An Evaluation of the Alkaline Pretreatment and Hydrolysis on the Production of Bioethanol using Durio zibethinus (Durian) Husks

Athea Gene Buca[1], *Joanna Grace* Dayanan[1], *Heinzson John* Lim[1], *Karlo Isagani* Mosqueda[1] and *Lalaine Joan* Limbago[1*]

[1]Mapúa Malayan Colleges Mindanao, College of Engineering and Architecture, Davao City, 8000 Philippines

Abstract. Growing energy crisis and alarming environmental concerns make the development of biofuels an essential alternative to fossil fuels. Durian, a tropical fruit native to Southeast Asia, is a lignocellulosic biomass suitable for use as feedstock. Despite the increasing interest in bioethanol production, studies on the use of durian husks as feedstock remain limited. In this study, alkaline pretreatment and alkaline hydrolysis using sodium hydroxide (NaOH) were used to produce bioethanol. The NaOH concentrations used for pretreatment/hydrolysis were 1.5M/0.5M, 2.0M/1.0M, and 2.5M/1.5M. The samples were fermented, distilled, and analyzed. The distillates were qualitatively measured using refractometry and quantitatively measured using gas chromatography. The highest results were obtained from the 2.5M/1.5M alkaline group, which produced 44.67 mL of distillate and bioethanol concentration of 82.59%. The amount of distillate and bioethanol concentration increased as the pretreatment and hydrolysis concentrations increased. One-way ANOVA revealed a significant difference among the alkaline concentration groups in bioethanol production. However, Tukey Post-Hoc test showed that the difference in distillate volume and bioethanol concentration between the samples from 2.0M/1.0M and 2.5M/1.5M alkaline groups were not significant. Therefore, the results from the 2.0M/1.0M alkaline group are considered to deliver the optimum product of 42 mL distillate with 79.83% bioethanol concentration.

1 Introduction

Rising global temperatures, declining fossil fuel reserves, worsening air quality, and inevitable emissions have intensified the energy crisis worldwide. Therefore, it is imperative to develop alternative sources of clean and renewable energy. Bioethanol, one of the most widely produced biofuels, contains shorter carbon chains, resulting in lower emissions, making it a promising alternative energy source. Moreover, bioethanol exhibits favorable combustion characteristics, including short ignition timing, high octane number, and enhanced flame speed.

* Corresponding author: ljvalfeche@mcm.edu.ph

First-generation bioethanol is produced using edible crops such as corn, potato, and cassava. However, a major concern associated with these feedstocks is the competition between land use for bioethanol production and the global food supply, which raises serious food security issues [1]. This limitation has prompted the need for alternative non-edible feedstocks for bioethanol production.

The Philippines is among the countries that actively support biofuel utilization, including the production of bioethanol. However, challenges remain in sustaining sufficient resources to produce bioethanol. Given the archipelagic nature of the country, there is a growing need to explore new and alternative lignocellulosic feedstocks, particularly those derived from biomass waste such as agricultural residues, trash, and leaves. Prioritizing localized resource assessments is essential, particularly in areas with similar geographical conditions [2].

Therefore, second-generation bioethanol production is being promoted. Second-generation bioethanol uses lignocellulosic, non-edible feedstocks composed of carbohydrates, a bioethanol precursor that is convertible to sugar. Durian, an exotic fruit found in Southeast Asian countries, is a lignocellulosic material that can be used as a feedstock to produce second-generation bioethanol.

Despite the increasing interest in second-generation bioethanol, studies on the use of durian husks as a feedstock remain limited, particularly in the Philippine context. Existing research has mainly focused on other agricultural residues or durian waste in different countries, with little emphasis on optimizing alkaline pretreatment conditions for durian husk waste [3]. The effects of varying alkaline pretreatment concentrations on bioethanol yield are not yet well established, highlighting the need for targeted investigations to support locally sustainable bioethanol production

This study investigated the production of bioethanol from durian husks through alkaline pretreatment and hydrolysis. Varying pretreatment/hydrolysis concentrations of sodium hydroxide (NaOH) from 1.5M/0.5M, 2.0M/1.0M, and 2.5M/1.5M were employed to evaluate their effects on bioethanol yield and concentration. This study investigates the combined use of alkaline pretreatment and hydrolysis on durian husks to optimize bioethanol production. By evaluating the effects of different NaOH concentrations on sugar release and ethanol yield, this study addresses gaps in integrating pretreatment and hydrolysis for efficient conversion of durian waste into biofuel.

