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Valorization from Cacao Pod Husk to Bio-ethanol: A Sustainable Source for Renewable Biochemicals

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Abstract:
Ethanol is a versatile compound used as an antiseptic or disinfectant, a primary ingredient in alcoholic beverages, and fuel additive in biodiesel. Given the growing demands for alternatives, researchers are increasingly exploring sustainable and eco-friendly sources of raw materials and processes of ethanol synthesis for a better bio-ethanol recovery from waste. This study aims to synthesize bio-ethanol from cacao pod husk. The method includes eco-friendly and non-toxic processes, including a 10-day delignification, acid-hydrolysis and fermentation. These processes were applied to determine the optimal precursor amounts to maximize the synthesis of bio-ethanol. Delignification yielded the highest amount of removed lignin at day 5, 6 and 7; 13.13, 13.00 and 13.13 milligrams, respectively. For acid-hydrolysis, 1 millimolar of sulfuric acid is sufficient to digest the delignified pod husk producing 50 mL of cacao pod hydrolysate containing fermentable sugars. Further, 10 mg of baker's yeast yielded the highest ethanol concentration from 50 mL of cacao pod husk, 1.02 ± 1.2 (% w/v). This three-pronged synthesis efficiently maximized the synthesis of bio-ethanol from cacao pod husk. This study establishes a framework for bio-ethanol production from cacao pod husk. Future research should focus on improving natural delignification duration, optimizing the conditions in acid hydrolysis, refining for yeast selection, and assessing the process feasibility for commercialization of bio-ethanol from waste.
Keywords: *Acid-hydrolysis, Delignification, Fermentation, Precipitation.*

INTRODUCTION:

The adoption of alternative bio-fuels efficiently lowers the carbon footprint. Among these, bio-ethanol stands as sustainable option (Niculescu et al, 2019). This versatile organic compound is utilized as an antiseptic, consumed as an alcoholic drink and fuel additive (Hans et al., 2021). As one of the future vital fuel, bioethanol comes primarily from crops such as sugarcane, corn, and switchgrass. As the global demand increases, researchers are exploring eco-friendly and sustainable raw materials and processes of producing ethanol (Calle and Walter, 2006). In the Philippines, bio-ethanol consumption elevated by 13 % since 2022 (USDA Foreign Agricultural Services report, 2022). The country's reliance of fuel heavily depends on imported oil for energy. This dependence can be greatly reduced by biodiesel production so that greater energy security can be built for the country and also to alleviate the damaging effects of oil price fluctuations upon the economy of the Philippines.

Significant developments have emerged in the process and raw materials utilized to produce bio-ethanol. The industry has recently shifted bio-ethanol synthesis from a first-generation feedstock, such as corn and sugarcane, to lignocellulosic biomass, the second-generation feedstock derived from plant material, fungi, and bacteria (Soccol et al., 2011). This type of biomass provides a sustainable option for ethanol recovery. Recent research initiatives focus on lignocelluloses conversion efficiency processes, such as pretreatment and enzymatic hydrolysis transformation.

Further advancements of these processes are crucial for expanding the use of biomass and lignocellulosic materials for biofuel generation (Limayem & Ricke, 2012). Enzymatic hydrolysis and acid hydrolysis have been well recognized as procedures for digesting lignocellulosic biomass into fermentable sugars suitable for bioethanol production (Selig et al., 2009; Mäki et al., 2012). However, these processes are expensive and energy intensive, limiting their commercial viability where energy and resource efficiency are determining factors. Added to these, agricultural residues of the country are often not uniform in nature.

Among the lignocellulosic biomasses, cacao pod husk is promising and sustainable feedstock. According to the Philippine Cacao Industry Roadmap (Garcia et. al, 2020), while 1% of cacao production's waste was attributed to the province of Cagayan, the majority comes from Davao region. Bio-waste including cacao pod husk, shell and skin amounted to 1.8421 metric tons in 2020 from cacao production (Garcia et al., 2021). Some of this biowaste were turned into products like fuel briquettes and flour derived from cacao pod husk (Tuates Jr. et al., 2020).

