International Journal of Research
Available at https://pen2print.org/index.php/ijr
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 06 Issue 01
January 2019

Bioethanol from Water Hyacinth by White Rot Fungi in Biodegradation

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Abstract:

Bioethanol is one of the most potential liquid fuels since the natural resources from fossil fuels become limited. Nowadays renewable energy become as an alternative energy to reduce fossil fuels consumption. Therefore, the study of water hyacinth and water lettuce was chosen as potential source of biofuel due to its abundant and easy to cultivate in nature. The study aims to investigate the potential of floating aquatic macrophytes to provide enough supply for bioethanol production. Study on feasibility of biological pretreatment on water hyacinth and water lettuce using white rot fungi by monitoring lignin and hemicellulose biodegradation. The potential of bioethanol production was determined by sugar consumption rate and percentage of fermentable sugar by Saccharomyces cerevisiae yeast. The experiment was fabricated with two tanks contain macrophytes which Eichhornia crassipes and Pistia stratiotes. Weight of both species was recorded for consecutive three days to monitor the optimum growth rate. Both species were pretreated with white rot fungi. The parameters involved are sugar content, lignin by Klasson method and hemicellulose by Chesson method. Water hyacinth was fermented with different yeast concentration and DNS method was used for sugar determination. The results showed that P. stratiotes has higher growth rate than E. crassipes. However, E. crassipes more feasible for lignin and hemicellulose biodegradation compared to P. stratiotes. Sugar consumption rate was influenced by yeast concentration and fermentable sugar of water hyacinth filtrate which recorded up to 70%.

Keywords
Water hyacinth, Growth rate, lignin, Hemicellulose, Biodegradation, White rot fungi, Fermentation

Introduction

Bioethanol as an alternative energy source that has received special attention worldwide due to depletion of fossil fuels. It also has an important role to reduce global warming by reducing carbon emission from the burning process. Besides that, ethanol also can be used for electricity generation if in large quantity and continuous production. In India, sugar cane molasses is the main raw material for ethanol production while United Stated is using corn for ethanol production (Mino, 2010).

The conventional method for lignin degradation process is by using sulphuric acid under high temperature and pressure condition (Chaturvedi & Verma, 2013). This method involves more and high potential hazardous chemical constituents in the process. In terms to reduce chemical waste in production of bioethanol, an alternative method was created toward biological process rather than chemical treatments. The alternative method is lignin biodegradation such as white rot fungi lignin degradation process (Wan & Li, 2012). This method is safer and can reduce the sources of pollutants from the bioethanol production process. Besides, this method can also reduce the cost of production because chemical processing is quite costly.

The goal of this study was to study the growth rate of two species of macrophytes (*Eichhornia crassipes* and *Pistia stratiotes*) in identical condition of nutrients supply. It was to investigate feasibility of hemicellulose and lignin biodegradation by white rot fungi on water hyacinth and water lettuce. The degradation of both plant components can affect the potential of extractable sugar from the plants. Furthermore, it was to study the effect of yeast concentration on the rate of sugar consumption and limitation of sugar

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 06 Issue 01
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conversion to bioethanol by common *Saccharomyces cerevisiae*.

Water hyacinth is a common type of lignocellulosic plant with high breeding rate, which can survive and give advantage to sewage treatment plant such as waste stabilization pond. While enhancing the effectiveness of the treatment, the plant can produce ethanol that can use as car fuel. The characteristic of rapid growth of this plant is very important which it is possible to fulfill the demand of the daily usage in Malaysia. White rot fungi also has an abundance source that is good in lignin biodegradation. Biodegradation of lignin is very crucial in the process of maximizing the production of bioethanol. Production ethanol from water hyacinth will not disturb food production because it is not food resources to human. Besides that, using ethanol as car fuel will give good impact to environment by reducing carbon emission.

1. Literature review

Extraction of bioethanol from abundance sources, lignocellulosic biomass such as water hyacinth can reduce food supply consumption. The ability of water hyacinth to produce ethanol is due to its sugar content. Higher sugar content in plant indicates higher ability to produce bioethanol. Several pretreatment processes can be used in converting sugar to ethanol. This pretreatment process is very important to breakdown hemicellulose and lignin which can produce more sugar extraction. Usually, the pretreatment process is by using acid pretreatment which is easy and effective to breakdown the lignin (Chaturvedi & Verma, 2013). After the pretreatment, the pretreatment substance will go through fermentation process. The fermentation is to convert sugar to ethanol.

2.1 Bioethanol

Bioethanol fuel has an important role in the field of environment to migrate global warming and conserve fossil fuel. It is an alcohol made of carbohydrates by fermentation process. Production of bioethanol from biomass or waste is one way to reduce both consumption of crude oil and environmental pollution (Chandel et al., 2007). Lignocellulasic biomass (corn, sugar, molasses, etc.) derived from non-food sources, such as grasses and trees, are also being developed as a feedstock for ethanol production. The physical and chemical characteristic of Bioethanol is similar to ethanol; just their different are resources of production. Bioethanol in its purest form is a colourless clear liquid with mild characteristic odour that boils at 78°C and freezes at -112°C.

