml of the flask was mixed. A drop of Tween 80 (Gupta et al., 2010) to enhance the growth of fungi and 10 mg of glucose was added to the substrate. The sample was further sterilized at 121°C for 30 min. Then, agar plugs of each fungi on agar medium plates were punched using cork borer and put into the substrates, covered with cotton and aluminum foil and incubated in a dark chamber cabinet for undergoing 30 days of biodelignification. Each test was conducted in triplicates as to compare with the control (non-inoculated samples).

Samples were taken every ten days for a period of 30 days in order to do the composition analysis of lignin-hemicellulose-cellulose and sugar content. The collected sample was mixed with 100 mL of distilled water, and filtered using whatman No. 1 filter paper (0.45 µm pore size) in order to remove all the suspended solids. The solids retained on the filter paper was dried until constant weight was obtained for the determination of lignin, hemicellulose, and cellulose.

2.5 Determination of Lignin

According to Klason Method, 1g of the sample was soaked with 5 mL of concentrated acid, 72% (v/v) sulfuric acid for 2 hr. Next, the sample was turned into a black color due to the reaction of the concentrated acid with the plant structure. Then, the solution was diluted with distilled water to 3% (v/v) acid concentration. The diluted solution was refluxed for 4 hr at 105°C. The diluted solution was filtered with 0.45 µm filter paper after it cooled down to room temperature. The solid remained on filter paper was transferred into an oven at 105°C until constant weight. Then, the sample was weighed as A and was further ignited in the furnace for 3 hr at 575°C. After that, the sample was weighed after the sample was cooled down to room temperature and recorded as B. The content of lignin was measured as follows:

$$Acid\ Insoluble\ Lignin\ (\%) = \frac{A-B}{Initial\ weight}\ x\ 100\% \qquad (2.1)$$

2.6 Determination of Hemicellulose and Cellulose

For determination of hemicellulose and cellulose contents, 1 g of sample (A) was weighed and mixed with 150 mL of distilled water and was refluxed for 1 hr at 100°C. Then, it was filtered with 0.45 µm filter paper, and the solids retained were transferred into the oven at 105°C until constant weight (B). The reducing weight of sample was measured in percentage (%). Then, 5% (v/v) sulfuric acid was added to the sample. The mixture was transferred into water bath with temperature of 100°C for 1 hr. The sample was further mixed with 300 mL distilled water and was filtered with 0.45

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

μm filter paper. The solids retained were transferred into the oven at 105°C until constant weight (C). Next, 10 mL of 72% (v/v) sulfuric acid was added to a dried sample and soaked at room temperature for 4 hr. Then, it was mixed with 400 mL of distilled water and was filtered with 0.45 μm filter paper, whereas the solids remained on the filter paper were transferred into the oven at 105°C until constant weight (D). Therefore, the determination of hemicellulose and cellulose content of the sample as the following formula:

$$Hemicellulose, \% = \frac{B-C}{A}\ x\ 100\% \quad (2.2)$$

$$Cellulose, \% = \frac{C-D}{A}\ x\ 100\% \quad (2.3)$$

2.7 Analysis

The compositional analysis for water hyacinth biomass was measured as follows, whereas all procedures were performed in duplicate to ensure the results were producible and accurate. Every ten days within 30 days, the sample was taken out for lignin, hemicellulose, and cellulose composition of samples determination. The acid-insoluble lignin or Klason's lignin content was determined using a slightly modified version of the corresponding TAPPI test method (2002). Meanwhile, as defined by Maryana et al. (2014), a modified procedure was used as the reference method for the hemicellulose and cellulose determination of the substrate.

III. RESULTS AND DISCUSSIONS

The presence of cellulose, hemicellulose, and lignin content was evaluated for the biodegradation of lignocellulosic biomass obtained from the water hyacinth plant.

3.1 Identification of Fungi

The result was obtained from FIRST Base Laboratory Sdn. Bhd. by sent out freshly pure cultured fungi on agar medium. This phylogenetic analysis was referred to with known fungi from the NCBI Genbank database. It shows M3 belongs to the *Trichoderma citrinoviri*de strain. Meanwhile, M8 belongs to the *Schizophyllum commune,* and M12 belongs to *Pestalotiopsis sp.* These three fungi were ascomycete, a division of the fungi kingdom and from the subkingdom of dikarya. The ascus is known as a fungi sac and microscopic sexual structures from ascospores. Other than that, microscopic structures such as hyphae were formed when enough nutrient supply for M3, M8, and M12. The hyphae were then formed mycelium to biodegrade lignocellulosic biomass.

