Optimizing the conditions of deep eutectic solvents pretreatment for the production of bioethanol from Water hyacinth using Response Surface Methodology (RSM)

Sukunya Areeya [1], *Frauke* Weber [2], *Malinee* Sriariyanun [1], *Atthasit* Tawai [1] and *Suksun* Amornraksa [1*]

[1]Biorefinery and Process Automation Engineering Center, Department of Chemical Engineering and Management, The Sirindhorn International Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand
[2]Faculty of Chemistry, Aalen University, Aalen, Germany
[*]corresponding author: suksun.a@tggs.kmutnb.ac.th

Abstract. Water hyacinth reproduces rapidly, causing numerous issues across various sectors, including irrigation, agriculture, and invasion to native plant species and ecosystems. As a lignocellulosic biomass, water hyacinth holds potential as a raw material in chemical and biotechnological applications. However, its resistance to enzymatic breakdown during hydrolysis necessitates pretreatment to enable the efficient production of bioproducts from its components. This study aimed to determine the optimal Deep Eutectic Solvent (DES) pretreatment conditions for water hyacinth using the Box-Behnken design under the Response Surface Methodology (RSM) in order to maximize the sugar recovery rate. The DES pretreatment conditions, using a mixture of choline chloride and monoethanolamine (ChCl:MEA) in a 1:4 molar ratio, were optimized by varying the solid-to-liquid ratio (1:5 to 1:15, w/w), temperature (90–130 °C), and reaction time (60–240 min). The RSM model estimated the optimal pretreatment conditions to be a solid-to-liquid ratio of 1:9.58 (w/w) at 90 °C for 60 min, resulting in a maximum sugar recovery rate of 44.22%. The impact of pretreatment on chemical structure, as revealed by Fourier Transform Infrared (FTIR) spectroscopy and X-ray diffraction (XRD) analyses, confirmed the efficiency of the DES pretreatment (ChCl:MEA). Therefore, this study highlights the potential of water hyacinth as a source for producing value-added compounds..

1 Introduction

In times of the search for alternative energy options approaches that tackle more than one problem seem favorable. The use of water hyacinth as biomass for generating bioethanol fits this concept. Water hyacinth (*Eichhornia crassipes*) is an invasive aquatic plant that spread all over the world on five continents [1]. It originated from Brazil, the Amazon basin and the Ecuador region [2] before invading over 50 countries [1]. With a grow rate of up to 140 tons of dry matter per hectare per year water hyacinth is counted to be one of the most productive plants [3]. Imported originally as ornamental plants for gardens, it is necessary to face the severe ecological and socio-economic effects caused by the excessive spreading of the plant [1]. As an alien plant without natural enemies and competition in most places, the massive growth endangers waterbodies and their affected ecosystem [2]. The invasion has an influence on the physic-chemical characteristics of the water by changing the temperature, pH and nutrient levels [3]. Even in times of drought, the seeds are able to survive for months or years [2]. This makes it hard to get control of the excessive growth and invasion. Nevertheless, water hyacinth has been applied for various beneficial purposes, such as removal of heavy metals and inorganic and organic components from water [2,4]. Additionally, the rapid growth of water hyacinth with limited needs for nutrient supplies and lands make it become potential raw materials for lignocellulose biomass for production of biofuels and biochemicals with low cost [5].

Lignocellulose biorefining process is a multiple-step process including pretreatment, hydrolysis, fermentation and product recovery. Hydrolysis step has a main function to break the lignocellulose into monomeric sugars that are subsequently fermented to produce biofuels and biochemicals. The goal of pretreatment is to reduce the size of the biomass and promote vulnerability of lignocellulose for enzymatic saccharification by removing lignin and hemicellulose. [6]. The process to generate bioethanol from biomass involves chemical pretreatment which allows lignin to be solubilized and dissolves hemicellulose. One sustainable pretreatment method involves deep eutectic solvents (DES) [7]. Their properties are similar to ionic liquids at the advantage of lower cost and less toxicity [6]. One of the key elements of successful pretreatment is the removal of lignin or modification of its structure [8]. DES helps to remove the lignin while the cellulose stays unaffected [7].