2.2 Lignocellulose

Lignocellulose refers to plant dry matter or biomass which is called as lignocellulosic biomass. These types of plant are the most abundantly available material on the earth in the production of biofuel, mainly bioethanol. Lignocellulosic biomass was used as sources of energy since 20th century ago. It showed an increase trend of interest in biomass as a precursor to liquid fuel mainly for bioethanol by fermentation of lignocelulosic biomass. This can be used as additive in the blending of petrol or gasoline for car fuels which can decrease the usage of fossil fuel. Lignocellulose is composed of carbohydrates polymers such as cellulose, hemicellulose and aromatic polymer (lignin).

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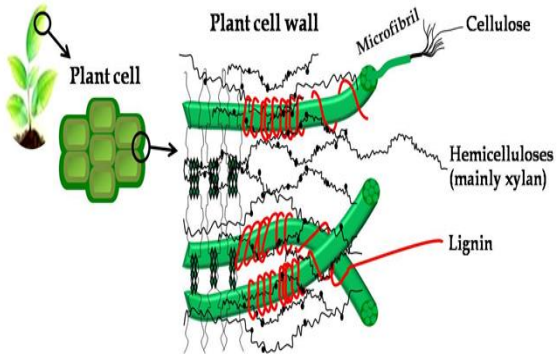

Figure 2.1 Lignocellulose contain Lignin, Cellulose and Hemicellulose
(Source: http://www.intechopen.com

Lignin is an organic substance binding the cells, fibers and vessels which constitute wood and the lignified elements of plants, as in straw. It is also complex polymer of aromatic alcohols and commonly derived from woods. It is formed from an excellent strength and durability tissue and becomes cell walls of almost all dry land plant. Lignified tissues such as wood are similar to fiber-reinforced plastics in which lignin represents the plastic binder and cellulose reinforcing fibers (Hsu, 1996). According to Brigham et al. (1996) lignin are irregular phenyl-propane polymers that represent approximately 20% and 25% of hardwood or softwood tree stems.

Hemicelluloses are complex, breached carbohydrate polymers that are formed from different monomeric sugars attached through different linkages. Substituent and noncarbohydrate components occur on hemicellulose on ethier the main chain or on the carbohydrate branches. The complex structure of hemicellulose is thought to confer a wide range of biophysical and biomechanical properties on the plants tissues in which it occurs on products made from these tissues (Brigham et al., 1996).

These carbohydrates polymers contain different sugar monomers (six and five carbon sugar) and they are tightly bound to lignin. The complex structure of Lignocellulose which is the cellulose is surrounded by a monolayer of hemicellulose and embedded in a matrix of hemicellulose and lignin. Lignin specifically creates a barrier to enzymatic attack while the highly crystalline structure of cellulose is insoluble in water while the hemicellulose and lignin create a protective sheath around the cellulose.

2.3 Water hyacinth as lignocellulose biomass

There are two types of water floating macrophytes chosen in this study. *Eichhornia crassipes* and *Pistia stratiotes* are plant water that easily grows in Malaysia. They are suitable for productions of bioethanol because of their rate of growth are very fast (Rezania et al., 2015). Each of them has advantages and disadvantages criteria that have to be studied in order to choose the most economical species for Malaysia climate.

Table 2.1: The percentage composition of Eichhornia crassipies (Nigam, 2002)

Organic Components	Percentage (%)
Hemicellulose	48.7
Cellulose	18.2
Lignin	3.5
Crude protein	13.3

2.4 Pretreatment techniques

There are many types of biological, physical and chemical technologies available for the pretreatment of lignocellosic biomass (Singh & Bishnoi, 2013). Combination pretreatment techniques that use several techniques in one process of bioethanol production are also common (Hsu, 1996). Some pretreatment methods seem to be economically more feasible than the others. However, the environmental concern and production of inhibitors to fermenting yeasts during these pretreatment processes are the major hurdles that have to be overcome for

commercial production of the bioethanol (Khuong, et al., 2014). So it is very important that an efficient, cost effective and environmentally friendly pretreatment method is considered.

2.5 White rot fungi

Lignin modification and degradation have been most extensively studied in basidiomycetes, in which a number of enzymes and mechanisms involved in lignin attack have been elucidated. White-rot basidiomycetes (notably *Phanerochaete chrysosporium*) are the most frequent wood-rotting organisms, because of their ability to degrade lignin, hemicelluloses, and cellulose, often giving rise to cellulose-enriched white material (Bugg, Ahmad, Hardiman, & Rahmanpour, 2011).

