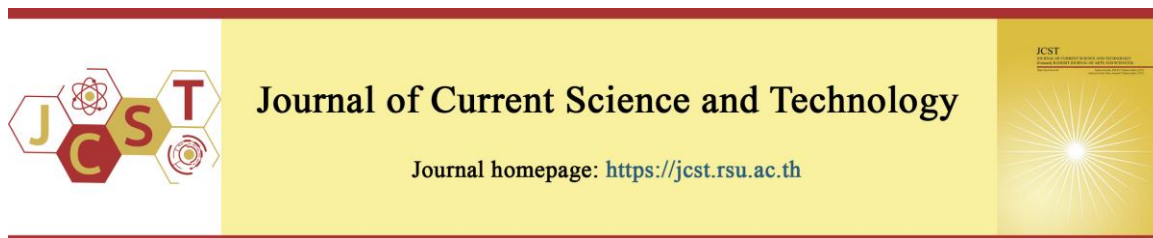


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Green Conversion of Macroalgae *Ulva lactuca* to 5-Hydroxymethylfurfural in Deep Eutectic Solvent Catalyzed by ChCl and HCl

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Abstract

In this research, sustainable production of 5-hydroxymethylfurfural (5-HMF) from macroalgae *Ulva lactuca* (MUL) was methodically studied in a closed system in the presence of a deep eutectic solvent (DES). Herein, the DES consisted of choline chloride (ChCl) and hydrochloric acid (HCl) in acetonitrile solvent, which could be practically applied in relevant reactions such as hydrolysis, isomerization, and dehydration. The roles and benefits of the DES and the types of organic solvents utilized in the reactions were described in preliminary detail. Polymeric humins, as undesirable by-products, were favorably formed via further condensation and/or polymerization of 5-HMF when excessive amounts of HCl or ChCl were utilized under harsh conditions in the catalytic system. Important parameters, such as HCl amount, ChCl amount, reaction time, and temperature, were scrupulously investigated to determine the optimum conditions for 5-HMF production. As anticipated, a maximum yield of 5-HMF (93.9%) was achieved at 120°C for 90 min using 2.4 mmol of HCl and 28 mmol of ChCl. The long-term reusability of ChCl in the catalytic system was also tested under optimum conditions, and the results showed that spent ChCl could be successfully recrystallized and reused four times with only slight reductions in 5-HMF yield. These studies pave the way for future advancements in green catalytic processes for the specific production of high value-added chemicals. This research offers an alternative route for sustainable production of 5-HMF from MUL feedstock, and is also potentially applicable to practical bio-refinery processes.

Keywords: 5-hydroxymethylfurfural, macroalgae *Ulva lactuca*, deep eutectic solvents, choline chloride, bio-refinery process

1. Introduction

Due to environmental issues, the increasing demand for energy and the continuous depletion of fossil fuels, it is essential to search for and utilize renewable resources (Suekkhachat et al., 2018). Biomass can be considered one of the most attractive options for sustainable production of renewable energy and chemicals (Wattana et al., 2020). 5-Hydroxymethylfurfural (5-HMF) is an interesting high value-added chemical that can be applied as a platform chemical in the bio-based economy for the further production of green bio-plastic and/or bio-fuel (Hurtado et al., 2025). Selective synthesis of 5-HMF can generally be performed via dehydration of fruit sugar in the presence of mineral acid catalysts such as sulfuric acid (H_2SO_4), nitric acid (HNO_3), and hydrochloric acid (HCl) (Prigyi et al., 2026). However, undesirable products (humins) are easily formed via further polymerization/condensation of the 5-HMF structure when unsuitable conditions such as excessive amounts of acid catalyst, long operating times or high operating temperatures are used in the synthetic system, resulting in significant reduction of 5-HMF yield (Bi et al., 2025). Thus, it is essential to identify a suitable method for selective synthesis of 5-HMF with effective suppression of humin formation.

