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Research Article

Optimization of pretreatment in delignification of hyacinth biomass for ethanol production

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Abstract

Water hyacinth (*Eichhornia crassipes*) is one of the potential feedstocks for bioethanol production due to their higher cellulose content. It needs the optimization of pretreatment to remove lignin and release cellulose and hemicellulose from lignocellulosic complex. Pretreatment was done using sulfuric acid, sodium hydroxide, and calcium hypochlorite as soaking agents. Experiment was carried out at 121 °C for 15 minutes. Pretreatment with 1% sulfuric acid decreased lignin by 6.74%, 4.0% sodium hydroxide reduced lignin content by 11.23%, whereas pretreatment with 0.3% calcium hypochlorite removed lignin by 8.30% from original lignin content lignocellulosic substrate. Therefore, sodium hydroxide pretreatment showed highest efficacy in delignification processes, followed by calcium hypochlorite, and sulfuric acid pretreatments.

Introduction

Ethanol is valuable as industrial solvent and is used in the preparation of medicines, resins, flavoring extracts, perfumes, varnishes, and shellac etc. Ethanol is a natural cleaner fuel and causes less environmental problems than petroleum (Saricks et al. 1999). This enables preparation of gasoline–ethanol blends resulting in gasohol for internal combustion engines that has higher octane value, replaces lead in gasoline, and high heat of vaporization impede self-ignition in the diesel engine (Balat and Balat, 2009). There has been increasing interests in conversion of biomass to fuel grade ethanol due to various reasons including alternative green energy sources, the rise in oil prices, and minimizing greenhouse gas emissions caused by the use of fossil oil (Huang et al. 2009).

Progress in treatmens of grass lignocelullosic biomass could leads to environmentally friendly renewable biofuels (Tocco et al. 2021). A number of environmental and economic benefits are claimed for bio-fuels. Bioethanol is by far the most widely used bio-fuel for transportation worldwide. Using bioethanol blended gasoline fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission. Bioethanol is essentially a clean fuel and has several clear advantages over petroleum-derived gasoline in reducing greenhouse gas emissions and improving air quality in metropolitan areas.

Production of bioethanol from biomass is one way to reduce both consumption of crude oil and minimize environmental pollution (Balat et al. 2008). Biomass derived from agricultural and forest residues along with herbaceous materials and municipal wastes have become the primary feedstocks for bioethanol production in the past several years (Limayema and Ricke, 2012). These raw materials are classified into three categories: simple sugars, starch, and lignocellulose. The biofuels produced from lignocellulosic materials, so-called second-generation bioethanol are energetic, economic and has environmental advantages in comparison to bioethanol produced from starch or sugar (Alvira et al. 2010). However, non-food plant sources like lignocellulosic biomass are far more abundant and cheaper.

Among various types of available lignocelluloses, water hyacinth (*Eichhornia crassipes*) is a potential resource available in many tropical regions of the world (Ganguly et al. 2012). It is a native Indonesian grass and considered as a noxious weed in many parts of the world as it grows very fast and rapidly depletes nutrient and oxygen from water bodies thereby adversely affecting flora and fauna. Moreover, due to high evapotranspiration it accelerates shortage of water all over the places where it grows. Water hyacinth is mainly removed by three methods hand cutting/pulling, mechanical removal and biological control.

Water hyacinth which has the advantage of being widely available is one of the potential feedstock options for bioethanol production, partly due to their higher cellulose content and compositional uniformity (Ganguly et al. 2012). Optimization of the fermentation process requires an efficient pretreatment to remove lignin and to release cellulose and hemicellulose from lignocellulosic complex of plant fiber. Therefore, determination of optimal pretreatment protocol for fermentation is essential for the successful lignocellulosic ethanol production process (Suhardi et al. 2013). The delignification can be classified into biological, physical, chemical, and physico-chemical pretreatments, depending on different forces or energy consumed in the process.

Various pretreatments of the water hyacinth by using chemical substances such as sulfuric acid, sodium hydroxide, and calcium hypochlorite were evaluated in this work. These pretreatments were compared for their efficacy of lignin removal from water hyacinth substrate.

Method

Substrate

Water hyacinth was collected manually from local freshwater pond affected by this weed. The biomass was cut into pieces, and dried under sunshine. The dried biomass was ground to fine particle size of ±60 mesh and stored in airtight plastic jars to avoid free moisture effect. This, was then used for pretreatment process. Lignin contents of substrate were estimated by Kappa method (SNI 0494, 2008).