Phanerochaete chrysosporium is one of white rot fungus that lives abundantly on dead wood. It has special ability to degrade the abundant aromatic polymer lignin without degrade the white cellulose in the same substrates. *P. chrysosporium* releases extracellular enzymes to breakdown the complex three dimensional structure of lignin into simple components that can be utilized by its metabolism. The extracellular enzymes are nonspecific oxidizing agents (hydrogen peroxide, hydroxyl radicals) used to cleave the lignin bonds (Martinez, 2004).

Degradation of lignin and pollutants is made possible by the production of extracellular enzymes. Components such as lignin peroxidase and manganese peroxidase take part in the remediation of various pesticides, polyaromatic hydrocarbons, carbon tetrachloride and various poisons (Bugg, Ahmad, Hardiman, & Rahmanpour, 2011). The process of lignin breakdown is carried out by means of cleavage reactions. These extracellular enzymes release free radicals to initiate spontaneous break down to phenyl propane units in the Secondary metabolism or stationary phase (Hammel and Kenneth, 2008).

2.6 Fermentation

There are four major steps in producing bioethanol from water hyacinth biomass. The first step is the pretreatment of biomass to break down lignin– hemicelluloses–cellulose complex to make it more susceptible for hydrolysis. The second step is hydrolysis to break down the cellulose and hemicelluloses into monomer sugars. The third step is fermentation of these sugars to ethanol. The final step is product recovery and concentration by distillation (Hu et al., 2008).

3.0 Research Methodology

3.1 Growth rate

3.1.1 Preparation of cultivation tank

This study was conducted at algae cultivation area located at Desa Bakti Wastewater Stabilization Pond. Two tanks (volume is 304 liter per tank) and several small container were set up for cultivation. The tanks and container filled with wastewater which was pumped from the effluent of the wastewater treatment pond. It filled up with wastewater except for the small container.

3.1.2 Cultivation of water hyacinth

In this study, two types of floating aquatic macrophytes were selected. The first one is *Eichhornia crassipes* labelled as type A. The characteristic of this type A is that, it have big spongy stem which enable them to float on water. The second type is *Pistia stratiotes* labelled as type B that have character with similar looks as lettuce or cabbage which their leaves are water proof and filled with air, so they can float on water. The mother plants were collected in Universiti Teknologi Malaysia (UTM)

which normally exists on water that is contaminated with high nutrients such as wastewater stabilization pond. Before propagate them in the tank, the mother plant was cleaned to remove all suspended solids and insect etc.

Figure 3.1 Water hyacinth and water lettuce

3.1.3 Growth rate of plants

All plants in the tanks were taken out and dried for a while. Then each type of plants was weighed and recorded the mass increment. The weight was measured using balance. The length of root was also recorded. The leaves were measured and the growth of the leaves was recorded. The length of both roots and leaves was measured using a ruler. This procedure was repeated every 3 days for 3 weeks and the data was recorded.

3.2 Biodegradation

3.2.1 Preparation of dry water hyacinth

Water hyacinth and water lettuce were harvested from the ponds in UTM and brought to the laboratory. All plants were rinse with tap water to remove dirt and any outside material that attached to water hyacinth. They were chopped into small pieces and placed inside a beaker. Then, they were put in the oven for overnight at 105°C for drying process. The dried sample was kept in the zipped plastic bag to maintain dry.

3.2.2 Preparation of water hyacinth powder

The dried sample was blended by using dry blender to get water hyacinth into a powder form. Then, the water hyacinth powder was kept again into the zipped plastic bag and ready for pretreatment process. This procedure is very important because the total area of water hyacinth was increased and reaction time in the pretreatment process will shorten. In other word, it increases the efficiency of the pretreatment process and increase rate of process.

3.2.3 Preparation of white rot fungi

White rot fungi (*P. chrysosporium*) was isolated from decayed woods by Central lab of Universiti Teknologi Malaysia (UTM). In propagation process, Potatoes Dextrose Agar (PDA) plates are used. The preparation of the PDA media was started with sterilize everything that were used in the process. The process needs the best aseptic technique as possible in the lab. Laminar flow was used to avoid contamination happened in the propagation of fungi.

Propagation process of fungi started with 3.12g of dry potato dextrose agar and was mixed with 80ml of distilled water until no scum visible in the bottle. Cork borer, measuring cylinder and 4 set of petri dish were sealed with heat resistance plastic. All sealed equipments and media were sterilized for 15 minutes at 121°C under 15 Psi pressure by using autoclave machine. All sterilized equipment and media were placed in laminar flow chamber for cooling down. 20ml of media was poured into each petri dish. Cork borer was used to transfer a fungal disc from stock plate into the prepared media. All petri dish were sealed with para-film to avoid contamination and placed in an incubator.