Many types of ionic liquids such as ethylmethylimidazolium chloride, butylmethylimidazolium chloride, 1-methyl-3-(butyl-4-chlorosulfonyl) imidazolium chlorosulfate and others, have been systematically utilized in the selective dehydration of fruit sugar to 5-HMF (Baruah et al., 2025). Herein, high 5-HMF yields in the range of 60–80% could be obtained under mild conditions including synthetic temperatures (80–120°C) and synthetic time (60–180 min). Unfortunately, because operating costs and environmentally friendly processes must also be considered for possible application in practical bio-refinery process, several drawbacks of ionic liquids have been identified such as high toxicity, high cost, low stability, and poor biodegradability (Askar et al., 2025). Deep eutectic solvent (DES) has been reported as an emerging green solvent based on several characteristic properties such as good biodegradability, low melting point, low toxicity, low cost, and others (Kargar & Hosseini, 2026). Since the main components of DES consist of hydrogen bond donor (inorganic acid) and hydrogen bond acceptor (quaternary ammonium salt), their applications for selective synthesis of 5-HMF from catalytic conversion of biomass-derived sugars are promising for addressing the above problems (Maneechakr et al., 2023). To date, many studies have

focused on using fruit sugar as a feedstock for 5-HMF production. To the best of our knowledge, the utilization of real biomass for 5-HMF production has not been widely studied due to complex decomposition and reactions (Singh et al., 2023). Recently, the highest yield of 5-HMF (52.0%) derived from microalgae biomass (*Chlorella sorokiniana*) was reported using 0.5% w/v sulfuric acid catalyst with dimethyl sulfoxide/lithium chloride/water at 150°C for 2 h (Wang et al., 2024). In addition, the possible reusability of DES system should be considered in current study (Sunar et al., 2026).

This research aimed to synthesize 5-HMF from the catalytic conversion of macroalgae *Ulva lactuca* (MUL) under a DES system in the presence of choline chloride (ChCl), HCl and acetonitrile solvent. It should be noted that MUL is one of the most interesting and promising biomass feedstocks for biofuel/biochemical production because it can grow much faster than conventional crops, leading to greater availability with a sufficiently high yield (Farobie et al., 2024). Moreover, since MUL biomass specifically contains large amounts of carbohydrates/polysaccharides with a low lignin yield, it should be considered as a notable feedstock for 5-HMF production. Four synthetic parameters, namely temperature, time, and the loading amounts of ChCl and HCl, were methodically studied to determine the optimum conditions for 5-HMF production with high productivity. The possible reusability of the DES system based on ChCl re-crystallization was evaluated for four cycles without direct application of a regeneration process. This research is highly anticipated for further application in practical bio-refinery processes.

2. Objectives

This research mainly aimed to synthesize the high value-added compound 5-HMF from MUL biomass using a green DES system. The objectives were to determine the optimum conditions for selective production of 5-HMF by investigating various important factors such as co-catalyst amount (HCl and ChCl), temperature, and time. The effects of organic solvent type, ChCl re-crystallization and reusability were also investigated after the optimum conditions were obtained.

3. Materials and Methods

3.1 Biomass Feedstock

MUL was collected from Lombok Island in Indonesia and utilized as biomass feedstock in this

research (Farobie et al., 2022). The dried MUL was crushed and sieved to a size of 0.25 mm, followed by moisture removal at 105°C for 12 h based on the ISO 18134 method. Proximate analysis of MUL was performed to determine the moisture, volatile matter, ash and fixed carbon using the American Society for Testing and Materials (ASTM) D5142-04 method, while ultimate analysis was carried out to determine the elemental composition using an elemental analyzer (OXFORD model 7021). The results of proximate and ultimate analyses are summarized in Table 1.