3.2 Lignocellulosic composition of Raw Water Hyacinth

It was found that raw water hyacinth consisted of 18.48% cellulose, 47.66% hemicellulose, and 15.63% lignin during this study. Madian et al. (2018) found that untreated water hyacinth had 31.71% cellulose, 19.08% hemicellulose, and 3.9% lignin. Whereas, Zhang et al. (2015) and Das et al. (2016) have reported that raw water hyacinth had 18.07 ± 0.2 and 31.44% cellulose, 28.21 ± 0.11%, and 44.68% hemicellulose and 7.03 ± 0.09% and 19.99% lignin respectively. Cheng et al. (2014) demonstrated that the raw water hyacinth had 24.15% cellulose, 27.23% hemicellulose, and 12.39% lignin. These differences might originate from the growth state of biomass (Ma et al., 2010), different sources (Carina and Cecilia, 2007), and drying methods of lignocellulosic biomass (Singh and Bishnoi, 2013). Other harvesting times and nutritional conditions in the plant habitat also contribute to the differences in biomass composition (Gunnarsson and Petersen, 2007; Reales-Alfaro et al., 2013).

3.2.1 Lignin

Lignin is an essential component found in plant cell walls that limits the biodegradation process by particular fungi. The reduction of lignin content leads to the exposure of the crystalline structure of cellulose and hemicellulose and consequently will release fermentable sugar for bioethanol production (Sun and Cheng, 2002). Based on the results of

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

the current study, all fungi significantly increased the loss of lignin in water hyacinth substrate within 30 days of incubation. The highest degradation occurred after the loss of lignin reached 10.57% from the starter to 15.63% in water hyacinth substrate by *Trichoderma citrinoviri*de strain. M3 followed by *Schizophyllum commune* M8 (11.78%) and *Pestalotiopsis sp* M12 (12.03%), as shown in Fig. 4. The results are in line with previous studies in which white-rot fungi evaluated lignocellulosic biomass, whereas lignin degradation was attributed to the maximum production of ligninolytic enzymes after 15 days incubation (Singh et al., 2011; Deswal et al., 2014).

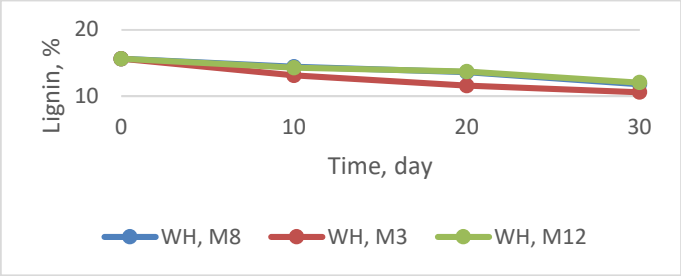

Figure 4 Lignin degradation trends for water hyacinth

3.2.2 Cellulose

Besides the delignification, the availability of cellulose is also an essential criterion for evaluating the efficiency of biological pre-treatment, which will be able to convert fermentable sugar through fermentation process as ideal substrate for the production of bioethanol (Deswal et al., 2014). Fig. 5 shows the biodegradation of cellulose content in water hyacinth obtained from *Trichoderma citrinoviri*de strain. M3, *Schizophyllum commune* M8, and *Pestalotiopsis sp* M12 for a period of every (10 days) up to the final day (30 days). The results show that, water hyacinth started with the presence of 18.48% cellulose content and was degraded to 10.51% cellulose content by *Trichoderma citrinoviri*de strain. M3, which is the highest degradation, followed by 16.32% and 17.54% of cellulose content with *Schizophyllum commune* M8 and *Pestalotiopsis* sp M12. The results show that when the degradation time increases, delignification and hemicellulose removal become intensified, which improved the cellulose's digestibility (Nazarpour et al., 2013; Ghasemzadeh et al., 2018). The results obtained are well within the range of composition as reported in the previous studies, where hemicellulose shows the degrading by 59-61% (Velmurugan & Muthukumar, 2011).

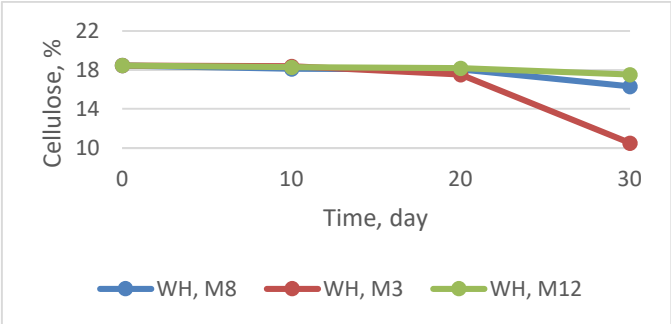

Figure 5 Cellulose degradation trends for water hyacinth

3.2.3 Hemicellulose

The biodegradation of hemicellulose content in water hyacinth using the fungal strains of *Trichoderma citrinoviri*de strain. M3, *Schizophyllum commune* M8, and *Pestalotiopsis sp* M12 shown in Fig. 6. It is found that, all fungal species offer similar hemicellulose degradation patterns, which gradually decreased using water hyacinth as substrate. Based on the obtained results, water hyacinth started with 47.66% hemicellulose content and was degraded to 36.45% by