Water hyacinth is a promising biomass as it was shown to be high in cellulose and hemicellulose while

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being low on lignin [9]. So far, various pretreatment methods like acid, alkali, biological and physical were tested for water hyacinth, [10] however the DES application is kept nearly untouched. Umai et al worked with a DES synthesized out of choline chloride and urea to optimize the delignification of water hyacinth, achieving a lignin removal of 64.32% (w/w) [11]. Previous work from Sun et al. (2023) studied the pretreatment of Pennisetum giganteum (PG) using different types of DES [13]. The results showed that among these DESs, ChCl/MEA exhibited the highest potential for PG biomass pretreatment, achieving the highest lignin removal of 79.8 % while retaining 89.5 % of cellulose. Furthermore, this DES pretreatment reached a 95.6 % glucose yield and 88.0 % xylose yield [12]. To optimize the efficiency of DES pretreatment, response surface methodology (RSM) was used. It is a statistical tool for the optimization of parameters in processes. Based on quadratic polynomial equations an experiment plan is developed with minimal runs of experiments. It allows us to observe interactions between the pretreatment parameters and to give the accurate predictions of optimal condition comparing to other statistical methods [13].

This study aimed to determine the optimal pretreatment conditions using RSM with a Box-Behnken design to assess the effectiveness of DES pretreatment on water hyacinth using choline chloride and monoethanolamine (ChCl/MEA). The efficiency of DES pretreatment was evaluated through chemical characterization by using Fourier Transform Infrared (FTIR) spectroscopy and X-ray diffraction (XRD) to understand the effect of ChCl/MEA on water hyacinth biomass.

2 Materials and Method

2.1 Materials

Chlorine chloride (ChCl) was purchased from Sigma Aldrich, St. Louis, USA. Monoethanolamine (MEA) was acquired from TCI (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), with a purity of at least 99%. All reagents used in this research were of analytical grade. The commercial cellulase enzyme, Ctec2, was procured from Sigma Aldrich, St. Louis, USA. Distilled water was sourced from the chemical department's processing laboratory.

2.2 Preparation of Water hyacinth

Water hyacinth biomass utilized for feedstock in this study was collected from the local freshwater river in Nonthaburi province, Thailand. The biomass was chopped and dried at 60 °C overnight to reduce moisture content, then ground using a food grinder to decrease particle size. The material was subsequently sieved through a 20-mesh (0.841 mm) aluminium screen to achieve uniformity. The resulting powder was sealed in an airtight container and stored at room temperature until further use.

2.3 Preparation of deep eutectic solvents

A DES, ChCl/MEA, was prepared by combining two components: choline chloride (ChCl) as the hydrogen bond acceptor (HBA) and monoethanolamine (MEA) as the hydrogen bond donor (HBD), in a molar ratio of 1:4. The synthesis process was carried out using a magnetic stirrer set at 100 rpm and maintained at 80 °C for 20 min, following the method by Sun et al. (2023) [12]. After synthesis, the DES was stored in a sealed reagent bottle and kept in a vacuum desiccator containing silica gel to prevent moisture absorption [14].

2.4 RSM experiment design

RSM is a statistical tool commonly used to efficiently design experiments with a minimal number of trials, thereby reducing time and costs [13]. It involves mathematical and statistical techniques to develop empirical models based on experimental data aligned with a specific design strategy [15]. RSM helps assess the influence of different variables on the response and optimizes the independent parameters. Among its approaches, the Box–Behnken Design (BBD) is frequently employed to analyze the effects of experimental factors in various studies [13,16-17]. The designed experimental conditions based on the Box–Behnken design (BBD) with three independent variables were conducted using 8 factorial points, 6 axial (star) points, and 3 duplicate center points.

The experimental results were analyzed using a second-order polynomial equation (quadratic model), as shown in Equation (1) [15], to assess the influence of individual variables on the response and identify the optimal processing conditions [13]. Variables with a statistically significant effect on the response were identified based on a confidence level exceeding 95%, corresponding to a p-value below 0.05. [18].

$$Y=\beta_0+\beta_1 A+\beta_2 B+\beta_3 C+\beta_{11} A^2+\beta_{22} B^2+\beta_{33} C^2+\beta_{12}AB+\beta_{13} AC+\beta_{23} BC \quad (1)$$

In this equation, y represents the response, while A, B, and C denote the independent variables. $ß_0$ is the constant term, β_1, β_2, and β_3 are the linear coefficients, β_{11}, β_{22}, and β_{33} are the quadratic coefficients, and β_{12}, β_{13}, and β_{23} represent the interaction coefficients. [13].