3.2 Synthesis and Separation of 5-HMF Product

The catalytic conversion of MUL to 5-HMF in the presence of DES was carefully performed in a closed system using a laboratory bottle (50 mL) equipped with a Type-K thermocouple. In a typical synthesis, 1 g of MUL powder and a certain amount of ChCl (0 to 35 mmol) were mixed with 30 mL of acetonitrile solvent, followed by the addition of HCl catalyst (0 to 3.6 mmol). For the synthetic process, the as-prepared batch reactor was immersed in an oil bath, and heated at 60 – 140°C for 30 – 180 min with a stirring speed of 500 rpm.

For the separation process, after synthesis was completed, the reaction was stopped and cooled to

approximately 70°C. Then, the remaining MUL powder was quickly separated from the 5-HMF solution by centrifugation at 5000 rpm. Thereafter, when the solution was cooled to room temperature (25 – 30°C), the 5-HMF product dissolved in ACN in the upper layer was separated in situ while ChCl was recrystallized and submerged in the bottom layer. Herein, crude 5-HMF product could be obtained via distillation using a rotary evaporator at 50 – 60°C. The separation and reuse processes under the DES system are summarized in Figure 1.

3.3 Analysis of 5-HMF Product

The amount of 5-HMF product produced from MUL conversion in the DES system was analyzed using high-performance liquid chromatography (HPLC, Agilent 1200 Series). The operating conditions of HPLC in this study were specifically determined using a UV detector at 284 nm with a NUCLEODUR 100-5 C18 ec column (5 µm, 150 × 4.6 mm) at an oven temperature of 35°C. Herein, a mobile phase of acetonitrile to water (10:90) was used at a flow rate of 0.4 mL/min while the injection volume of 20 µL was fixed. The yield of 5-HMF product was quantified using an external standard method (Niakan et al., 2022).

Table 1 Proximate and ultimate compositions of MUL.

Proximate analysis	wt. %	Ultimate analysis	wt. %
Moisture	2.65	Carbon	41.22
Volatile matter	58.76	Hydrogen	6.78
Ash	37.98	Nitrogen	2.45
Fixed carbon ^a	0.61	Sulfur	4.13
		Oxygen	45.42

^aAmount of fixed carbon was calculated from mass difference

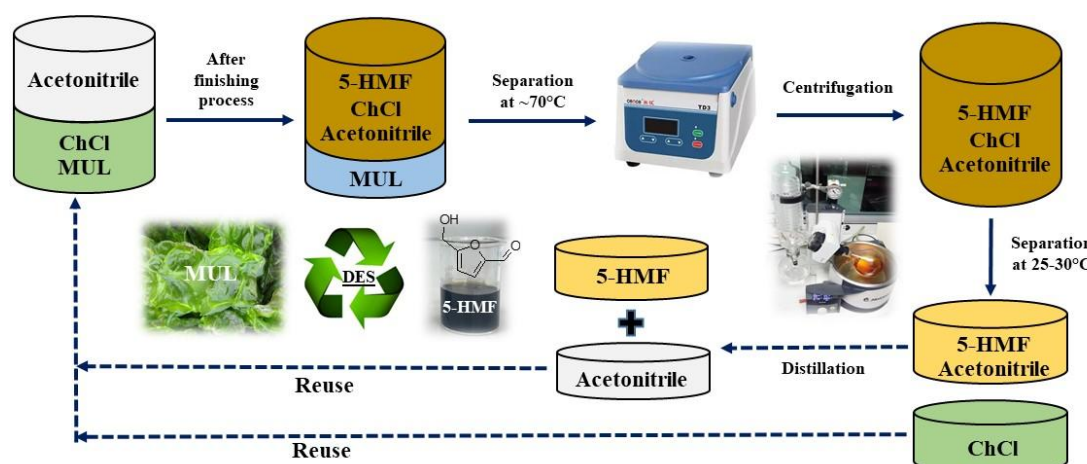


Figure 1 Schematic diagram for separation and reusable processes of DES.