Sulfuric Acid Pretreatment

The substrate at a solid loading of 10% (w/v) was pretreated in autoclave with sulfuric acid at concentrations of 0; 0.5; 1; 1.5; 2; and 2.5% (w/v) as soaking agent for residence time of 15 minutes at temperature of 121 °C, and pressure of 15 psi. After cooling, the contents were filtered with two layers of muslin cloth and residues were washed several times in tap water to neutralize the pH followed by a final rinse in distilled water. After that, residue was dried by oven at 105 °C for 10 hours and then used for subsequent analysis.

Sodium Hydroxide Pretreatment

The substrate at a solid loading of 10% (w/v) was pretreated in autoclave with sodium hydroxide at concentrations of 0; 2; 4; 6; 8; and 10% (w/v) as soaking agent for residence time of 15 minutes at temperature of 121 °C, and pressure of 15 psi. After cooling, the contents were filtered with two layers of muslin cloth and residues were washed several times in tap water to neutralize the pH followed by a final rinse in distilled water. After that, residue was dried by oven at 105 °C for 10 hours and then used for subsequent analysis.

Calcium Hypochlorite Pretreatment

The substrate at a solid loading of 10% (w/v) was pretreated in autoclave with calcium hypochlorite at concentrations of 0; 0.1; 0.3; 0.5; 0.8; and 1% (w/v) as soaking agent for residence time of 15 minutes at 121 °C temperature, and 15 psi pressure. After cooling, the contents were filtered with two layers of muslin cloth and residues were washed several times in tap water to neutralize the pH followed by a final rinse in distilled water. After that, residue was dried by oven at 105 °C for 10 hours and then used for subsequent analysis.

Results and Discussion

Bioethanol production from lignocellulosic materials is processed through three major operations: pretreatment for delignification is necessary to liberate cellulose and hemicellulose before hydrolysis; hydrolysis of cellulose and hemicellulose to produce fermentable sugars including glucose, xylose, arabinose, galactose, mannose and fermentation of reducing sugars (Sarkar et al. 2012). The screening and optimization methodologies represent a valuable tool for optimization of nutrients and process parameters for ethanol production (Singh and Bishnoi, 2012). The pretreatment of any lignocellulosic biomass is a crucial step before hydrolysis in producing ethanol process.

Pretreatment objectives are disruption of plant cells, separating hemicellulos and lignin from cellulose, decreasing cellulose crystallinity, and minimizing fermentation inhibitor formation (Li and Takkellapati, 2018). The lignocellulosic biomass delignification was investigated in many studies. The challenges in future research are optimization of operating parameters and assessment of total cost of biofuel production from lignocellulose biomass at large scale by using different pretreatment methods (Cheah et al. 2020). It is important to ensure that throughout lignocellulosic bioethanol production processes, favorable features such as maximal energy saving, waste recycling, wastewater recycling, recovery of materials, and biorefinery approach are considered.

Pretreatment enhances the hydrolysis of cellulose. In this step the bundles of lignocelluloses should be opened in order to access the polymer chains of cellulose and hemicellulose. Thus, the pretreatment process is possibly the most important factor in the ethanol production process because it influences waste treatment, cellulose conversion rates and hydrolysis of hemicellulose sugars and their subsequent fermentation (El-Zawawy et al. 2011).

In this work, physical pretreatment with size reduction and heating was applied on substrate. The sample was milled to ±60 mesh in size and heated at temperature of 121 °C for residence time of 15 minutes and pressure of 15 psi without soaking agent. This treatment reduced 3.55% lignin content in substrate. Lignin content decreases from 10.41% for raw sample to 10.24% for treated sample. Pretreatment at temperature of ± 120 °C was applied as optimum conditions in preparation sugar beet pulp (SBP) (Zheng et al. 2013). Acid pretreatment at this temperature increased the enzymatic digestibility of SBP from 33% to 93%.

The process causes hemicellulose degradation and lignin removal, thus increasing the potential of cellulose hydrolysis (Ganguly et al. 2012). It can be assumed that the porous surface was due to the partial removals of lignin and hemicellulose, which improved subsequently the accessibility of the enzyme onto the lignocellulosic substrate (Zhu et al. 2009).

Each pretreatment has a specific effect on the cellulose, hemicellulose and lignin fraction, thus different pretreatment methods and conditions should be chosen according to the process configuration selected for the subsequent hydrolysis and fermentation steps (Alvira et al. 2010).