The coefficients and interaction effects of the parameters in Eq. (1) were determined, and Analysis of Variance (ANOVA) was applied to assess the significance of the three independent variables [13]. These variables included pretreatment temperature (A, °C), reaction time (B, min), and the solid-to-liquid ratio (C, w/w), with value ranges set at 90–130 °C, 60–240 min, and 1/5–1/15 w/w, respectively. A total of 17 experimental runs were recommended by the design, varying the process variables at different levels (Table 1). To identify the optimal conditions for maximizing biomass recovery from water hyacinth pretreatment, this study utilized Design-Expert 7.0.0 software (Stat-Ease Inc., Minneapolis, MN, USA) to analyze the influence of each variable across the 17 experiments and predict the corresponding recovery rates.

2.5 Deep eutectic solvents pretreatment

The ground and dried water hyacinth underwent ChCl/MEA pretreatment using a stirring mantle under the conditions specified in Table 1. Following the DES treatment, the samples were left to cool at room temperature to stop the reaction process [19]. The solid portion was rinsed with 50 mL of distilled water per gram of biomass to eliminate any remaining DES. The washed solution was then adjusted to a neutral pH prior to enzymatic saccharification and then filtered through 125 mm filter paper (chm Cat No. F1001 125). Solid fraction was dried overnight at 60 °C in a hot air oven and subsequently stored in an airtight container for further saccharification and analysis (Figure 1) [20].

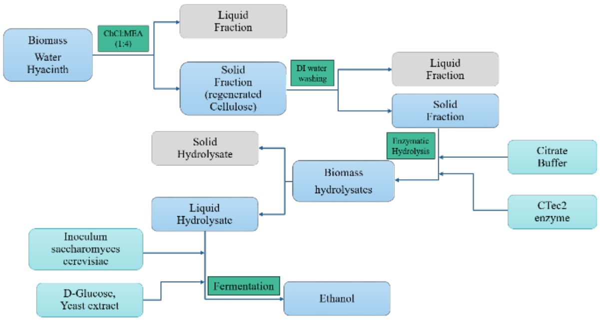

Fig. 1. Process flow of pretreatment, enzymatic saccharification, and fermentation process of water hyacinth biomass.

Table 1. Experimental design of response surface methodology for pretreated water hyacinth biomass.

Run	S/L ratio (w/w)	Temperature (°C)	Time (min)	Sugar yield (%)
1	5	120	150	22.494
2	20	120	150	26.628
3	12.5	95	150	26.265
4	5	70	150	23.769
5	20	95	240	28.778
6	12.5	70	240	25.055
7	12.5	70	60	17.352
8	12.5	95	150	29.165
9	12.5	120	60	20.488
10	5	95	240	18.371
11	12.5	95	150	24.350
12	20	95	60	21.771
13	20	70	150	32.126
14	5	95	60	26.191
15	12.5	120	240	25.722
16	12.5	95	150	25.970
17	12.5	95	150	21.794

2.6 Chemical characterization of raw and pretreated sugarcane leaves

2.6.1 Fourier-transform infrared spectroscopic analysis (FTIR)

FTIR spectroscopy was employed to precisely determine the chemical composition of biomass and to identify functional groups. In this study, an FTIR spectrometer (Spectrum 2000, Perkin-Elmer, USA) was used to examine structural changes in untreated and pretreated water hyacinths. Each sample was analyzed in triplicate, with spectra recorded in transmission mode across a wavenumber range of 400 to 4000 cm^{-1}, using 16 scans per minute at a resolution of 4 cm^{-1} to observe shifts in wavenumber [21].

2.6.2 X-ray diffraction analysis

The structure of lignocellulose, especially of the amorphous and crystalline structure can be evaluated using X-ray diffraction (XRD) analysis [15]. The XRD analysis provides information on the crystalline regions of cellulose within the biomass. In this study, the crystallinity of both untreated and pretreated water hyacinth was examined using a powder XRD instrument (Model: SmartLab Studio II, Rigaku, Japan), operating within a 2θ range of 5° to 80°, at a scan speed of 5°/min, and utilizing Cu-Kα radiation (λ = 0.154 nm) as the monochromatic source [14]. The crystallinity index (CrI) was determined via the peak intensity method, calculated as the ratio between the maximum peak intensity at 2θ = 22.0 (I_{002}) and the minimum intensity at 2θ = 18.0 (I_{am}), which lies between the 001 and 002 diffraction peaks. [22].

$$\text{Crystalinity Index, CrI } (\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \qquad (2)$$

2.7 Enzymatic saccharification

The process of enzymatic saccharification is well known for breaking the bonds of the cellulose and hemicellulose components in lignocellulosic biomass [23]. A 2.5% (w/v) of solid loading was used to hydrolyze the untreated and pretreated water hyacinth with 50 mM citrate buffer (pH 4.8 ± 0.2) and the cellulase enzyme Ctec2 (30 FPU/g biomass) at 50 °C and 150 rpm for 72 h [16].