3.2.4 Pretreatment process

Firstly, 10g of substrates powder was weighed and put into a 100 ml glass beaker. Six beakers with water hyacinth powder and six beakers with water lettuce powder were prepared. Every beaker was dropped with a drop of Tween 80 and added 10 mg of glucose powder. Tween 80 was added into the substrates mixture to enhance the growth of fungi in the limited glucose condition (Jacques et al., 1980). All Substrates mixture was wetted with 20 ml of distilled water. Then, all mixtures were sterilized at 121°C for 15 minutes in an autoclave.

After autoclaved, the substrates were cooled down to the room temperature for a few hours. Then, 5 fungal discs (4mm diameter) were added into 3 water hyacinth beakers and 3 water lettuce beakers by using cork borer and were labeled as 5WH and 5WL respectively. Two fungal discs were added to the remaining beakers and labelled as 2WL and 2WH. All beakers were stored in a cabinet for biodelignification process. Sample was taken every 10 days until 30 days for glucose determination, lignin degradation and hemicellulose content.

After 10 days, the mixture was taken out from cabinet and mixed with 100 ml distilled water and grinded by using laboratory mortar and pestle. Then, it was filtered to remove all suspended solid with Whatman No 1 filter paper with 0.45 µm pore size. The filtrate was used for glucose determination and the solid retain on filter paper was oven dry overnight for lignin and hemicellulose determination.

3.2.5 Determination of sugar content

The equipment used is test tubes, pipets and spectrophotometer DR6000. The reagents that require in DNS method are 1% Dinitrosalicylic Acid Reagent Solution and 40% Potassium sodium tartrate solution (Miller, 1959).

DNS method procedure started with 3 ml of DNS reagent was added to 3 ml of glucose sample in a lightly capped test tube. In terms to avoid the loss of liquid due to evaporation, cover the test tube with a piece of paraffin film if a plain test tube is used. The mixture heated at 90º C for 15 minutes to develop the red-brown colour. 1 ml 40% (w/v) potassium sodium tartrate (Rochelle salt) solution was added into the test tube to stabilize the colour. After cooling to room temperature in a cold water bath, the absorbance with a spectrophotometer at 575 nm were recorded.

Calibrated curve was made to convert absorbance value into the desire concentration value. Same procedure was followed and the sample was substituted with standard sugar solution with different known concentration. Then the data was plotted into a graph and the slope equation will be used as the conversion formula.

3.2.6 Determination of hemicellulose

The method used to determine hemicellulose in this study is gravimetric method which is Chesson method (Chesson, 1978). The oven dry water hyacinth sample was grinded into a powder form. 1g of sample was weighed and mixed with 150 ml of distilled water. Then, it was refluxed for 2 hours at 100°C. After reflux, it was filtered with 0.45 um filter paper and the solid retain was put in oven at 105°C for overnight. Weight lost was calculated in percentage. The weight lost from this procedure is soluble protein. Same procedure was repeated with the same sample by using 5% (v/v) sulphuric acid to get another weight loss. The weights lost from this procedure are soluble protein plus hemicellulose. In order to get hemicellulose, weight lost from second procedure minus weight lost from first procedure.

International Journal of Research
Available at https://pen2print.org/index.php/ijr
e-ISSN: 2348-6848
p-ISSN: 2348-795X
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3.2.7 Determination of lignin

The grinded oven dry water hyacinth sample was weighed to 330 mg and soaking with 5 ml of concentrated acid which is 72% (v/v) sulphuric acid. The soaking process takes 2 hours and the water hyacinth powder turns to black colour because of the reaction of concentrated acid with the plant structures. After the soaking process, the solution was diluted to 3% (v/v) acid concentration by adding distilled water to the water hyacinth solution. The diluted solution underwent reflux for 4 hours with 125°C temperature. After cooled, it was filtered with 0.45 um filter paper and the solid retain was put in oven at 105°C for overnight. The next day, the weight of the dry filtered sample was weighed and put it in a furnace and ignites for 3 hours at 575°C. Then, it was cooled down and the sample was weighed and recorded.

Acid insoluble lignin (%) = weight after oven dry-weight after ignite initial weight x 100%

3.3 Fermentation process

Same procedure as stage two was done in order to get the filtrate. Type of plants and number of fungal disc were chosen by the result of sugar content in stage two. The pH of filtrate was adjusted to 5.5 with diluted hydrochloric acid. DNS method was done for initial sugar content in the filtrate. Sample was transferred into three 250 ml conical flasks with 200ml working volume for fermentation process. Each conical flask was labelled with different concentration of yeast. Three variation of yeast concentration were used which are 1g/L, 2g/L and 3g/L. Sampling was taken every 2 hours until 10 hours for sugar reduction by DNS method.