Sulfuric Acid Pretreatment

Dilute acid pretreatment is the most studied process for bioethanol production from lignocellulosic biomass (Sindhu et al. 2011; Harun and Danquah, 2011; Kim et al. 2014). The application of dilute acid pretreatment to lignocellulose biomass can achieve some level of success in that it can provide satisfactory cellulose conversion with certain material. The process is affected by some parameters such as acid concentration, residence time, temperature, and pressure of operating procedures. However, dilute sulfuric acid was used as soaking agents for pretreatment with variation for concentration in this experiment. Residence time of 15 minutes, at temperature 121 °C, and pressure 15 psi were applied for the system.

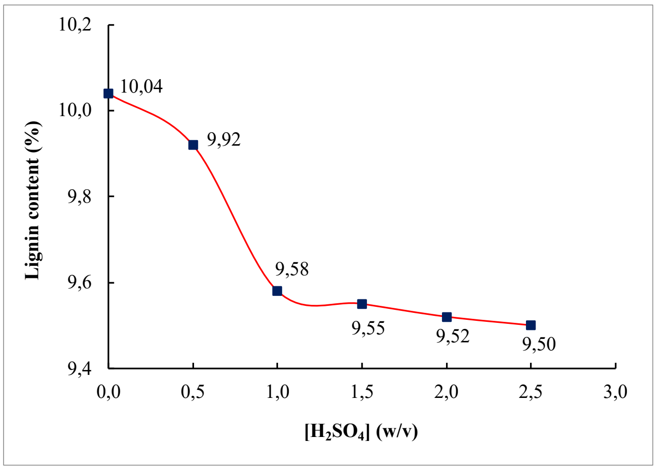

Fig.-1. Influence of Sulfuric Acid Concentrations on Lignin Reduction in Water Hyacinth Substrate

Relationship between acid concentration and lignin reduction is shown in Fig.-1. Percentage of lignin reduction increases in accordance with sulfuric acid concentration. The highest lignin reduction was achieved when using sulfuric acid of 2.5% (w/v) in which remaining lignin content is 9.50%. Lignin reduction from 9.58% to 9.50% is too small or insignificant change for acid concentration in 1.0 – 2.5% range. The increase of acid concentration exceeds 1.0% enhanced insignificantly the lignin reduction. Performance of sulfuric treatment on the lignin reduction showed the maximum at concentration of 1.0%. It can reduce lignin content significantly from 10.04% to 9.58% of substrate mass that corresponds to lignin reduction of 6.74% compared to original lignin content.

Therefore, acid concentration of 1.0% is recommended to apply in the pretreatment step for delignification of water hyacinth. This result is very close to that found in previous experiment with microalgae as feedstock. It showed that the highest bioethanol concentration was achieved when the pretreatment step was performed with 15 g/L of microalgae using 1% (v/v) of sulfuric acid at temperature of 140 °C for 30 minutes (Harun and Danquah, 2011). By using HNO_3 as soaking agent in pretreatment, a maximum enzymatic digestibility was achieved for lignocellulosic materials in a condition of 0.65% HNO_3, 158.8 °C and 5.86 minutes (Kim et al. 2014).

Acid pretreatment is low cost, high toxic by-product, and applicable to a wide range of biomass. Diluted acid is used to limit inhibitors generation, and enhancement of biofuel production with solubilization of hemicellulose, condensation, and precipitation of solubilized lignin (Cheah et al. 2020). It means that acid pretreatment is better applied in a condition of dilute solution or lower concentration. Using acid in higher concentration can destroy the cellulose structure that will reduce the fermentable glucose yield in the next step. Moreover, acid pretreatment with higher concentration spends so much demineralized water for substrate neutralization.

The main reaction that occurs during acid pretreatment is the hydrolysis of hemicellulose. Solubilized hemicelluloses can be subjected to hydrolytic reactions producing monomers, furfural, HMF, and other volatile products in acidic environments (Quintero et al. 2011). During acid pretreatment, solubilized lignin will quickly condensate and precipitate in acidic environments (Liu and Wyman, 2003). The solubilization of hemicellulose and precipitation of solubilized lignin are more pronounced during strong acid pretreatment compared to dilute acid pretreatment.

Sodium Hydroxide Pretreatment

Alkali pretreatment was applied for many delignification processes in bioethanol production from lignocellulosic feedstock (Guragain et al. 2011). Delignification is required to alter biomass structure as well as chemical composition, so that hydrolysis of carbohydrate to monomeric sugars can be achieved more rapidly with higher yields (Sindhu et al. 2011). Different parameters such as concentration, temperature, pressure, and pretreatment time can be investigated in alkali pretreatment for delignification. However, sodium hydroxide pretreatment with only concentration parameter was employed in this work.