Approximately 3000 metric tons of cacao pod husk are discarded annually (Business World, 2022). There is significant potential to use cacao pod husk (CPH) and other lignocellulosic source as substrates for bioethanol, presenting an opportunity for sustainable biofuel production, particularly in the Philippines as cacao-cultivating country. Despite the promise, there is research gap regarding the effective and low-cost conversion of cacao pod husk into bioethanol. Key challenges include the best methods to pretreat, hydrolyze, ferment, and purify bioethanol from CPH, and to then optimize the process for large-scale industrial applications. Cacao pod husk is a lignocellulosic biomass, rich in cellulose, hemicellulose, and lignin, and is resistant to enzyme action for breakdown and fermentation (Hozman-Manrique et. al., 2023). Therefore, while lignocellulosic biomass is an attractive raw material to synthesize bio-ethanol, converting it to fermentable sugars requires effective pre-treatment actions for disintegration of the lignocellulosic hard structure. Pretreatment technologies, including physical, chemical, and biological methods have been investigated for other agricultural wastes, but their specific application for CPH remains underexplored. Extensive studies on appropriate specific chemical pretreatments conditions for cacao pod husk, such as sulfuric acid hydrolysis or alkaline treatment are lacking (Bajpai, 2018). Thus, inexpensive and green procedures for pretreatment of CPH that will enhance hydrolysis and fermentation steps is vital. Following pretreatment, hydrolysis is a crucial process for the breakdown of complex polysaccharides such as cellulose and hemicellulose into fermentable sugars. Enzymatic hydrolysis is a classic method for other lignocellulosic materials but is usually costly and non-efficient mainly because of the nature of biomass (Vasić et al., 2021). To date, optimisation of enzymatic hydrolysis pertaining to CPH remains uninvestigated, making to acid or alkali hydrolysis more economical options (Hernández-Mendoza et al., 2021). The effects of CPH's unique chemical composition on hydrolysis remains unclear, thus cannot replicate the process at a larger scale. Beyond hydrolysis, fermentation using microorganisms, e.g. Saccharomyces cerevisiae, is another important process converting sugars liberated from CPH into ethanol.

With these research gaps, the present study provides a three-step method of bio-ethanol synthesis from cacao pod husk. Specifically, this study aims to develop a novel process for synthesizing bio-ethanol from common cacao pod husks. The proposed method follows a straightforward three-step approach: delignification, hydrolysis and fermentation. Recognizing the large volume of cacao pod husk waste generated in the Philippines and its underutilization, this study aims to fill a methodical gap by a three-step optimized procedure for bioethanol synthesis.

MATERIALS AND METHODS:

Research Design

An experimental research design was employed in this study to systematically identify optimal conditions for natural delignification, acid-hydrolysis, and fermentation, which would ensure reproducibility and accuracy of results. Each of the steps of the experiment comprises three replicates.

Locale of Study

The study took place at the Cagayan State University-Lasam Campus Cacao Processing Center.
Raw Material Collection and Preparation

The conduct of the study uses 2 kg grams of fresh cacao pod husk obtained from Cagayan State University-Lasam Cacao Processing Center; 1 box filter paper (Whatmann no.1), 3 stirring rods (50 mm), 3 funnels (50 mm), three 100 mL beakers, ten 50 mL beakers, three 100 mL graduated cylinders, four 250 mL Erlenmeyer flask, one 100 mL volumetric flask, 1 set of distillation set-up (2 iron stand, 1 condenser, boiling flask, rubber tubings, thermometer, collecting glass), 1 box of cotton purchased, 100 grams sodium hydroxide, 100 grams of potassium iodide, 500 mL iodide solution, 50 grams baker's yeast, 1 hot plate with magnetic stirrer, 500 mL of concentrated sulfuric acid, 21 petri dish, 100 grams food grade sodium lauryl sulfate, 500 mL benedict's solution, five 500 mL beaker, 10 mL pipette and aspirator, marker and tape, 1 digital platform balance, three alcohol thermometer, 1 acid-base burette, 100 grams phenolphthalein, 1 digital pH meter one dessicator, 1 cacao grinder, 21 cork and crucible, one wash bottle, 120 V Miller and 21 round plastic containers with cover purchased from Reman Supplies Bambang, Manila.
Preparation of Cacao Pod Husk

Fresh cacao pod husk using UF-18 variety was collected at the cacao processing center of Cagayan State University-Lasam Campus. The fresh cacao pod husk was pulverized using a cacao grinder until coarse texture is achieved. Further, the pod husk was subjected to miller to turn the coarse powder to fine form.