The findings of this study are expected to contribute to the advancement of cleaner and more sustainable energy sources by promoting the utilization of biomass waste and reducing environmental pollution.

2 Literature Review

The increasing demand for sustainable energy has driven extensive research on bioethanol as an alternative fuel source. First-generation bioethanol derived from edible crops has been widely studied and commercialized. However, concerns regarding food security and land-use competition have prompted a shift toward second-generation bioethanol production from lignocellulosic biomass [1]. Lignocellulosic materials are abundant, renewable, and non-edible, making them attractive feedstocks for sustainable bioethanol production.

The promotion of biofuels has been institutionalized in several countries through government policies. In the Philippines, the National Biofuels Board (NBB) of the Department of Energy (DOE) implemented the Philippine Biofuels Law, which promotes the use of sustainable feedstocks for biofuel production. According to the Philippine Statistics Authority Region XI, Durian is among the top five fruit crops produced in the Davao region. In 2019, durian production increased at an annual rate of 13.9% over the past 3 years [4]. This high production rate generates a significant amount of biodegradable waste, particularly

durian peels, which are often discarded. Waste generated from durian peels has strong potential for utilization and conversion into value-added products.

Similar concerns have been reported in other Southeast Asian countries. In Indonesia, increased durian production has led to the accumulation of durian peel waste, which is commonly burned, releasing harmful substances into the atmosphere [5]. The high lignocellulosic content of durian peels renders them a promising feedstock for bioethanol production. In Malaysia, durian seeds and husks are considered agricultural waste, however, they are rich in carbohydrates in the form of starch and lignocellulose, making them suitable for bioethanol production [6].

Early studies have demonstrated the feasibility of converting durian biomass into fermentable sugars and ethanol using acid or enzymatic hydrolysis. However, these methods often result in limited delignification and relatively modest bioethanol yields [7]. To improve conversion efficiency, recent studies have applied alkaline pretreatment, particularly using sodium hydroxide (NaOH), to enhance lignin removal and increase carbohydrate accessibility. Alkaline pretreatment has been shown to significantly improve sugar recovery from durian waste, leading to better conditions for subsequent hydrolysis and fermentation processes. Alkaline pretreatment improves the digestibility of durian biomass by removing lignin and increasing cellulose accessibility, but it faces several challenges. The rigid structure of durian peels limits enzymatic sugar release, and optimizing NaOH concentration, temperature, and reaction time is critical to avoid carbohydrate degradation [8].

Enzymatic hydrolysis is widely employed in bioethanol production to release sugar monomers from structural carbohydrates, such as cellulose and hemicellulose. This causes depolymerization of the cellulose into fermentable sugars; however, for enzymatic hydrolysis to become feasible, solid loading of at least 10% w/v is needed, suggesting that lab-scale experiments may not be applicable [9]. The major drawbacks of enzymatic hydrolysis are the long sample treatment time that may last 5-12 hours, low analyte recoveries, and enzyme handling and storage difficulties [10].

The drawbacks of utilizing acid pretreatment and enzymatic hydrolysis highlight the importance of developing a more efficient and effective method for producing bioethanol using lignocellulosic biomass. Despite the technical challenges associated with lignocellulosic bioethanol production, durian waste shows strong potential as a sustainable bioethanol feedstock and holds promise in the renewable energy market. Continued research and optimization of pretreatment and fermentation techniques are essential to fully realize the potential of durian waste for large-scale bioethanol production in the future.

This study investigated the combined application of alkaline pretreatment and hydrolysis of durian husks to optimize bioethanol production. While previous research has examined alkaline pretreatment to remove lignin or hydrolysis to release fermentable sugars, there is a limited systematic study integrating both processes and evaluating the effects of varying sodium hydroxide (NaOH) concentrations.

Alkaline pretreatment and hydrolysis can be performed at lower temperatures and pressures, do not depend on the lignin content, and the materials needed are more accessible than those required for acid hydrolysis [11]. By examining how different alkaline pretreatment conditions influence subsequent hydrolysis and bioethanol yield, this study aims to provide a comprehensive understanding of the conversion of durian waste into bioethanol, thereby addressing existing gaps in the process optimization of lignocellulosic feedstocks.