Influence of sodium hydroxide concentration on lignin reduction in water hyacinth substrate is plotted in Fig.-2. In terms of remaining lignin content, ±8.93% of substrate weight, minimum was obtained by using sodium hydroxide of 10% (w/v) under 121 °C for 15 minutes in autoclave. This lignin reduction achieves 13.77% of original lignin content.

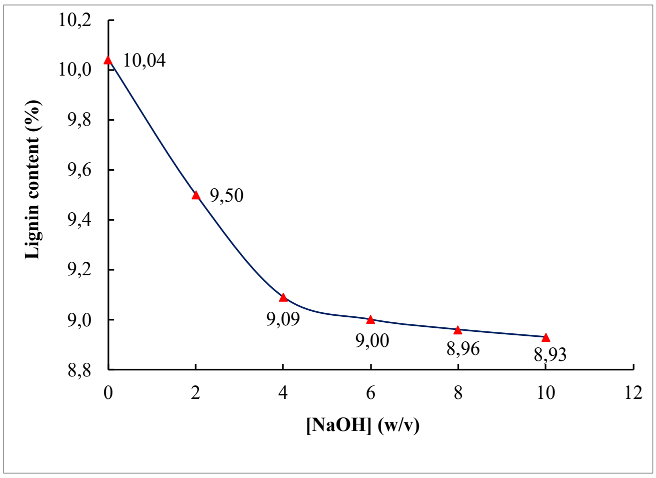

Fig.-2. Influence of Sodium Hydroxide Concentrations on Lignin Reduction in Water Hyacinth Substrate

Percentage of lignin content decreases with respect to the enhancement of sodium hydroxide concentration used as soaking agent in pretreatment. Increasing the alkali concentration from 4% to 10% (w/v) gave a small change of the lignin content in substrate from 9.09% to 8.93%. The lignin reduction of 0.16% shows insignificant change. Increasing the alkali concentration above 4% does not influence the lignin reduction significantly.

Pretreatment performance has a significant enhancement in reducing lignin content of substrate with sodium hydroxide at concentration of 4%. Lignin content decreases from 10.04% to 9.09% of substrate weight that corresponds to 11.23% of original lignin content. This result is comparable to that observed in previous work (Singh and Bishnoi, 2013). In the experiment, highest delignification observed at 90 minutes with 5.0% NaOH concentration. Alkali treatment with 4% NaOH was also applied for delignification of sugarcane bagasse (Asgher et al. 2013). It is therefore advised to use sodium hydroxide of 4 – 6% (w/v) in alkali pretreatment for delignification of lignocellulosic biomass. Using higher concentration will spend so much distilled water to clean and neutralize the substrate.

During alkaline pretreatment, the first reactions taking place are solvation and saponification. This causes a swollen state of the biomass and makes it more accessible for enzymes and bacteria. At “strong” alkali concentrations dissolution, “peeling” of end groups, alkaline hydrolysis, and degradation and decomposition of dissolved polysaccharides can take place (Hendriks and Zeeman, 2009).

Calcium Hypochlorite Pretreatment

Reducing lignin content though oxidative pretreatment is an alternative method, which is also be able to use in the process of making bioethanol from lignocellulosic biomass. An oxidative pretreatment consists of the addition of an oxidizing compound, like hydrogen peroxide or peracetic acid (Quintero et al. 2011) and ozone (García-Cubero et al. 2009) to the biomass, which is suspended in water.

Calcium hypochlorite was used for pretreatment in this experiment at temperature of 121 °C for 15 minutes with concentration variations. In previous study, the feedstock material is treated with water and either by air or oxygen at temperatures above 120 °C. Influence of applied concentration on lignin reduction is shown at Fig.-3.

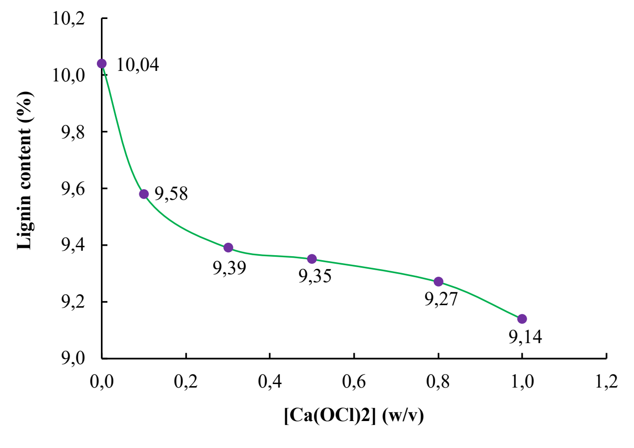

Fig.-3. Influence of Calcium Hypochlorite Concentrations on Lignin Reduction in Water Hyacinth Substrate