4. Results and Discussion

4.1 Applicable Roles of HCl and ChCl

Figures 2 and 3 present the applicable roles of HCl and ChCl amounts in the selective production of 5-HMF under DES at 100°C for 60 min. It was found that increasing the HCl amount from 0.6 to 2.4 mmol promoted higher yields of 5-HMF, from 17.1% to 52.7%. The same trend was observed when higher ChCl amounts from 14 to 28 mmol were applied. This may be due to high catalytic reactivity via specific reaction pathways including hydrolysis, isomerization and/or dehydration (Ameen et al., 2025). The possible reaction pathways for conversion of MUL to 5-HMF and other chemicals are shown in Figure 4. Firstly, the specific conversion of cellulose into glucose molecules occurred through acid hydrolysis at nucleophilic site. Then, fructose molecules were generated via glucose isomerization process (Mao et al., 2023). During this step, ChCl could particularly interact with the fructose structure through electrostatic force in the presence of strong nucleophile or chlorine ion and hydrogen bond donor or HCl. In this case, oxonium ions were generally formed and further converted into related intermediates (Maneechakr & Karnjanakom, 2020). Herein, the dehydration procedure was promoted while 5-HMF product could be produced in the reaction system. In addition, an excessive amount of acid catalyst likely promoted the protonation of the carbonyl group on the 5-HMF structure, enhancing its electrophilic properties, resulting in further conversion into humins (Velasco Calderón et al., 2022).

As observed in Figure 2, a further increase above the above-mentioned amount of HCl resulted in

a slight reduction of 5-HMF yield, due to the further conversion of 5-HMF to humins via polymerization and/or condensation (Zhang et al., 2024). In other words, the polymerization of 5-HMF molecules with the formation of humins was highly favored under acidic conditions with an excessive amount (> 2.4 mmol of HCl). Herein, the color of the liquid product changed from light brown to dark brown. Also, the ability of ChCl to recrystallize might be decreased to some extent when the HCl amount was increased. In the case of an excessive ChCl amount (35 mmol), the yields of 5-HMF decreased from 52.7 to 45.8%, suggesting that 5-HMF molecules could possibly be adsorbed on the ChCl structure via dipole-dipole interaction. In contrast, higher viscosity could possibly be generated in the reaction system, leading to inhibition of mass transfer and relevant reactions (Guo et al., 2025). It should be noted that non-significant formation of 5-HMF product could be found in the absence of HCl or ChCl (0 mmol), resulting in an inability to overcome the activation energy under the current operating conditions. In addition, the pH values detected in reaction systems with different amounts of added acid were in the range of 4.2 to 6.5, which was appropriate for catalytic conversion of MUL to 5-HMF and other chemicals. These results confirm that the synergistic effect between HCl and ChCl in DES at optimum amounts was very important for selective production of 5-HMF. Thus, 2.4 mmol of HCl and 28 mmol of ChCl were selected as optimum amounts for the next step of the study.

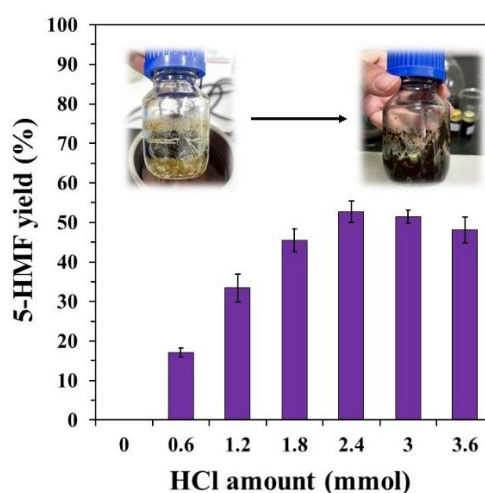


Figure 2 Effect of HCl amount (0 to 3.6 mmol) for catalytic conversion of MUL to 5-HMF under DES. Synthetic conditions: 1 g of MUL, 28 mmol of ChCl, 30 mL of acetonitrile, temperature at 100°C and time for 60 min.