3.3.1 Determination of sugar

The fermented solution was taken out and put in centrifuge tube about 45 ml. It was centrifuged at 6000 rpm for 10 minutes. After centrifuge, yeast cell was settled down at the bottom of the centrifuge tube. The supernatant was collected by using syringe and filtered by using nylon syringe filter with 0.45 um. The filtered sample undergoes DNS method procedure and the absorbance value was recorded.

4.0 Results and discussion

4.1 Growth rate

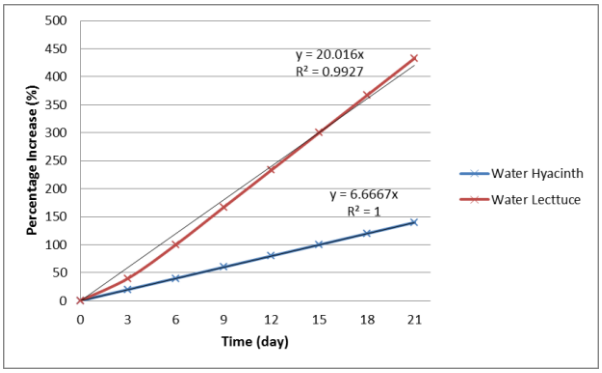
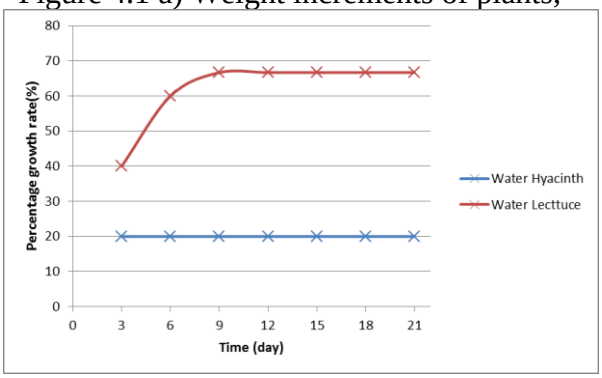


Figure 4.1 a) Weight increments of plants,



b) Growth rates of plants

Figure 4.1a showed the percentage increment from the start until the last day (21st day). From the graph, weight of water hyacinth gradually increased and form a linear regression line which is y = 6.6667x and R^2 = 1. This means that water hyacinth grew about 6.7% in a day. Weight of water lettuce also gradually increased and a linear regression line showed an estimation equation. The equation is y = 20.016x and R^2 = 0.9927 which mean that water lettuce grew about 20% a day. The value of

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Volume 06 Issue 01
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regression is approaches and near to 1 which means the equation is acceptable and valid to use for estimation.

Every plant will achieved maximum growth rates at a certain time until the weight increment is maintain and remain constant for every day. Figure 4.1b shows the relationship between percentage growth rate and time for both species. Based on the graph, the growth rate of water hyacinth is maintained and remained same from day 3 until day 21 which is 20% increment for every 3 days. Growth rate for water lettuce gradually increased from 40% on day 3 and 60% on day 6 and maintained at 67% on day 9 until day 21. This phenomenal is acclimatization process which is the plant needs a period of time to adapt to new environment such as nutrient condition, temperature, toxicity and so on. Every plant needs a period of time to acclimatize to the surrounding but no need for water hyacinth because it was found live in the tank that already adapted to the condition in this experiment.

The analysis above showed that different growth rate between water hyacinth and water lettuce. It can be seen clearly from the tanks that water lettuce already fulfill the space of the tank in day 21 while tank contains water hyacinth still have some empty space on the final day of the phase of the experiment. Therefore, water lettuce is preferable species to propagate and have ability to provide enough supply for bioethanol production due to its rapid growth rate.

4.2 Biodegradation

The parameters measured in this study were sugar concentration, hemicellulose and lignin. All parameters represent degradation process of water hyacinth plant structure by White Rot fungi. In this part, water hyacinth and water lettuce were used as substrate.

4.2.1 Hemicellulose

Figure 4.2 represents hemicellulose content in water hyacinth and water lettuce plants in different number of fungal disc from the start until day 30 in 10 days gap. From the graph, hemicellulose in both species gradually decreased in the ranges from 47.6% to 31.1%. All graphs shows quite similar pattern of hemicellulose degradation by white rot fungi.