Converting agricultural residues into bioethanol offers a promising solution that mitigates waste disposal issues while contributing to renewable energy generation. By investigating durian waste as a feedstock, this study supports circular economy practices, reduces reliance on fossil fuels, lowers greenhouse gas emissions, and advances sustainable development and energy security.

3 Materials and Methods

3.1 Data Collection

The procedures mentioned in this study were conducted solely in the City of Davao. The raw materials were obtained from local durian vendors at the Bankerohan Public Market. Laboratory experiments, such as pretreatment, fermentation, and distillation, were performed in the Chemistry Laboratory of Mapúa Malayan Colleges Mindanao. Hydrolysis and gas chromatography were performed at the Center of Green Nanotechnology Innovations for Environmental Solutions (CGNIES) at the University of Mindanao, Matina Campus.

This study was quantitative in nature, and a quasi-experimental design was utilized by the researchers. A quasi-experimental design is commonly used to establish a cause-and-effect relationship between the dependent and independent variables. In quasi-experimental studies, changes are made to the independent variables; however, unlike true experiments, they do not rely on random assignments. There are three different approaches under quasi-experimental design, but the researchers used the natural experiment approach. Thus, the researchers conducted different trials with several samples applied at different concentrations used in pretreatments and hydrolysis to gather the necessary data for the study. The data gathered were compared and analyzed using the instruments and tools specified and discussed in the succeeding sections.

3.2 Research Procedure

3.2.1 Collection and Drying of Durian Husks

The researchers gathered the durian husks from the Bankerohan Public Market. The collected husks were washed to remove dirt and other materials. After ensuring that the husks were clean, they were dried in an oven at 120°C for 8 h and sun-dried for 2 weeks [12]. This ensured that the husks were completely dry and that no moisture remained. After drying, the durian husks were ground. This was done using a heavy-duty grinder until the husks were separated and durian fibers were observed.

3.2.2 Pretreatment Process

An alkaline pretreatment method was used. Sodium hydroxide (NaOH) pellets were dissolved in water for this purpose. A 500 mL solution of NaOH with concentrations of 1.5M, 2.0M, and 2.5M were prepared. After preparing NaOH solutions of different concentrations, 25 g of durian husk fibers were soaked in each NaOH solution. The solutions with the durian fibers were then left to soak for 24 hours while continuously being stirred at 600 rpm. After 24 hours, the fibers were filtered and washed with distilled water to neutralize the pH. In the next step, the pretreated durian fibers were dried in an oven at 200°C for 12 h.

3.2.3 Hydrolysis

In the alkaline hydrolysis process, three samples of pretreated durian fibers weighing 25 g were prepared, along with three 500 mL NaOH solutions with concentrations of 0.5M, 1.0M, and 1.5M. The weighed pretreated samples were then added to the NaOH solution. For hydrolysis, each pretreated fiber sample was added to its designated NaOH solution: durian fiber pretreated with 1.5 M NaOH was hydrolyzed in 0.5 M NaOH, fiber pretreated with 2.0 M NaOH was hydrolyzed in 1.0 M NaOH, and fiber pretreated with 2.5 M NaOH was

hydrolyzed in 1.5 M NaOH. The NaOH and fiber solutions were then placed in a 40 kHz frequency ultrasonic instrument for 5 min. After sonication, the mixtures were placed in a water bath for 90 min at 60°C.

3.2.4 Fermentation

The hydrolyzed samples were placed in a 4-liter container and a 0.3 g/L of the yeast strain was prepared. The mixture was stirred at 150 rpm to fully mix. The yeast was then placed in the container with a small hole to release the carbon dioxide gas generated during the process. The fermentation process was performed for 7 days. After 7 days, the fermented samples were filtered to separate the solids from the liquids. The solids were discarded and considered as waste. The filtered liquid was stored in an airtight container and used in the distillation process.

3.2.5 Distillation

A distillation flask, hot plate, condenser, and collecting flask were used in the distillation process. In the distillation process, the fermented solution was placed in a distillation unit. A temperature of 78°C was maintained for 2 hours. The three samples for each NaOH with different concentrations were distilled. The collected products were placed in an airtight glass container.

3.2.6 Characterization of Distillate

After distillation, the collected distillate was measured. The first bioethanol analysis was conducted using refractometer. In this step, a handheld refractometer was used for each distillate, and the Brix was measured. The Brix measured should be at least 17° to confirm the presence of alcohol in the distilled sample. Gas chromatography was conducted to further confirm the presence and purity of bioethanol in the product.