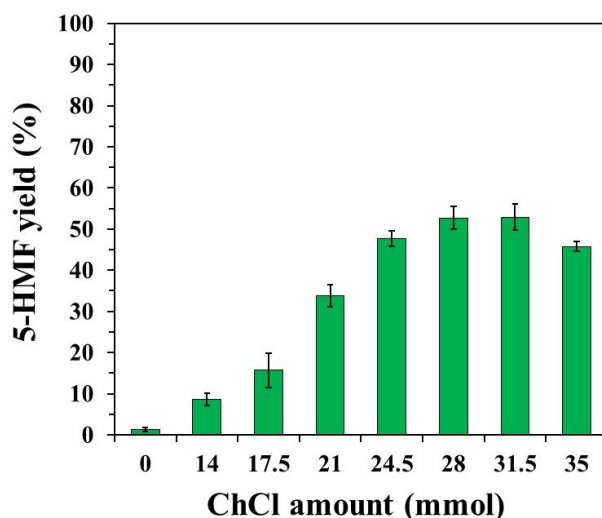


Figure 3 Effect of ChCl amount (0 to 35 mmol) for catalytic conversion of MUL to 5-HMF under DES. Synthetic conditions: 1 g of MUL, 2.4 mmol of HCl, 30 mL of acetonitrile, temperature at 100°C and time for 60 min

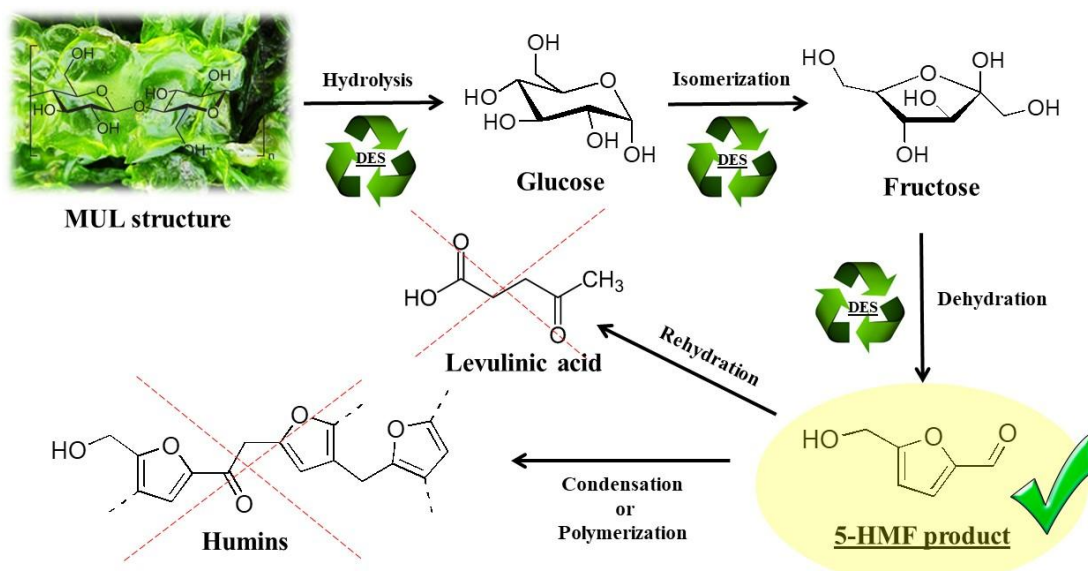


Figure 4 Possible reaction pathways for conversion of MUL to 5-HMF and other chemicals under DES.

4.2 Effect of Reaction Time and Temperature

Figures 5 and 6 show the effects of reaction temperature and time on the selective production of 5-HMF under DES at 60 – 140°C for 30 – 180 min. As expected, since the specific reactions for catalytic conversion of MUL to 5-HMF product in the presence of DES involved an endothermic process, the 5-HMF yield was enhanced to some extent when reaction temperature was increased from 60 to 120°C. The same phenomenon was observed when the reaction time was increased for 5-HMF production with higher

yield. Unfortunately, unsuitable conditions such as reaction temperature (> 120°C) and reaction time (> 90 min) resulted in significant reduction in the formation efficiency of 5-HMF product. Meanwhile, undesired products such as levulinic acid and humins could likely be further generated via rehydration and polymerization (Guo et al., 2021). From the above-mentioned results, a maximum yield of 5-HMF (93.9%) was satisfactorily achieved from the selective conversion of MUL under DES with optimum conditions at 120°C for 90 min.