Delignification of Pod Husk

The researcher collected three sets of 50 grams of collected fresh cacao pod husk, labeled as samples A, B and C, and placed them in food-grade plastic containers. The researcher also weighed 20-gram food grade sodium lauryl sulfate and dissolved it in a 100 mL beaker of distilled water. To simulate natural delignification, 5 grams of pod husk from the labelled sample were weighed and dissolved in 10 mL of sodium lauryl sulfate solution daily. A black precipitate from the mixture of pod husk and solution represents the amount of raw lignin. The researcher filtered and dried the precipitate using a desiccator for 24 hours before measuring the mass to measure the amount of removed lignin. This procedure was reiterated for 10 days for samples A, B, and C. The sample with the maximum lignin elimination on a specific day was selected for the next step.

Acid-Hydrolysis of Delignified Pod Husk

The researcher prepared 1, 2, 3, 4, and 5 millimolar (mM) sulfuric acid solutions using a volumetric flask, an aspirator, a pipette, and distilled water. Subsequently, 10 grams of naturally delignified pod husk were dissolved in each solution within a pre-heated 500 mL beaker at 60°C for 60 minutes. The resulting mixture was then cooled and filtered using Whatman No. 1 filter paper to eliminate insoluble constituents. The filtrate, pod hydrolysate, of each solution was analyzed for reducing sugars using 20 mL of Benedict's solution. The process was repeated three times for each sulfuric acid concentration. The solution with the highest amount of reducing sugars was selected for the following step.

Fermentation of Reducing Sugars

The researcher prepared five solutions of 10, 20, 30, 40, and 50 mg of food grade baker's yeast in an Erlenmeyer Flask. Each flask received 50 mL of pod hydrolysate and was covered with cotton and tape to avoid aerobic contamination. The fermentation was allowed to proceed for seven days, and the process was repeated three times for each yeast concentration.

Distillation of Raw Bio-ethanol

To remove impurities and thus filter the bio-ethanol substrate, the researcher conducted a distillation process utilizing the setup, which included a condenser, rubber tubing, an ice-cooled basin, a collecting tube, thermometer, cork stopper, and heat source. The crude bio-ethanol was put in the flask and heated to 75-80°C. The distilled bioethanol was collected in a 100 mL graduated cylinder for measurement of volume.

Determining the Concentration of Bio-ethanol

The researcher employs tri-iodomethane precipitate method to determine the concentration of bio-ethanol. A 10 mL of distilled bio-ethanol was mixed with 2g of sodium hydroxide in a flask. Subsequently, 10 mL iodide solution was added to the mixture. The amount of yellow precipitate formed corresponded to the ethanol concentration. The residue was filtered, dried, and weighed. The process was repeated three times.

Statistical Treatment

With the aid of university statistician, the researcher determined the means and standard deviations for each treatment groups using social statistica online calculator version 20. A test of normality was preliminarily conducted prior to the conduct of quantitative computations to ascertain normality of the data. To determine significant differences of means, one-way analysis of variance with Tukey's Post Hoc Test was employed at alpha level of 0.01.

RESULTS AND DISCUSSION:

Delignification of Cacao Pod Husk

Figure 1 illustrates the mean amount of removed lignin in milligrams and its corresponding standard deviation during the ten-day natural delignification. The figure discloses that the amount of removed lignin varies from day 1 to 2, with means of 11.33 ± 0.11, 11.63 ± 0.11 mg, respectively. Subsequently, a small rise was detected on days 3 and 4, with means 12.26 ± 0.20 and 12.66 ± 0.35 mg, respectively. From day 5 to 7, the amount of removed lignin stabilized, reaching a plateau, as seen in Figure 1. A decline of removed lignin was observed from day 8 to 10, which may be attributed to a decrease in lignin consumption by natural delignifiers. The natural delignifiers have progressively reduced their lignin consumption, as observed in Figure 1. Lignin compresses cellulose, which are crucial constituents in acid hydrolysis as well as in the synthesis of fermentable sugars (Pacubat, 2022). Efficient lignin elimination is vital for the liberation of cellulose, which is the main substrate for acidic hydrolysis (Palmqvist and Hahn-Hägerdal, 2000). While other studies have utilized pre-treatment chemicals like sodium hydroxide, potassium perchlorate, and concentrated acids for delignifying softwood and hardwood trees, this study proves the effectiveness of natural delignifiers for cacao pod husk.