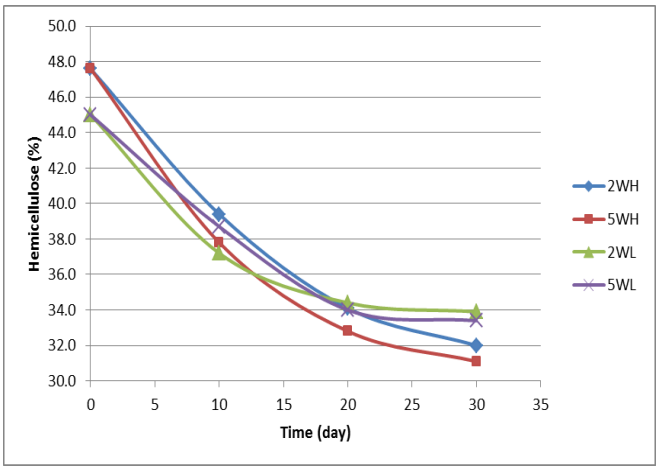

Figure 4.2 Hemicellulose degradation trends

The graph showed that water hyacinth started with 47.6% hemicellulose content degraded to 31.1% by white rot fungi with 5 fungal discs at the beginning. Experiment with 2 fungal discs degraded to 32% of hemicellulose which mean lower degradation. While water lettuce started with 45% hemicellulose content degraded to 33.4% by same species of fungi with also 5 fungal discs at the beginning. Experiment with 2 fungal discs degraded to 33.9% of hemicellulose which also mean lower degradation. Therefore, the number of fungal disc affects the rate of hemicellulose degradation but not significantly.

International Journal of Research
Available at https://pen2print.org/index.php/ijr
e-ISSN: 2348-6848
p-ISSN: 2348-795X
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Figure 4.3 Hemicellulose degradation in 3 phases

Figure 4.3 shows four sets of histogram that represents hemicellulose reduction in different phases of time for water hyacinth and water lettuce with different fungal dosage. The rate of degradation was separated into three phases of time which is 0-10 days, 10-20 days and 20-30 days. As can be seen, all set of histogram shows same pattern which gradually decreased. The histograms of hemicellulose degradation showed similar pattern to biological degradation was studied by Kamra et al., (1993) which degrade sugarcane bagasse by white rot fungi. 5WH demonstrated the highest hemicellulose degradation in the first phase with 20.6% degradation in 10 days. Whereas, 5WL demonstrated the lowest hemicellulose degradation in 0-10 days phase with 14% degradation in 10 days. 5WL should have higher degradation rate than 2WL due to fungal dosage which 2WH and 5WH as the reference. This might be due to contamination of white rot fungi in the experiment.

Total hemicellulose degradation by white rot fungi in 2WH, 5WH, 2WL and 5WL are approximately 32.8%, 34.7%, 24.7% and 25.8% respectively. The total degradation was calculated from day 0 until day 30. It can be seen from the total hemicellulose degradation in 30 days, the highest degradation was demonstrated by 5WH experiment which water hyacinth powder with 5 fungal discs as the fungal dosage. Therefore it can be concluded that water hyacinth plant is more feasible for biological pretreatment by using white rot fungi in hemicellulose degradation than water lettuce.

4.2.2 Sugar

Figure 4.4 represents sugar concentration in filtrate of water hyacinth and water lettuce that was biologically pretreated by white rot fungi with different fungal dosage. The concentration of sugar in the filtrate represents extractable sugar in water hyacinth and water lettuce plants. It can be seen that the trends of sugar concentration in the filtrate gradually increased from day 10 until day 30. All graph lines showed almost similar pattern of sugar increment due to the process of biological pretreatment using white rot fungi.

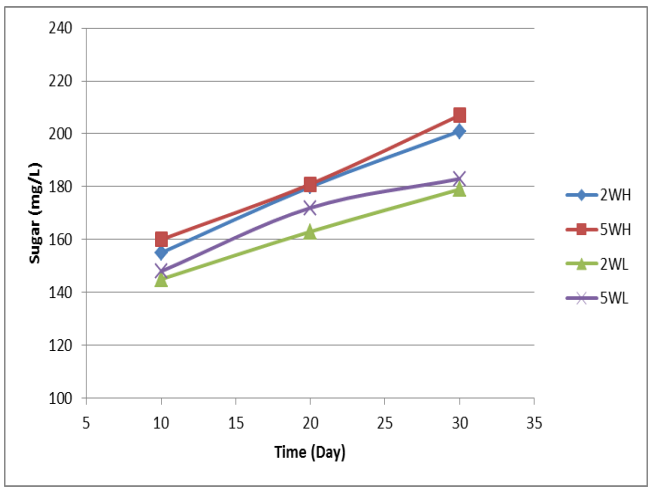

Figure 4.4 Sugar increment trends

All lines in the graph do not intercept each other which mean that the lines were formed nicely and the reaction in all experiment almost similar. The concentration of sugar was nicely arranged on all three days of sampling which is day 10, 20 and 30. The

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e-ISSN: 2348-6848
p-ISSN: 2348-795X
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order that can be seen in the graph is 5WH, 2WH, 5WL and 2WL with high to low sugar concentration order. This shows that water hyacinth plants have more extractable sugar than water lettuce species and sugar increment rate was influenced by number of fungal dosage.