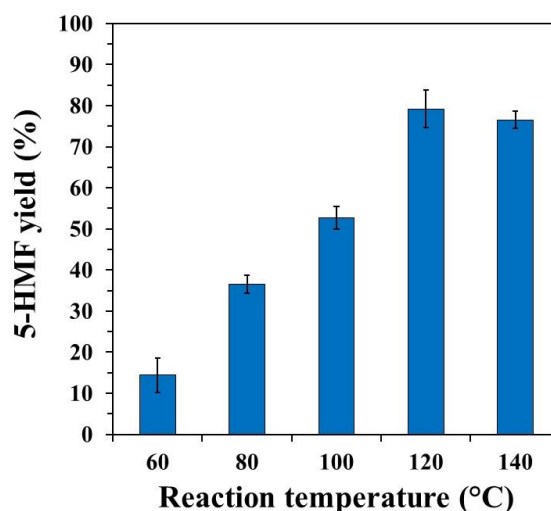


Figure 5 Effect of temperature (60 - 140°C) for catalytic conversion of MUL to 5-HMF under DES.
Synthetic conditions: 1 g of MUL amount, 2.4 mmol of HCl, 28 mmol of ChCl, 30 mL of acetonitrile and time for 60 min.

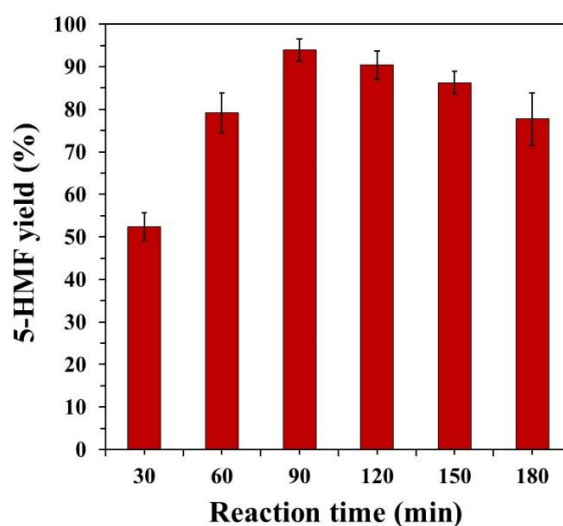


Figure 6 Effect of time (30 - 180 min) for catalytic conversion of MUL to 5-HMF under DES.
Synthetic conditions: 1 g of MUL amount, 2.4 mmol of HCl, 28 mmol of ChCl, 30 mL of acetonitrile and temperature at 120°C.

4.3 Effect of Organic Solvent

Different organic solvents such as acetonitrile, tetrahydrofuran, methyl sulfoxide, isobutyl methyl ketone and hexane were utilized for catalytic conversion of MUL to 5-HMF under DES with the optimum conditions, and the obtained results are shown in Figure 7. Interestingly, methyl sulfoxide showed the best efficiency for 5-HMF production with a yield of 96.7% followed by acetonitrile (93.9% of 5-HMF yield) and tetrahydrofuran (84.5% of 5-HMF yield). In contrast, poor activity in 5-HMF formation was found with the application of isobutyl methyl ketone (30.6% of 5-HMF yield) and hexane