2.8 Reducing sugar analysis by using DNS assay

The reducing sugar concentration was measured using the 3,5-dinitrosalicylic acid (DNS) method as described by Miller [24]. In this assay, 50 µL of the hydrolysate supernatant was mixed with 150 µL of DNS reagent. The mixture was vortexed, heated at 95 °C for 5 min in a water bath, and then cooled on ice for another 5 min. Afterward, 1 mL of deionized water was added, and the absorbance was recorded at 540 nm using a UV-Vis spectrophotometer (T80+ UV/Vis Spectrometer, PG Instrument Ltd., USA).

3 Results and Discussions

3.1 Results and Discussions

In this study, RSM was utilized to optimize the process parameters of DES pretreatment for maximizing total sugar yield (g/g) and enhancing the recovery rate of water hyacinth. Multiple regression analyses were applied to evaluate the experimental data. BBD [25], a common RSM approach, was used with three input variables: pretreatment temperature (A), reaction time (B), and solid-to-liquid ratio (C). A total of 17 experimental runs were performed, and the recovery rate was measured as the response. Analysis of variance (ANOVA) was employed to assess the significance of the quadratic model. A high F-value and a low p-value ($p<0.0001$) indicated strong model significance across all variables, whereas a low F-value would suggest the presence of random variation or noise in the results. [22]. The ANOVA results confirmed the model's significance (Table 2), presenting an F-value of 27.28 with the model's regression coefficient (R^2) at 0.9550. Based on p-values ($p<0.05$), the linear terms (A, B, and C), the interaction term AC, and all quadratic terms (A^2, B^2, and C^2) were statistically significant at a 95% confidence level. The interaction parameters AB and BC were eliminated because they were not significant according to the significance criterion ($p < 0.05$), in order to reduce model complexity and improve model accuracy [25]. The optimal conditions for DES pretreatment of water hyacinth, as suggested by the model, were a temperature of 90 °C for 60 min at a solid-to-liquid ratio of 1:9.58 (w/w), resulting in a maximum recovery rate of 44.22%. Experimental results under these optimal conditions yielded a reducing sugar concentration of 0.31 g/g and a recovery rate of 45.46%. The reducing sugar obtained in this study (0.31 g/g) was 22.53% higher than that reported in previous research (0.253 g/g) [24], which used 3% KOH pretreatment of water hyacinth. The predicted recovery rate (44.22%) was compared with the validated experimental value (45.46%), with an error percentage of 2.80%. Therefore, this model demonstrates good accuracy and potential to predict the optimized conditions and recovery rate of the pretreatment process of water hyacinth using ChCl/MEA.

Table 2. ANOVA results for the response surface quadratic model (response: recovery rate (%)).

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	65.43	7	9.35	27.28	<0.0001	**
A - Temperature	30.23	1	30.23	88.22	<0.0001	
B - Time	5.50	1	5.50	16.05	0.0031	
C – S/L ratio	7.62	1	7.62	22.24	0.0011	
AC	2.94	1	2.94	8.57	0.0168	
A^2	14.12	1	14.12	41.22	0.0001	
B^2	2.98	1	2.98	8.70	0.0162	
C^2	2.09	1	2.09	6.10	0.0356	
Residual	3.08	9	0.34			
Lack of fit	3.08	5	0.62			
Pure error	0.000	4	0.000			
Cor Total	68.51	16				

**Significant, NS not significant

Figure 2 illustrates a 3D surface plot showing how the three independent variables, such as S/L ratio, temperature, and reaction time, influence the recovery rate during enzymatic saccharification of pretreated water hyacinth. A notable interaction was observed between the S/L ratio and temperature, which had a significant effect on the recovery rate (Fig. 2a). In contrast, the interaction between temperature and time did not show a meaningful impact on the response (Fig. 2b). The regression equation that models the predicted recovery rate (Y) as a function of temperature (A), reaction time (B), and S/L ratio (C) is presented below.