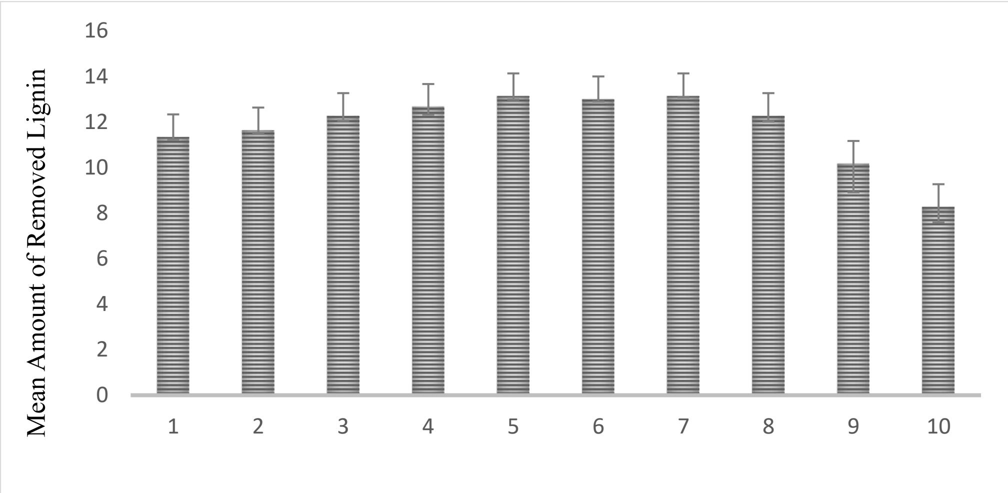

Figure 1. Amount of removed lignin in 10-day observation.

Acid-hydrolysis of Cacao Pod Husk

Figure 2 presents the mean and standard deviation of reducing sugars concentrations determined using Benedict's test. The mean reducing sugar concentrations derived from 1 to 5 millimolar were 3.66 ± 0.41, 3.73 ± 0.25, 3.53 ± 0.29, 3.93 ± 0.22, 3.53 ± 0.25 mg/L, respectively. Unlike previous methods using strong and concentrated acids (Ire et al. 2016), this study demonstrated that diluted sulfuric acid effectively digests the lignocellulosic component of pod husk in producing reducing sugars The study findings revealed that the mean fermentable sugar concentration among treatment groups have no significant difference. These results indicate that cellulose hydrolysis in delignified cacao pod husk is catalyzed effectively by dilute sulfuric acid. This also agrees with the previous research conducted by Lenihan et al. (2009) who reported that saccharification of lignocellulosic biomass can successfully be done through dilute acid hydrolysis. The employment of diluted sulfuric acid is considered a softer practice in acid hydrolysis than concentrated acid. This relatively lesser degree of harshness is likely to produce less corrosion effect on materials, lower amounts of inhibitory by-products (such as furfural and hydroxymethylfurfural), and hence may tend to have a lower overall environmental impact.

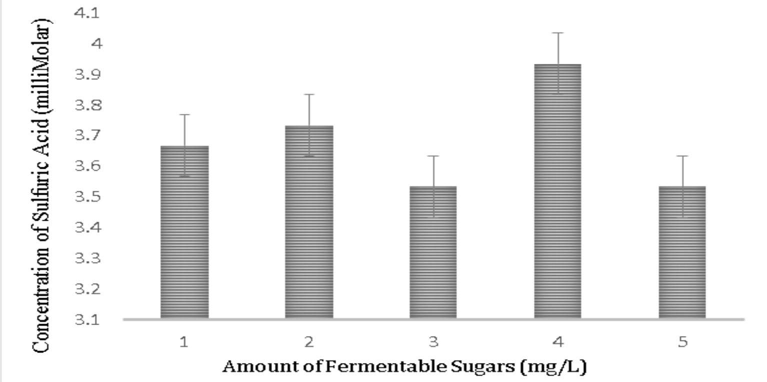

Figure 2. Amount of reducing sugars from acid-hydrolysis of cacao pod husk.