(8.8% of 5-HMF yield), probably due to their non/low polarities. Based on these results, the strong polarity of the organic solvent was very important for selective production of 5-HMF (Hu et al., 2026). Recently, Tran et al. (2026) reported that polarity of organic solvent could effectively promote the interaction and stabilization of immediate vicinity of hydroxyl groups together with the carbocation process during reactions, leading to high catalytic activity in the chemical conversion process. In contrast, an increase in hydrocarbons in organic solvent structure resulted in drastic decrease in polarity, for instance, the application of hexane created unfavorable conditions

for ChCl dissolution in reaction system, resulting in a reduction in catalytic reactivity. It should be noted that although methyl sulfoxide provided the best result based on 5-HMF yield but spent ChCl after the complete reaction could not be recrystallized again in DES system, leading to an inability to perform a reuse test. Therefore, acetonitrile was selected as a suitable solvent for 5-HMF production in this study because it could effectively preserve the spent ChCl crystal.

4.4 ChCl Reusability

Before testing ChCl reusability, the spent ChCl crystal was regenerated by gentle washing with

isobutyl methyl ketone to separate some molecules of intermediates and/or humins adsorbed on the ChCl structure. For analysis by Fourier transform-infrared (FT-IR) spectroscopy (PerkinElmer spectrum 100 FT-IR), as shown in Figure 8A, the main vibration peaks of the FT-IR spectra were observed at about 1085 and 3390 cm^{-1} , attributed to the presence of C-N and O-H functional groups on the ChCl structure, respectively (Amesho et al., 2023). Herein, the FT-IR spectra of fresh and spent ChCl presented similar patterns, indicating that the regeneration and separation processes were acceptable without significant contamination.

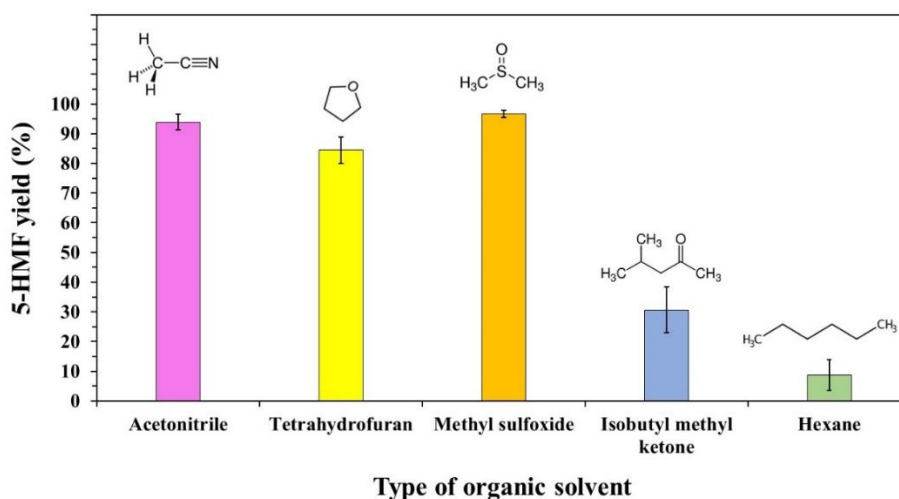


Figure 7 Effect of organic solvent for catalytic conversion of MUL to 5-HMF under DES.

Synthetic conditions: 1 g of MUL amount, 2.4 mmol of HCl, 28 mmol of ChCl, 30 mL of organic solvent, temperature at 120°C and time for 90 min.