$$Y=38.77-1.94A-0.83B-0.98C-0.86A\times C+1.83A^2+0.84B^2-0.7C^2 \quad (3)$$

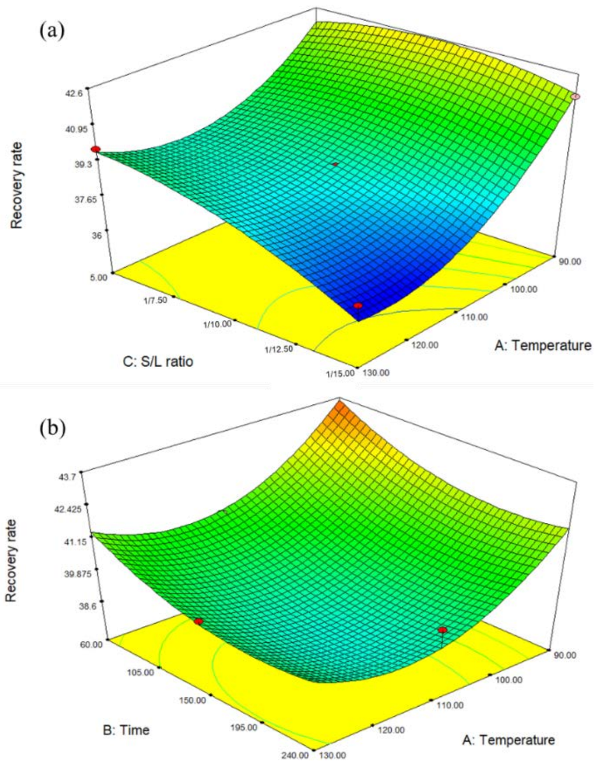

Fig. 2. Three-dimensional plot of the effects of (a) S/L ratio and temperature on sugar yield at optimal time (150 min) and (b) temperature and time on sugar yield at optimal S/L ratio (1/5)

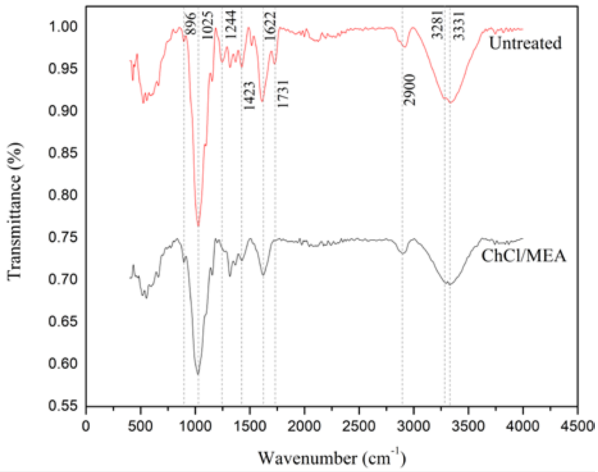

Fig. 3. Fourier-transform infrared spectroscopy (FTIR) spectra of untreated and pretreated water hyacinth biomass.

3.2 Effect of Structural changes in biomass after DES pretreatment using FT-IR Spectroscopy

FTIR analysis is commonly used to reveal bond breakage and stretching vibrations, offering insights into the structural integrity of lignocellulosic biomass [27]. This type of biomass is mainly composed of cellulose, hemicellulose, and lignin, where cellulose exhibits a crystalline structure, while lignin is largely amorphous [22]. In this study, FTIR spectroscopy was employed to investigate the structural alterations in untreated and DES-pretreated water hyacinth at optimized condition (temperature of 90 °C, a reaction time of 60 min, and a solid-to-liquid ratio of 1:9.58 (w/w)) through the spectral range of 400−4000 cm^{-1} (Figure 3). The absorption band near 896 cm^{-1} corresponds to β-glucosidic linkages, indicating CH deformation specific to cellulose [28,29]. A sharp peak at 1025 cm^{-1} is linked to C-O-C stretching vibrations in the pyranose rings found in cellulose and hemicellulose [30]. Additionally, a band at 1244 cm^{-1} appeared exclusively in the DES-pretreated sample, suggesting that remnants of chemical modifications in lignin persisted post-treatment [31,32]. A spectral band near 1423 cm^{-1} is associated with asymmetric C=O stretching vibrations, indicating the presence of carbonate compounds in biomass ash [33]. The peak at 1622 cm^{-1} corresponds to H−O−H bending vibrations, which become more pronounced and shift slightly due to component interactions [34]. The band at 1731 cm^{-1} reflects complex ester linkages between hemicellulose and lignin, such as acetyl, feruloyl, and p-coumaroyl groups [35]. After pretreatment, this 1731 cm^{-1} peak diminished, suggesting cleavage of those ester bonds. Absorption in the 3500−2800 cm^{-1} range typically indicates the presence of cellulose (strong signal), hemicellulose (strong), or lignin (weak) in lignocellulosic biomass [36]. Following ChCl/MEA treatment, a slight increase in intensity around 2900 cm^{-1} was observed, attributed to enhanced C−H stretching in cellulose [30]. Additionally, the bands at 3281 and 3331 cm^{-1} were linked to hydrogen-bonded O−H stretching on the cellulose surface [23].