Highest lignin reduction was achieved when calcium hypochlorite concentration at 1.0%. This treatment reduces lignin content from 10.4% to 9.14%, which decreases around 10.74% of raw lignin content. The highest change of lignin reduction appears at 0.3% calcium hypochlorite that reduces lignin around 8.30% of original lignin content from 10.04% to 9.39%. The use of calcium hypochlorite with concentration above 0.3% showed a small change on lignin content from 9.39% to 9.17%.

During oxidative pretreatment, several reactions can take place, like electrophilic substitution, displacement of side chains, cleavage of alkyl aryl ether linkages or the oxidative cleavage of aromatic nuclei (Hendriks and Zeeman, 2009). Even, losses of hemicellulose and cellulose can occur in the use of unselective oxidant. A high risk on the formation of inhibitors exists, as lignin is oxidized and soluble aromatic compounds are formed (Quintero et al. 2011).

Therefore, it is recommended to use oxidant, calcium hypochlorite with concentration maximum of 0.3% in oxidative pretreatment for delignification of lignocellulosic biomass. Delignification with higher concentration can caused the cellulose and hemicellulose contents reduction that results in lower glucose yield at next step.

Pretreatment Performance on Delignification

Delignification can be done by physical pretreatment such as size reduction and heating. Size reduction may provide better results but very fine particle size may impose negative effects on the subsequent processing such as enzymatic hydrolysis. Effectiveness of pretreatment was determined the substrate structure and composition of lignocellulosic biomass as well as pretreatment conditions (Fuertez-Córdoba et al. 2021). Pyrolysis is an endothermic process where less input of energy is required. In this process the materials are treated at higher temperature, whereby cellulose rapidly decomposes to produce gaseous products such as H2, CO, and residual char. The decomposition is much slower and fewer volatile products are formed at lower temperatures. It may generate clumps during the subsequent steps involving liquid and may lead to channeling (Sarkar, et al., 2012).

Chemical pretreatment is the most applied method at commercial scale (Cheah et al. 2020). Moreover, sulfuric acid, sodium hydroxide, and calcium chlorite pretreatments were applied and compared for their effect on lignin removal from water hyacinth. Experiment was carried out at 121 °C for 15 minutes. Pretreatment with 1% sulfuric acid decreases 6.74% lignin, while with 4.0% sodium hydroxide reduce lignin content of 11.23%, and with 0.3% calcium hypochlorite removes 8.30% lignin from original lignin content.

Hydrolysis of hemicellulose can occur and solubilized lignin will quickly condensate and precipitate in acidic pretreatment. Solvation and saponification take place during alkaline pretreatment. Reactions like electrophilic substitution, displacement of side chains, cleavage of alkyl aryl ether linkages or the oxidative cleavage of aromatic nuclei can take place in the oxidative pretreatment. A high risk on the formation of inhibitors exists, as lignin is oxidized and soluble aromatic compounds are probably formed at higher concentration and temperature.

Alkali application is able to alter the structure of lignin by degrading ester and glycosidic side chains, cellulose swelling, and partial cellulose recrystallization (Kim et al. 2014). Previous studies on alkaline pretreatment have shown its effectiveness in a variety of modes and conditions with treatment times on the order of hours and days rather than minutes or seconds (Fuertez-Córdoba et al. 2021). In this work, sodium hydroxide pretreatment showed the highest performance in delignification process, followed by calcium hypochlorite, and sulfuric acid pretreatments. Pretreatment with sodium hydroxide as soaking agent is the best choice for delignification of water hyacinth.

Conclusion

In this work, delignification was done by physical pretreatment such as size reduction and heating. Moreover, sulfuric acid, sodium hydroxide, and calcium chlorite pretreatments were applied and compared for their effect on lignin removal from water hyacinth. Experiment was carried out at 121 °C for 15 minutes. Among them, sodium hydroxide pretreatment showed the highest performance in delignification process, followed by calcium hypochlorite, and sulfuric acid pretreatments. The best way for delignification of water hyacinth is pretreatment with sodium hydroxide as soaking agent.

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Conflict of Interests

The author (s) declares that there is no conflict of interest in this research and manuscript.

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