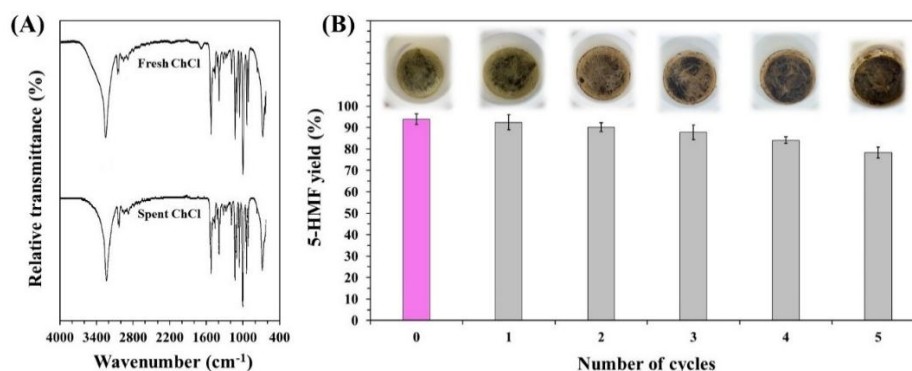


Figure 8 (A) FT-IR spectra of fresh ChCl before reaction and spent ChCl after reaction and regeneration, and (B) effect of ChCl reusability for catalytic conversion of MUL to 5-HMF under DES.

Synthetic conditions: 1 g of MUL amount, 2.4 mmol of HCl, 28 mmol of ChCl, 30 mL of organic solvent, temperature at 120°C and time for 90 min.

For reuse testing, as shown in Figure 8B, the catalytic efficiency for selective production of 5-HMF from MUL conversion under the DES system showed high stability and reusability for four cycles with only slight reductions of 5-HMF yield from 92.5% in the 1st cycle to 78.3% in the 5th cycle. This also indicates that ChCl reusability applied in this work was successfully achieved with slight deactivation in DES system. It should be noted that the re-crystallization capability of spent ChCl was obviously reduced when the 5th reuse cycle was tested, resulting from interaction effect between the hydroxyl group of the by-product and ChCl structure, leading to facile transformation from the pristine solid phase to a new liquid phase (Mankar et al., 2021). To solve this problem, a seeding technique could be considered as the most suitable and inexpensive method for ChCl re-crystallization (He et al., 2020). As clearly observed, this study provided a sustainable route for DES reuse process which could meet economic requirements for practical processes.

5. Conclusion

The selective synthesis of 5-HMF from MUL conversion under DES was successfully achieved under mild conditions in the bio-refinery process. The important effects of HCl and ChCl amounts, reaction temperature and time, type of organic solvent, and DES reusability were systematically investigated. Based on the obtained results, the proper application of DES in the presence of ChCl and HCl presented high catalytic reactivity for hydrolysis, isomerization and/or dehydration while humin formation could be suppressed. A maximum yield of 5-HMF (93.9%) was obtained under synthetic conditions of 120°C for 90 min using 2.4 mmol of HCl and 28 mmol of ChCl. Acetonitrile was presented as a suitable solvent for 5-HMF production in DES, in which spent ChCl could easily recrystallize and be reused after the complete reaction. The polymerization of 5-HMF into humins and/or rehydration of 5-HMF into levulinic acid likely occurred when harsh conditions such as an excessive amount of HCl and prolonged reaction time were applied in the DES system. The spent ChCl for 5-HMF production under DES could be reused for four cycles without a drastic reduction of 5-HMF yield. Based on these results, actual application could be expected in practical bio-refinery processes. These studies pave the way for future advancements in the development of other active catalysts for application in biomass conversion into renewable fuels/chemicals.

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7. Abbreviations

Abbreviation	Full Term
5-HMF	5-hydroxymethylfurfural
MUL	macroalgae <i>Ulva lactuca</i>
DES	deep eutectic solvent
ChCl	choline chloride
HCl	hydrochloric acid
H ₂ SO ₄	sulfuric acid
HNO ₃	nitric acid
ASTM	American society for testing and materials
FT-IR	Fourier transform-infrared

8. CRediT Statement

Panya Maneechakr: Supervision, Validation.
Niknurmee Yee-arsae: Experimental Investigation, Methodology, Formal analysis.
Parichat Phumee: Experimental Investigation, Methodology, Formal analysis.
Obie Farobie: Conceptualization, Resources.
Irwan Kurnia: Conceptualization, Validation.
Asep Bayu: Conceptualization.
Guoqing Guan: Supervision, Validation.
Surachai Karnjanakom: Project Administration, Funding Acquisition, Writing-Original Draft, Writing-Review & Editing.

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