3.3 Effect of DES pretreatments on cellulose crystallinity structure using XRD method

Cellulose crystallinity plays a crucial role in determining the efficiency of pretreatment processes. To assess this, XRD analysis was performed to examine the crystalline structure of cellulose, offering valuable insight into the effectiveness of the pretreatment. Figure 4 presents the XRD patterns for both untreated and DES pretreatment at optimized condition (temperature of 90 °C, a reaction time of 60 min, and a solid-to-liquid ratio of 1:9.58 (w/w)) of water hyacinth biomasses. The diffraction profiles revealed two prominent peaks at 22° and 18°. Notably, the pretreated biomass showed a more pronounced reduction in the peak intensity at 18°, indicating a higher degree of hemicellulose removal and structural disruption in the amorphous regions compared to the untreated sample [15]. The CrI values indicate that the pretreated water hyacinth biomass (49.31%) exhibits greater crystallinity compared to the untreated sample (35.28%). This increase in CrI after DES pretreatment is likely because of structural alterations in the biomass, specifically the removal of amorphous components like the lignin−hemicellulose matrix. This matrix typically surrounds the cellulose, and its removal during pretreatment exposes more of the crystalline cellulose regions, resulting in a higher CrI. [15].

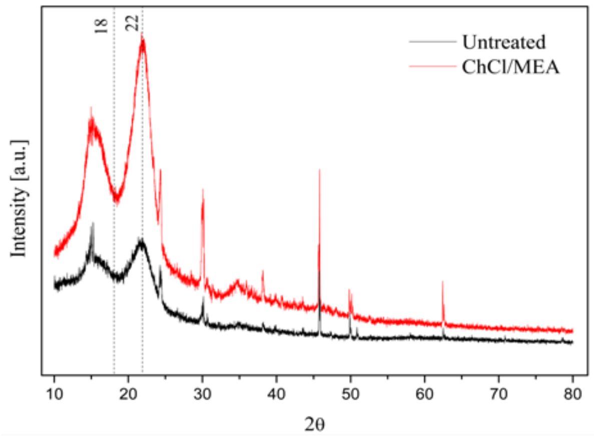

Fig. 4. Fourier-transform infrared spectroscopy (FTIR) spectra of untreated and pretreated water hyacinth biomass.

4 Conclusion

The mathematical model predicted the optimal pretreatment conditions with a regression coefficient (R^2) of 0.9550, identifying a solid-to-liquid ratio of 1:9.58 (w/w) at 90 °C for 60 minutes, which yielded a maximum recovery rate of 44.22%. Under these optimal conditions, the pretreatment significantly enhanced the amount of reducing sugars in enzymatic hydrolysis. The results of Fourier Transform Infrared (FTIR) spectroscopy and X-ray diffraction (XRD) confirmed the effectiveness of DES pretreatment on the water hyacinth biomass. Therefore, this study indicates that DES pretreatment under the identified optimal conditions can facilitate the valorization of water hyacinth waste for the production of value-added products within the biorefinery sector.

The authors would like to thank King Mongkut's University of Technology North Bangkok (Research Grant Contract No. KMUTNB-68-BASIC-27.