3.3 Data Analysis Methods

The study used Analysis of Variance (ANOVA) tests. ANOVA was used to compare the means of experiments containing three or more groups. ANOVA is a statistical treatment used to analyze the variation between the means of two or more groups and their treatments. Calculations were performed at a 0.05 alpha (α) value for the level of significance.

4 Results and Discussion

4.1 Refractometry in Brix

After the distillation process, a handheld refractometer was used to confirm the presence of bioethanol in the distillate. The refractive index of bioethanol was measured in Brix as shown in Table 1.

Table 1. Refractive index in Brix

Alkaline Pretreatment/Hydrolysis Group	Trial Number	Brix
1.5M/0.5M	1	17°
	2	19.5°
	3	19°
2.0M/1.0M	1	19.2°
	2	19.5°
	3	19°
2.5M/1.5M	1	19°
	2	19.5°
	3	20°

When Brix content is 17° or higher, it implies adequate sugar content for yeast to produce alcohol. It can be observed that the values for all alkaline pretreatment/hydrolysis groups ranged from 17° to 20° Brix indicating the presence of the alcohol.

4.2 Distillate

After the distillation process, the distillate was measured to determine the amount of distillate produced. Figure 1 shows the distillate yields under different alkaline pretreatment/hydrolysis conditions. The results revealed that the amount of distillate gathered from distillation was highest at 2.5M/1.5M alkaline group compared to 1.5M/0.5M and 2.0M/1.0M. For three trials at each concentration, it can be observed that for the 1.5M/0.5M alkaline group, a value of 31.00 ± 1.73 was obtained. For the 2.0M/1.0M alkaline group, a value of 42.00 ± 3.0 was achieved. Finally, for 2.5M/1.5M alkaline group, a value of 44.67 ± 3.06 was attained. This indicates that the amount of distillate is directly proportional to the concentration of alkaline used in this study, meaning that the higher the concentration of NaOH used in pretreatment and hydrolysis, the higher the yield.

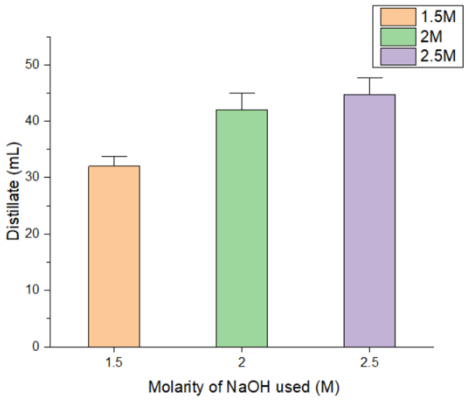

Fig. 1. Volume of distillate collected in different alkaline pretreatment concentrations

To further clarify the significant difference between the values, a One-Way ANOVA at a 0.05 level of significance was conducted. Table 2 shows the computed P-value of 0.0017, which was below the alpha (α) value of 0.05. A value below the level of significance indicates a difference between the three concentrations of NaOH used in terms of the distillate

collected. The increase in distillate volume can be attributed to the enhanced effectiveness of higher NaOH concentrations in disrupting the lignocellulosic structure, particularly through lignin removal and increased cellulose accessibility, which improves downstream hydrolysis and fermentation.

Table 2. ANOVA results comparing the yield of distillates

Alkaline Pretreatment/Hydrolysis Group	Mean	SD	F value	P-value	Remarks
1.5M/0.5M	31.00	1.73	22.140	0.0017	Significant
2.0M/1.0M	42.00	3.00			
2.5M/1.5M	44.67	3.06			

4.3 Bioethanol Concentration

To further test the presence of bioethanol and know the concentration of the bioethanol produced in the experiment, gas chromatography was conducted to quantitatively test the samples. Figure 2 shows the percentage of bioethanol was highest at 2.5M/1.5M alkaline group compared with 1.5M/0.5M and 2.0M/1.0M NaOH groups. From the results presented, the concentration of bioethanol produced from the 1.5M/0.5M alkaline group is 72.32% ± 7.19. For the 2.0M/1.0M alkaline group it can be observed that the concentration of bioethanol is at 79.83% ± 2.58 and for 2.5M/1.5M alkaline group, the value of the produced bioethanol concentration is at 82.59% ± 4.92.