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

*Trichoderma citrinoviri*de strain. M3. Meanwhile, *Schizophyllum commune* M8 degraded 42.15%, a lesser degradation rate than *Trichoderma citrinoviri*de strain. M3 was observed. However, *Pestalotiopsi*s *sp* M12 recorded the lowest degradation rate of hemicellulose of water hyacinth, with 44.35%. In the previous study, it is reported that hemicellulose breaks down occurred more quickly than cellulose because it is found to have branches with short lateral chains of different sugars (Nadir et al., 2019). The current finding aligns with the present study results, whereas the percentage of hemicellulose is found higher than cellulose in the water hyacinth biomass substrate.

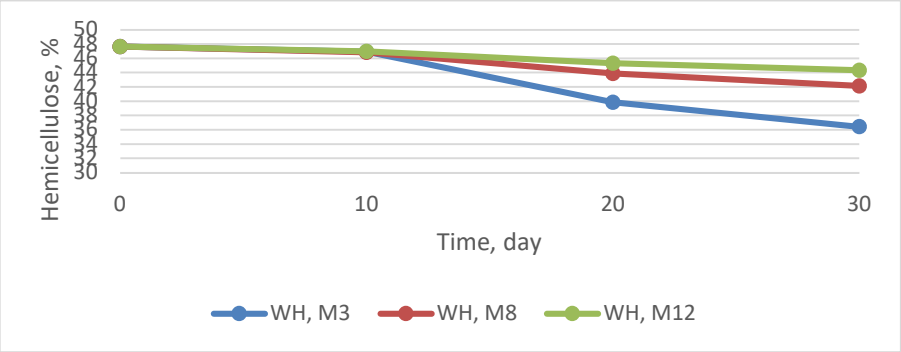

Figure 6 Hemicellulose degradation trends for water hyacinth

3.3 Biodegradation of lignocellulosic Biomass

Lignocellulosic components that represent about 80% of the dry weight of the plant biomass consist mainly of the significant amount of cellulose, hemicellulose, and lignin that is strongly bond with each other by non-covalent forces as well as covalent cross-links. Table 1 shows the weight and component loss of water hyacinth by *Trichoderma citrinoviri*de strain. M3, *Schizophyllum commune* M8, and *Pestalotiopsi*s *sp* M12 within 30 days. Different fungal species vary significantly in their capabilities to degrade lignin, hemicellulose, and cellulose in the plant cell wall (Deswal et al., 2014). Based on the results, all components declined over time. For the selective lignin-degrading fungal, a high selectivity value (SV) greater than 1.0, which was considered a delignification efficiency function (Hakala et al., 2004). Selectivity value is the ratio of lignin degradation to cellulose reduction, used to evaluate the selective lignin-degrading ability (Ghasemzadeh et al., 2018). Based on the result, all fungi caused the delignification on water hyacinth substrate. *Trichoderma citrinoviri*de strain. M3 had the highest degrading ability of cellulose, hemicellulose, and lignin on water hyacinth, followed by *Schizophyllum commune* M8 and *Pestalotiopsi*s *sp* M12. Hence, *Trichoderma citrinoviri*de strain. M3 had the highest value of SV, which is 1.01 (>1.0), and recognized as efficient lignin-degrading fungus for water hyacinth substrate biomass.

Till date, among the known species of fungi, the highest efficiency of biodegradation of lignocellulosic biomass belongs to *P. chrysosporium* with up to 31.9% lignin degradation, which is frequently used in many studies due to high growth rate and lignin biodegradation capabilities within the short incubation time (Chen et al., 2010). However, due to a selectivity value less than 1, it can simultaneously degrade cellulose and lignin. It defines that cellulose might be hydrolysed and consumed by P. chrysosporium during the pre-treatment of lignocellulosic biomass (Wan and Li, 2012). Therefore, the selectivity of fungi regarding lignin degradation depends on the lignocellulosic substrate, cultivation time, and other factors (Hakala et al., 2004; Hatakka and Hammel, 2010). The present results of this study are in accordance with the previous reports on the biodegradation of lignocellulosic biomass by variable fungal species (Gupta et al., 2010). *Pleurotus* sp. was reported to degrade 8.5% lignin of water hyacinth within 22 days (Mukherjee and Nandi, 2004). As shown by Kancelista et al. (2013), *Trichoderma* fungi strain is a group of hydrolytic enzymes that play an important role in the biodegradation of lignocellulosic biomass of cellulose, hemicellulose, and lignin. The degradation of substrate weight loss, lignin, cellulose, and hemicellulose increased with incubation time. Therefore, it can be directly correlated with the lignin-degrading ability via these three fungi used in the current experiment of this study.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