References

1. A. M. Villamagna, B. R. Murphy, Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review. Freshwater Biology, **55**, 282-298 (2010)
2. S. Rezania, M. Ponraj, A. Talaiekhozani, Shaza E. Mohamad, M. F. M. Din, S. Md Taib, F. Sabbagh, F. Md Sairan, Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. Journal of Environmental Management, **163**, 125-133 (2015)
3. P. P. D. Omo; V. Spena; S. L. Froscia, Assessment of a Mechanical Pretreatment to Enhance Biogas Production from the Noxious Weed Eichhornia Crassipes on Industrial Scale. in Proceedings of 5th International Symposium on Environment-Friendly Energies and Applications (EFEA), September September 24-26 (2018)
4. S. Pandey, N. Singh, A. K. Nirala, A. Gir, Dynamics of Water Weed Eichhornia Crassipes: A Review. IJRASET. **3** (2015)
5. G. K. Gaurav, T. Mehmood, L. Cheng, J. J. Klemeš, D. K. Shrivastava, Water hyacinth as a biomass: A review. Journal of Cleaner Production, **277**, 122214 (2020)
6. D. Jose, K. Rattanaporn, N. Kittiborwornkul, A. A. Adediran, and M. Sriariyanun, Biorefining Processes for Valorization of Lignocellulosic Biomass for Sustainable Production of Value-Added Products (CRC Press, Boca Raton, London, New York, 2023)
7. H. El-gendi, E. M. El-Fakharany, M. H El-Sayed, D.A. Alenazi, R. Jame, N. Alkhathami, A. S. Khera, M. Abdelaziz, A comprehensive overview of lignocellulosic biomass exploiting for sustainable bioethanol production: recent advances and emerging challenges towards commercial implementation. Egypt. J. Chem. **67**, 2053-2072 (2024)
8. O. M. Perrone, M. M. de S. Moretti, S. E. Bordignon, J. de C. Pereira, R. da Silva, E. Gomes, M. Boscolo, Improving cellulosic ethanol production using ozonolysis and acid as a sugarcane biomass pretreatment in mild conditions. BioResources. **13**, 100628 (2021)
9. T. Ruan, R. Zeng, X. Y. Yin, S. Zhang, Z.-H. Yang, Water Hyacinth (Eichhornia crassipes) Biomass as a Biofuel Feedstock by Enzymatic Hydrolysis. BioResources. **11**, 2372-2380 (2016)
10. B. K. Show, G. Shivakumaran, A. Koley, A. Ghosh, S. Chaudhury, A. K. Hazra, S. Balachandran, Effect of thermal and NaOH pretreatment on water hyacinth to enhance the biogas production. Environmental Science and Pollution Research, **30**, 120984–120993 (2023)
11. R. D. Umai, S. Jacob, V. Kumar, Deep Eutectic Solvent Pretreatment of Water Hyacinth for Improved Holocellulosic Saccharification and Fermentative Co-Production of Xylitol and Lipids Using *Rhodosporidium toruloides* NCIM 3547. Fermentation, **8**, 591 (2022)
12. Y.-Q. Sun, Y. Yuan, K.-X. Dai, Z.-L. Xiu, The pretreatment of the sustainable biomass feedstock of Pennisetum giganteum for biorefinery using deep eutectic solvents. Bioresource Technology, **384**, 129289 (2023)
13. M. Sulaiman, H. Mahmood, H. M. Khan, T. Iqbal, N. U. Khan, M. M. Abbas, M. N.-E.-Alam, M. E. M. Soudagar, Cellulosic rich biomass production with optimized process parameters by using glycerol pretreatment for biofuels applications. Energy Nexus, **14**, 100298 (2024)
14. K. Narayanan, P. Venkatachalam, E. J. Panakkal, P. Tantayotai, A. Tandhanskul, R. Selvasembian, S. Chuetor, M. Sriariyanun, Exploring Ternary Deep Eutectic Solvent Pretreatment in a One-Pot Process with Napier Grass for Bioethanol Production. BioEnergy Research, **17**, 2213–2225 (2024)
15. S. L. Sunar, R. K. Oruganti, D. Bhattacharyya, D. Shee and T. K. Panda, Pretreatment of sugarcane bagasse using ionic liquid for enhanced enzymatic saccharification and lignin recovery: process optimization by response surface methodology. Cellulose, **31**, 2151–2173 (2024)
16. S. Areeya, M. P. Gundupalli, B. Dharmalingam, B. Paramasivam, P. Tantayotai, P. Yasurin, E. J. Panakkal, Process Optimization of Deep Eutectic Solvent Pretreatment of Coffee Husk Biomass. E3S Web of Conferences, **428**, 01010 (2023)
17. D. Pradhan, S. Jaiswal, B. K. Tiwari, A. K. Jaiswal, Nanocellulose separation from barley straw via ultrasound-assisted choline chloride – Formic acid deep eutectic solvent pretreatment and high-intensity ultrasonication. Ultrasonics Sonochemistry, **110**, 107048 (2024)
18. B. Timoteo-Cruz, R. M. Gómez-Espinosa, R. Sánchez-Orozco, J. J. García-Sánchez, F. Ureña-Núñez and G. Roa-Morales, Pretreatment of Typha latifolia biomass with imidazolium ionic liquid as a strategy for sugar production. Biomass Conversion and Biorefinery, **14**, 4591–4604 (2024)
19. M. Pawłowska, M. Zdeb and M. Nieścioruk, Efficiency of Chemical Pretreatment of Sugar Beet Pulp Biomass Intended to Energy Production via Biological Processes, Energies, **16**, 574 (2023)
20. R. P. Martins, A. A.Schmatz, L. A. de Freita, M J. R. Mutton, M. Brienzo, Solubilization of

hemicellulose and fermentable sugars from bagasse, stalks, and leaves of sweet sorghum. Industrial Crops and Products, **170**, 113813 (2021)