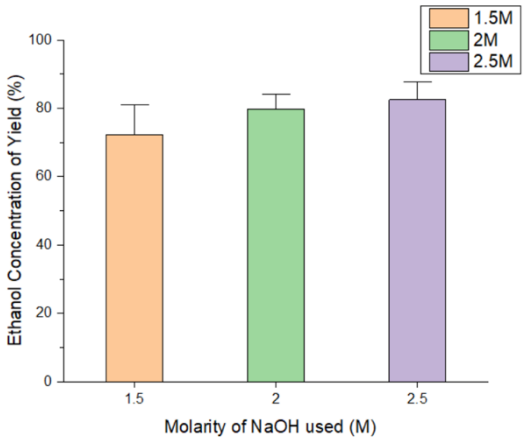

Fig. 2. Percentage of bioethanol in different alkaline pretreatment concentrations

With the resulting values, One-Way ANOVA was conducted to confirm the difference between the concentrations of the produced bioethanol. As shown in Table 3, the P-value is 0.0012 which was below the alpha (α) value of 0.05. Therefore, there is a significant difference between the bioethanol concentration produced from the different NaOH concentrations.

Table 3. ANOVA result comparing the concentration of bioethanol produced

Alkaline Pretreatment/Hydrolysis Group	Mean	SD	F value	P-value	Remarks
1.5M/0.5M	72.3744	7.19	9.139	0.0012	Significant
2.0M/1.0M	79.8305	2.58			
2.5M/1.5M	82.5908	4.92			

A post-hoc Tukey HSD test was conducted to confirm whether the differences in bioethanol concentrations were statistically significant, specifically comparing the bioethanol produced at different alkaline pretreatment and hydrolysis groups. The results showed significant differences between the 1.5M/0.5M alkaline groups and the 2.0M/1.0M and 2.5M/1.5M alkaline groups, with p-values of 0.016 and 0.001, respectively. However, the post-hoc test also indicated that there was no significant difference between bioethanol produced using pretreated 2.0M NaOH hydrolyzed in 1.0 M NaOH and pretreated 2.5 M NaOH hydrolyzed in 1.5 M NaOH, as the p-value (0.514) was greater than the alpha value of 0.05. This implies that 2.0 M/1.0M NaOH is the optimum concentration for maximizing bioethanol production under the conditions of this study.

According to a study on bioethanol production, bioethanol produced from durian seeds with a bioethanol content of 17.93 mL/L is achievable for a pretreatment concentration of 0.522 M NaOH. However, it has been found that durian husks are more lignocellulosic in comparison to its seed [6]. That said, this study's result using pretreated 2.0M NaOH hydrolyzed in 1.0 M NaOH of 44. 67 mL bioethanol with a concentration of 81.39% is greater than the other studies for bioethanol production from durian. Moreover, present studies shows that an alkaline concentration of 2.5M is the optimum concentration for alkaline pretreatments as concentrations higher than 2.5M did not give higher efficiency [13]. However, the significant difference between the bioethanol produced using 2.0M/1.0M and 2.5M/1.5M alkaline groups are not that significant; therefore, making 2.0M NaOH a better substitute to the optimum pretreatment concentrations found in present studies.

In the study of the production of bioethanol from coconut fibers using alkaline hydrolysis, untreated fibers have high lignin and cellulose content. This study presented results that indicate that alkaline pretreatment removed lignin from the biomass, imparting higher fiber reactivity, and the cellulose content important to produce bioethanol increased [14]. Alkaline hydrolysis also showed great assistance in increasing the fermentation performance in bioethanol production. Furthermore, the hydrophobicity of the substrate decreased with the use of alkaline pretreatment before hydrolysis [15]. In addition to that, in alkaline hydrolysis, a large portion of lignin was removed by breaking ester bonds cross linkage between lignin and xylan, increasing the porosity of the biomass [16].

5 Conclusion

Alkaline hydrolysis and pretreatment were used in this study to produce bioethanol. The NaOH concentrations used for pretreatment/hydrolysis were 1.5M/0.5M, 2.0M/1.0M, and 2.5M/1.5M. Based on the experimental results, alkaline pretreatment and alkaline hydrolysis were effective in producing bioethanol from durian husks. The higher the NaOH concentration in both pretreatment and hydrolysis, the higher the bioethanol yield and concentration. The bioethanol yield was highest at 44.67 ± 3.67 mL in the group pretreated in 2.5M NaOH and hydrolyzed in 1.5M NaOH compared to the other groups. The bioethanol concentration in the 1.5M/0.5M alkaline group was 72.37% ± 7.19 and 79.83% ± 2.57 for the 2.0M/1.0M alkaline groups. The 2.5M/1.5M alkaline group resulted to 82.59% ± 4.92 bioethanol concentration. The results showed that the yield and concentration were directly

proportional to the NaOH concentration used, which was also observed in the ANOVA, where there was a significant difference between the alkaline groups tested.