4.2.3 Lignin

Figure 4.5 represents lignin content in water hyacinth and water lettuce in different number of fungal dosage from the start until day 30 in 10 days gap. It can be seen from the graph, lignin in both species gradually decreased in the ranges from 18.8% to 15.2%. Most of lines in the graph showed similar trend by gradually decreasing in time except for 5WH experiment. Line 5WH experiment shows very different trend because it shows increment of lignin content from day 10 to day 20.

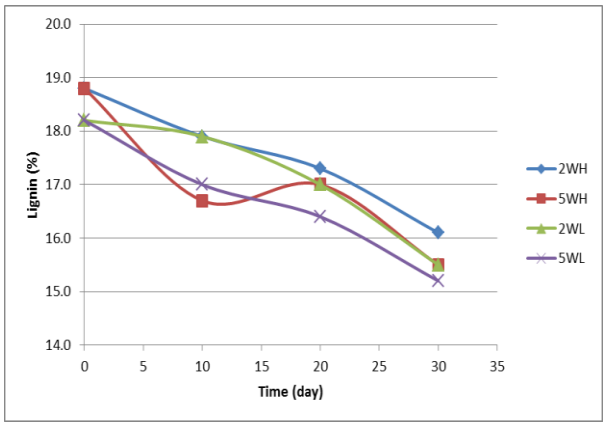

Figure 4.5 Lignin degradation trends

The graph shows that water hyacinth started with 18.8% lignin content and then degraded to 15.5% by 5 fungal discs of white rot fungi. Experiment with 2 fungal discs degraded to 16.1% of lignin content which mean lower degradation. While water lettuce started with 18.2% lignin content and degraded to 15.2% also by 5 fungal discs of same species of fungi. Experiment with 2 fungal discs degraded to 15.5% of lignin content which mean lower degradation happened. Therefore, the number of fungal disc affects the rate of lignin degradation.

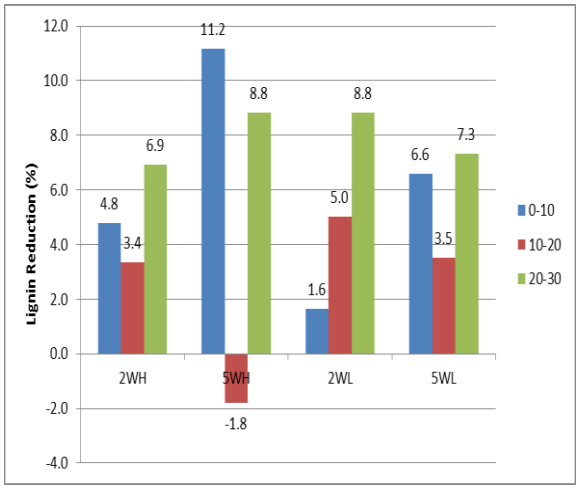

Figure 4.6 Lignin degradation in 3 phases

Figure 4.6 shows four sets of histogram that represents lignin degradation in different phases of time for water hyacinth and water lettuce with different fungal dosage. The rate of degradation was separated into three phases of time which is 0-10 days, 10-20 days and 20-30 days. As can be seen, all set of histogram shows different patterns and 5WH experiment show negative reduction of lignin on phase 10-20 days which mean that lignin increased. This happened because 5WH lignin content of day 10 sampling indicated the lowest lignin content with 16.7% and boost up the lignin degradation rate on phase 0-10 days to 11.2% degradation in 10 days. According to Kamra et al. (1993), lignin biodegradation by white rot fungi should have low biodegradation rate at the beginning and slowly increase the rate as shown by 2WL experiment.

Total lignin biodegradation by white rot fungi in 2WH, 5WH, 2WL and 5WL are approximately 14.4%, 17.6%, 14.8% and 16.5% respectively. The total degradation was calculated from day 0 until day 30 which the final day of sampling in this

experiment. The total lignin biodegradation in 30 days, the highest degradation was demonstrated by 5WH experiment. Therefore it can be concluded that water hyacinth plant is more feasible for biological pretreatment using white rot fungi in lignin biodegradation than water lettuce.

Overall, as can be seen in the discussion above that the graph pattern for both hemicellulose degradation and sugar content increments have a quite good negative correlation. Hemicellulose consists of other polysaccharides, principally xylans and mannans, which are closely associated with the cellulose filaments, and chemically linked with lignin (Sjostrom, 1993). According to Dhepe & Sahu (2010), hemicellulose degraded into xylose (major) and arabinose as the hemicellulose degradation pathways. Xylose and arabinose are under categories of sugar that classified as a monosaccharide of aldopentose type which means that it contains five carbon atoms. Therefore, every hemicellulose degradation process happened it will increase sugar concentration of the filtrate which hemicellulose breakdown into xylose and arabinose as additional sugar. In summary, number of fungal dosage affects the rate of degradation of hemicellulose and lignin. Water hyacinth shows it more feasible for both lignin and hemicellulose biodegradation by white rot fungi and produce more extractable sugar than water lettuce.