Table 1: Component losses of water hyacinth after 30 days incubation

Biomass Substrate	Fungi Species	Selective Value	Lignocellulosic Components Losses (%)			
			Cellulose	Hemicellulose	Lignin	Biomass Weight
Water Hyacinth	*Trichoderma citrinoviride* strain. M3	1.03	10.21	36.45	10.57	8.52
	Schizophyllum commune M8	0.72	16.32	35.79	11.78	7.52
	Pestalotiopsis sp M12	0.68	17.54	44.35	12.03	5.01

IV. CONCLUSION

Raw water hyacinth was found to be have 18.48% cellulose, 47.66% hemicellulose, and 15.63% lignin in this study. The three fungal species of *Trichoderma citrinoviride*, *Schizophyllum commune*, and *Pestalotiopsis* sp. were identified before evaluation was made based on the performance of biodegradation of lignocellulosic biomass of water hyacinth plant. It was found that, all fungi used in the study was able to cause delignification of water hyacinth substrate. Selectivity value (SV) was used as an indicator for the selective lignin-degrading fungi as SV more excellent than 1.0 was considered as a function of delignification efficiency. *Trichoderma citrinoviride* M3 showed highest degrading ability of cellulose, hemicellulose, and lignin on water hyacinth, followed by *Schizophyllum commune* M8 and *Pestalotiopsis* sp. M12. Therefore, *Trichoderma citrinoviride* M3 had the highest value of SV, 1.01 (>1.0), and recognized as efficient lignin-degrading fungus for water hyacinth biomass. As a result, during this study more attention has been emphasized in exploring *Trichoderma citrinoviride* M3 as a potential of good lignin-degrading fungi for future works.

REFERENCES

[1]. Arefin, M. A., Rashid, F. and Islam, A. (2021). A review of biofuel production from floating aquatic plants: an emerging source of bio-renewable energy. *Biofuels Bioproducts Biorefining*. 15(2): 574-591.

[2]. Carina,C.G. and Cecilia, M.P. (2007). Water hyacinth as a resource in agriculture and energy production: A literature review. *Waste Management*. 27:117-129.

[3]. Chen, S., Zhang, X., Singh, D., Yu, H. And Yang, X. (2010). Biological pretreatment of lignocellulosics: potential, progress and challenges. *Biofuels*. 1-177-199.

[4]. Cheng, J., Wang, X., Huang, R., Liu, M., Yu, C. And Cen, K. (2014). Producing ethanol from water hyacinth through simultaneous saccharification and fermentation with acclimatized yeasts. *Bioresoource*. 9(4):7666-7680.

[5]. Chesson, A. (1987). The maceration of linen flax under anaerobic conditions. *J. Appl. Bacteriol.* 45:219-230.

[6]. Das, S., Bhattacharya, A., Haldar, S., Ganguly, A., Gu, S., Ting, Y.P. and Chatterjee, P.K. (2015). Optimization of enzymatic saccharification of water hyacinth biomass for bio-ethanol: Comparison between artificial neural network and response surface methodology. *Sustainable Materials and Technologies*. 3:17-28.

[7]. Das, S., Ghosh, P., Paul, T., Ghosh, U., Pati, B.R. and Mondal, K.C. (2016). Production of bioethanol as useful biofuel through the bioconversion of water hyacinth (Eichhornia crassipes). *3 Biotech*.

[8]. Deswal, D., Gupta, R., Nandal, P. and Kuhad, R.C. (2014). Fungal pretreatment improves amenability of lignocellulosic material for its saccharification to sugars. *Carbohydr. Polym*. 99:264-269.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

[9]. Ghasemzadeh, R., Mosavian, M.T.H. and Karimi, A. (2018). Analysis of biological pretreatment rapeseed straw with white rot fungi for enzymatic hydrolysis. *Maderas Cienc. Technol*. 20(4):725-736.

[10]. Gunnarsson, C.C. and Petersen, C.M. (2007). Water hyacinth as a resource in agriculture and energy production. A literature review. *Waste Management*. 27(1): 117-129.