The post-hoc Tukey HSD results showed significant differences between the 1.5M/0.5M alkaline groups and the 2.0M/1.0M and 2.5M/1.5M alkaline groups, with p-values less than the alpha value. However, the post-hoc test also indicated that there was no significant difference between bioethanol produced using 2.0M/1.0M and 2.5M/1.5M alkaline groups, as the p-value (0.514) was greater than the alpha value of 0.05. This suggested that there was no significant difference between the bioethanol concentrations produced at 2.0M/1.0M and 2.5M/1.5M alkaline groups. This study suggests that the use of 2.0 M NaOH concentration for pretreatment and 1.0 M NaOH concentration for hydrolysis, is to be used on the succeeding studies for it yields statistically better results than that of the 2.5M/1.5M alkaline group.

In future studies, it is recommended to advance the experiments using the 2.0M/1.0M alkaline group. Other parameters can be explored such as extending the experiment period, specifically, the fermentation process, to monitor overlooked changes and effects. Other variations in the pretreatment and hydrolysis steps may be explored. Moreover, this procedure may also be applied to different lignocellulosic biomass available.

Acknowledgment

The authors would like to express their sincere gratitude to Mapúa Malayan Colleges Mindanao for providing access to essential research databases and facilities. The authors also acknowledge the invaluable support and guidance of their mentors and colleagues, whose insights and constructive feedback significantly contributed to the improvement of this study. Special appreciation is extended to the researchers whose prior work laid the foundation for the present research.

References

1. R. Muktham, S. K. Bhargava, S. Bankupalli, A. S. Ball, JSBS, **06** (2016)
2. A. W. Go, A. T. Conag, R. M. B. Igdon, A. S. Toledo, J. S. Malila, ESR, **23** (2019)
3. F. Hu, A. Ragauskas, BR, **05** (2012)
4. K. M. Gatdula, R. B. Demafelis, B. G. Bataller, Bioethanol (2021)
5. Y. Yuliusman, M. P. Ayu, A. Hanafi, A. R. Nafisah, *Activated carbon preparation from durian peel wastes using chemical and physical activation*, in Proceedings of the International Symposium on Sustainable and Clean Energy (ISSCE): Quality in Research 2019, 22-24 July 2019, Indonesia (2019)
6. T. H. Chriswardana, Y. Mulyaningsih, Y. Mulyaningsih, A. H. Bahar, M. Indra, CE, **05** (2021)
7. H. B. Aditiya, T. M. I. Mahlia, W. T. Chong, H. Nur, A. H. Sebayang, ER, **66** (2016)
8. D. Ariyanti, N. A. Sasongko, R. Maryana, R. A. Nugroho, S. Thamrin, D. Rimantho, Y. Wahyono, A. K. Das, O.Y. Garia, F. Tayon, IJAE, **45** (2024)
9. H. R. Velankar, C.Thulluri, A. J. Mattam, Advanced Biofuel Technologies (2022)
10. X. B. Yin, P. Wu, Y. Li, X. P. Yan, E&F, **24** (2012)
11. A. A. Shah, T. H. Seehar, K. Sharma, S. S.Toor, Hydrocarbon Biorefinery (2022)
12. P. Bhandari, What Is Qualitative Research Methods & Examples (2020) https://www.scirp.org/reference/referencespapers?referenceid=3087568

13. D. Q. Nguyen, L. N. M. Nguyen, T. T. A. Tran, H. N. Cao, T. K. P. Le, T. P. Mai, *Ultimate pretreatment of lignocellulose in bioethanol production by combining both acidic and alkaline pretreatment*. MATEC Web of Conferences (2019)
14. M. M. Cabral, A. K. Abud, C. E. Silva, R. M. R. Almeida, CR, **46** (2016)
15. F. Meng, H. Yang, Z. Shi, P. Zhao, J. Yang, BT, **358** (2022)
16. G. Chaudhary, L. K. Singh, S. Ghosh, BT, **124** (2012)