4.3 Fermentation

The parameter measured in this part of study is sugar concentration. This parameter represents sugar consumption rate that convert sugar into ethanol by yeast. The common yeast species used in this experiment is *Saccharomyces cerevisiae* that can be found in baker yeast. The substrate used for the fermentation process is water hyacinth filtrate has pretreated using white rot fungi in 30 days.

4.3.1 Sugar

Figure 4.7 shows a graph which represents the sugar reduction in water hyacinth filtrate in different yeast extract concentration from the start until 10 hours in 2 hours gap of sampling. All experiment was started with 189 mg/L of sugar as shown in table 4.5. It can be seen from the table, that sugar concentration in all flask reduced in the ranges from 189 mg/L to 54.7 mg/L. Sugar concentration was maintained and remained constant after a certain time of period that shows the process of converting sugar was stopped.

Table 4.1: Sugar concentration in fermented solution in 10 hours

Time (h)	Yeast Extract Concentration (g/L)		
	1	2	3
	Sugar (mg/L)		
0	189	189	189
2	86.4	81.9	69.7
4	69.7	69.7	61.0
6	55.4	57.5	56.1
8	56.4	56.1	54.7
10	56.8	58.2	56.8

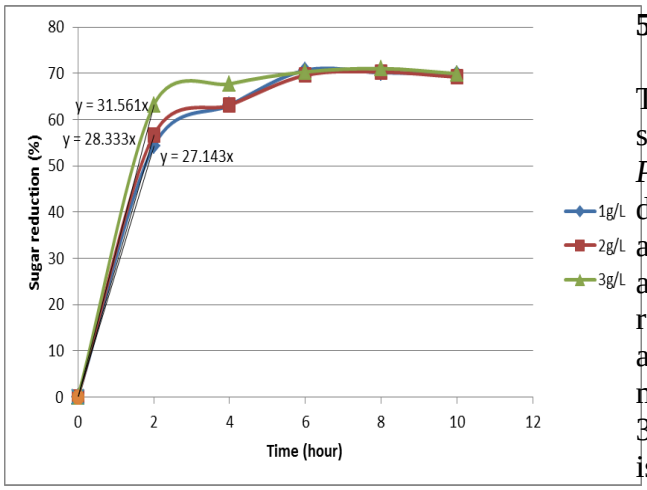

Figure 4.7 Percentage of sugar reduce versus time

As shown in figure 4.7, water hyacinth filtrate with 3g/L of yeast had the highest rate of percentage sugar reduction which approximately 31.6% sugar reduction in an hour followed by others with approximately 28.3% and 27.1% for 2g/L and 1g/L respectively. Therefore the concentration of yeast for fermentation process affected the rate of sugar conversion into ethanol. Increasing the yeast concentration which could boost up rate of sugar consumption.

The sugar consumption was stopped at about 70% after 6 hours of fermentation process by using *Saccharomyces cerevisiae*. This means fermentable of extract sugar from water hyacinth that undergoes biological pretreatment by white rot fungi was about 70%. 30% of extract sugar is unfermentable type of sugar and most probably is xylose. According to Schneider (1989), xylose is the major product of the hydrolysis of hemicellulose from any type of lignocellulosic biomass. Xylose is classified as unfermentable sugar by *S. cerevisiae* (Paulino et al., 2003). According to (Wohlbach et al., 2011), only genetically engineered yeast (*S cerevisiae*) which have xylose isomerization ability can consume xylose and convert it into bioethanol.

5.0 Conclusion

The growth rate of both water hyacinth species which are *Eichhornia crassipes* and *Pistia stratiotes* shows significantly different rates. Based on the analysis, the approximate growth rate of water hyacinth and water lettuce are 6.7% and 20% a day respectively. Furthermore, water hyacinth and water lettuce demonstrated the maximum growth rates by 20% and 67% in 3 days respectively. Therefore, water lettuce is preferable species to propagate and have ability to provide enough supply due to its rapid growth rate.

Biodegradation process happened on both species by using white rot fungi. However, water hyacinth (*E. crassipes*) was more feasible to degrade by white rot fungi due to performance of hemicellulose and lignin biodegradation rather than water lettuce. Hemicellulose breakdown affected to extractable sugar content of biomass. Furthermore, biodegradation rate of hemicellulose and lignin were influenced by number of fungal dosage.

The rate of sugar conversion into bioethanol was determined by sugar consumption. Based on the analysis, higher concentration of yeast shows higher sugar consumption rate which could represent bioethanol production rate. Fermentable sugar in water hyacinth using biological pretreatment by white rot fungi reach up to 70%.

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