[11]. Gupta, R., Mehta, G., Khasa, Y.P. and Kuhad, R.C. (2010). Fungal delignification of lignocellulosic biomass improves the saccharification of cellulosics. *Biodegradation*. 22(4):797-804.

[12]. Hadibarata, T. and Tachibana, S. (2009). Microbial degradation of crude oil by fungi pre-grown on wood meal. *Interdisciplinary Studies on Environmental Chemistry*. 317-322.

[13]. Hakala, T.K., Maijala, P., Konn, J. and Hatakka, A. (2004). Evaluation of novel wood-rotting polypores and corticioid fungi for the decay and biopulping of Norway spruce (picea abies) wood. *Enzyme and Microbial Technology*. 34(3-4):222-263.

[14]. Hatakka, A. and Hammel, K.E. (2010). Fungal biodegradation of lignocelluloses. *Industrial Applications*. 2: 319-340.

[15]. Kancelista, A., Janiewicz, U., Stempniewicz, Piegza, M. (2013). Application of lignocellulosic waste materials for the production and stabilization of Trichoderma biomass. *Polish Journal of Environmental Studies*. 22(4): 1083-1090.

[16]. Ma, F., Yang, N., Xu, C., Yu, H., Wu, J. and Zhang, X. (2010). Combination of biological pretreatment with mild acid pretreatment for enzymatic hydrolysis and ethanol production from water hyacinth. *Bioresource Technology*. 101(24):9600-9604.

[17]. Madian, H.R., Sidkey, N.M. Abo Elsoud, M.M., Hamouda, H.I. and Elazzy, A.M. (2018). Bioethanol production from water hyacinth hydrolysate by Candida tropicalis Y-26. *Arabian Journal for Science and Engineering*. 44:33-41.

[18]. Maryana, R. Ma'rifatun, D., Wheni, A. Satriyo, K.W. and Rizal W.A. (2014). Alkaline pretreatment on sugarcane bagasses for bioethanol production. *Energy Procedia*. 47: 250-254.

[19]. Maurya, D.P.M., Singla, A. and Negi, S. (2015). An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. *3 Biotech*. 5:597-609.

[20]. Mukherjee, R. and Nandi, B. (2004). Improvement of in vitro digestibility through biological treatment of water hyacinth biomass by two Pleurotus species. *International Biodeterioration and Biodegradation*. 53(1):7-12.

[21]. Nadir, N., Ismail, N.L. and Hussain, A.S. (2019). Fungal pretreatment of lignocellulosic materials. *IntechOpen*.

[22]. Nazarpour, F., Abdullah, D.K. and Abdullah, N. (2013). Evaluation of biological pretreatment of rubberwood with white rot fungi for enzymatic hydrolysis. *Materials*. 6(5):2059-2073.

[23]. Reales-Alfaro, J.G., Trujillo, L.T. Arzuaga, G., Castano, H. and Polo, A. Acid hydrolysis of water hyacinth to obtain fermentable sugars. CT&F Cienc. Technol. Futuro. 5(2):101-112.

[24]. Rezania, S., Ponraj, M., Talaiekhozani, A., Mohamad, S.E., Md Din, M.F., Taib, S. M., Sabbagh, F. and Sairan., F.M. (2015). Perspectives of Phytoremediation Using Water Hyacinth for Removal of Heavy Metals, Organic and Inorganic Pollutants in Wastewater. *Journal of Environmental Management*. 163: 125-133.

[25]. Sindhu, R., Binod, P. and Pandey, A. (2016). Biological pretreatment of lignocellulosic biomass – An overview. *Bioresource Technology*. 199: 76-82.

[26]. Singh, A. and Bishnoi, N. (2013). Comparative study of various pretreatment techniques for ethanol production from water hyacinth. *Ind. Crop. Prod*. 44:283-289.

[27]. Singh, D., Zeng, J., Laskar, D.D., Deobald, L., Hiscox, W.C. and Chen, S. (2011). Investigation of wheat straw biodegradation by Phanerochaete chrysosporium. *Biomass Bioenergy*. 35:1030-1040.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 2, Issue 8, May 2022
Impact Factor: 6.252

[28]. Sun, Y. and Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology*. 83:1-11.

[29]. Wan, C. and Li, Y. (2012). Fungal pretreatment of lignocellulosic biomass. Biotechnol. Adv. 30:1447-1457.

[30]. Zhang, Q., Weng, C., Huang, H., Achal, V. and Wang, D. (2015). Optimization of bioethanol production using whole plant of water hyacinth as substrate in simultaneous saccharification and fermentation process. *Frontiers in Microbiology*. 6:1411.