Fermentation of Reducing Sugars

Figure 3 exemplifies the bio-ethanol concentrations synthesized by fermentation using baker's yeast. It shows a declining trend of mean bio-ethanol concentrations from 10 to 50 mg of baker's yeast: 1.02 ± 1.21, 0.893 ± 0.90, 0.703 ± 0.55, 0.46 ± 0.17, and 0.257 ± 0.60 % w/v, respectively.

It was observed that as the amount of baker's yeast increases, synthesized bio-ethanol concentration decreases. This suggests an optimal ratio between baker's yeast and reducing sugars present in the pod hydrolysate. This finding aligns with the study of Ayuyo et.al (2016). As shown in table 3, 10 mg of baker's yeast yielded the highest mean bio-ethanol concentration, 1.02% as compared with other treatments.

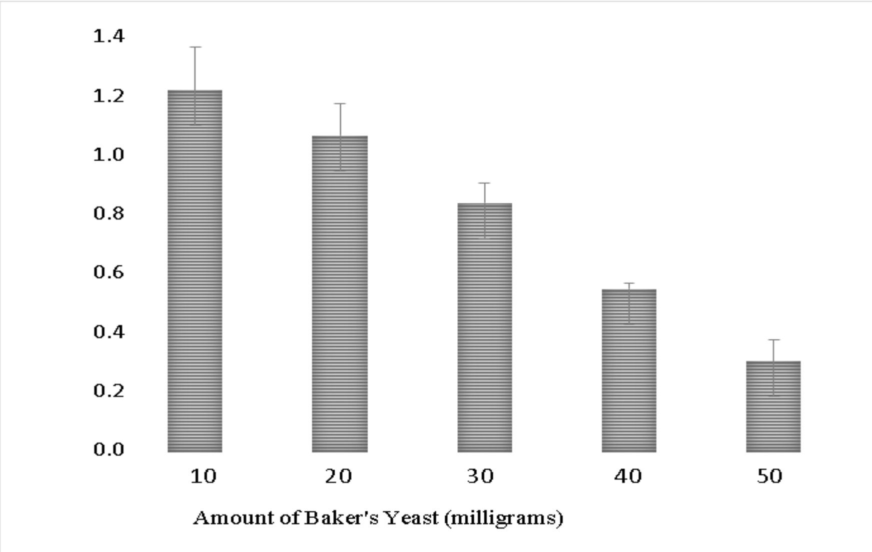

Figure 3. Concentration of synthesized bio-ethanol from varying concentrations of baker's yeast.

CONCLUSION:

The current study demonstrated that bio-ethanol is attainable from a sustainable and readily available agricultural waste product, which is cacao pod husk. To achieve this, the researcher improved the method to increase ethanol yield by using natural delignification and acid hydrolysis followed by fermentation with baker's yeast. The findings imply that hydrolysis was efficient with a 5-day natural delignification period and 1 mM concentration of sulfuric acid. Also, 10 mg of baker's yeast was demonstrated to be the best fermenting agent. This study presents a promising method of synthesizing bio-ethanol from cacao pod husks. On the other hand, more efforts should be directed to purification processes so that high-grade ethanol, more suitable for various purposes, is produced. Further process optimization and scale up potential exploration, this project seems to have the potential to reduce waste and escalate sustainable energy production. This research successfully showed the potentiality of producing bio-ethanol from a sustainable and abundant agricultural waste material, cacao pod husk. This advancement was achieved with green methods of natural delignification, followed by acid hydrolysis and fermentation with bakery yeast for the exploitation of ethanol yield. The research has been convincingly proven to show that it is a viable bio-ethanol production by an abundant agricultural the cacao pod husk. The process involved a combination of natural delignification, dilute acid hydrolysis, and fermentation with baker's yeast, which all seem very promising for converting such harmonious waste materials into bioenergy. Further research is suggested to optimize, as necessary, the entire process and make it economically viable, but more importantly, this study lays the groundwork for the production of bio-ethanol sustainably from cacao pod husk.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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