21. M. Ivanović, D. Grujić, J. Cerar, M. I. Razboršek ,L. Topalić-Trivunović, A. Savić, D. Kočar, Mitja Kolar, Extraction of Bioactive Metabolites from Achillea millefolium L. with Choline Chloride Based Natural Deep Eutectic Solvents: A Study of the Antioxidant and Antimicrobial Activity. Antioxidants, **11**, 724 (2022)
22. O. Govindaraj, G. N. Olaganathan, R. ASM, S. Uthandi, An eco-friendly EnZolv pretreatment optimization in banana fiber biomass using response surface methodology (RSM) and its structural characterization. Biomass Conversion and Biorefinery, **14**, 1329–1342 (2023)
23. P. Kahar, Synergistic Effects of Pretreatment Process on Enzymatic Digestion of Rice Straw for Efficient Ethanol Fermentation. Environmental Biotechnology - New Approaches and Prospective Applications, 65-87 (2013)
24. G.L. Miller, Anal. Chem. **14**, 426-428 (1959)
25. E.K. Tetteh, S. Rathilal, Response Surface Optimization of Biophotocatalytic Degradation of Industrial Wastewater for Bioenergy Recovery**,** **9**, 3 (2022)
26. R.H. Jadhav and A. Dey, Pre-Treatment and Characterization of Water Hyacinth Biomass (WHB) for Enhanced Xylose Production Using Dilute Alkali Treatment Method, Water, **17**, 301 (2025)
27. J.L. Morales-Martínez, M.G. Aguilar-Uscanga, E. Bolaños-Reynoso, L. López-Zamora, Optimization of chemical pretreatments using response surface methodology for second-generation ethanol production from coffee husk waste. BioEnergy Research, **14**, 815–827 (2021)
28. Z. Yao, G. Chong, H. Guo, Deep Eutectic Solvent Pretreatment and Green Separation of Lignocellulose. Appl. Sci. **14**, 7662 (2024)
29. K. C. Patil, R. H. Patil, Qualitative characterization of pretreated fruit waste to produce an effective fertilizer by using FTIR. 2022 IJCRT. **10**, 12 (2022)
30. J. E. H. Rodríguez, D. E. Rincón, D. F. H. Rojas, I. G. C. Orjuela, L. M. Socolovsky, D. G. E. Rondón, C. L. L. Calderón, Effects of Hydrolysis and Bleaching Conditions on the Efficiency of Cellulose Microribbons Extraction from Coffee Parchment Through a Design of Experiments, Cellulose (2022)
31. W. Wang, C. Wang, Zahoor, X.Chen, Q. Yu, Z. Wang, X. Zhuang, Z. Yuan, Effect of a Nonionic Surfactant on Enzymatic Hydrolysis of Lignocellulose Based on Lignocellulosic Features and Enzyme Adsorption, ACS Omega. **5** 15812–15820 (2020)
32. R. Vârban, I. Crișan, D. Vârban, A. Ona, L. Olar, A. Stoie, R. Ștefan, Comparative FT-IR Prospecting for Cellulose in Stems of Some Fiber Plants: Flax, Velvet Leaf, Hemp and Jute. Appl. Sci. **11**, 8570 (2021)
33. J. Kalembkiewicz, D. Galas, E. Sitarz-Palczak, The Physicochemical Properties and Composition of Biomass Ash and Evaluating Directions of its Applications, Pol. J. Environ. Stud. **27**, 6 (2018)
34. A. Mudzakir, A. B. D. Nandiyanto, E. Heraldy, J. Efendi, K. M. Rizky, M. Widyaningsih, L.S. Anggiat, Dissolution Study of Lignin from Coconut Fiber as Lignocellulosic Biomass Using Eutectic Based Ionic Liquids, in Proceedings of the 9th Mathematics, Science, and Computer Science Education International Seminar (MSCEIS 2023)
35. Z. Zhu, R. Simister, S. Bird, S. J. McQueen-Mason, L. D. Gomez, D. J. Macquarrie, Microwave assisted acid and alkali pretreatment of Miscanthus biomass for biorefineries, AIMS Bioengineering **2**, 449-468 (2015)
36. S. Machmudah, Wahyudiono, P. N. Trisanti, H. Setyawan, S. Madhania, K. Sambodho, S. Winardi, T. Adschiri, M. Goto, Hydrothermal alkaline treatment of lignocellulosic biomass for microcrystalline cellulose generation at subcritical water, South African Journal of Chemical Engineering. **51**, 45-56 (2025)