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Deep Eutectic Solvents – Versatile Chemicals in Biodiesel Production

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Abstract

Due to booming economies, a sharp increase in energy requirements is anticipated worldwide, primarily in the transportation sector. Biodiesel's aptness in substituting petro-diesel conveniently has pushed research toward more on economically and environmentally benign production technologies. Deep eutectic solvents (DESs), a sub-class of ionic liquids have gained wide popularity in various fields of biodiesel production because of process competence and environmental benevolence. This present study specifically summarizes the application of DESs as a catalyst, cosolvent and extracting solvent in biodiesel production. Sequentially, we first discuss the extraction of lipids, valuable compounds, feedstock pre-treatment, transesterification, and ester purification. Overall DESs in all roles show good performance and recyclability. Based on the findings of this study, we highlight areas where deep eutectic solvents require more research and identify future possibilities. It is suggested that a fully DESs-based biodiesel production scheme is possible.

Highlights

- Role of DESs in biodiesel production is reviewed.
- Acidic strength of HBD part influences catalyzing abilities.
- Choline Chloride based DESs show excellent solvent-abilities.
- Future research areas for growth in DESs based biodiesel production are outlined.

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Abbreviations

A	Acetamide
AA	Acetic acid
ACPO	Acidulated crude palm oil
ATPB	Allyl triphenyl phosphonium bromide
BTMAC	Benzyl trimethyl ammonium chloride
BTEAC	Benzyl triethyl ammonium chloride
BTU	British thermal units
CA	Citric Acid
ChAc	Choline Acetate
ChCl	Choline chloride
CSA	Camphor-10-sulphonic acid
CTAB	Cetyl tetrabutyl ammonium chloride
DEEAC	N, N-diethylenethanol ammonium chloride
DEG	Diethylene glycol
DESs	Deep Eutectic Solvents
EG	Ethylene glycol
FFAs	Free fatty acids
FAAEs	Fatty acid alkyl esters
FAMEs	Fatty acid methyl esters
Fruc	Fructose
GHG	Greenhouse gas emissions in (gram CO_2equi/MJ biodiesel)
Gly	Glycerol
Guc	Glucose
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond Donor
ILUC	Induced land usage changes
LGCPO	Low-grade crude palm oil
Lipozyme CALB L	Liquid lipase B from Pseudozyma antarctica

Lipozyme TL 100L	Lipase from Thermomyces lanuginosus
MA	Malonic acid
MTPB	Methyl triphenyl phosphonium bromide
NADES	Natural deep eutectic solvents
ODD	Oil deodorizer distillate
OECD	Organisation for Economic Co-operation and Development
OX	Oxalic acid
PTSA	para-Toluene sulphonic acid
PG	Propylene glycol or 1, 2 propanediol
SC	Super critical
SPO	Sludge palm oil
STEM	Science, Technology, Engineering and Management
TBAC	Tetra butyl ammonium chloride
TEG	Triethylene glycol
TFA	2, 2, 2 Trifluoroacetamide
U	Urea

1 Introduction

Road-based vehicles emit around three-quarters of transport CO_2 emissions [1]; of these, diesel engines power more than 80% of all transportation fleet worldwide. In the period 1990-2018, while there has been a significant reduction (20%) in energy-related CO_2 emissions, the transportation sector has increased its emissions by ~ 27% [2]. There is a constant increase in energy demands of the developing nations and is anticipated to continue in the next decades (Figure. 1). Diesel consumption globally in the transportation sector is predicted to rise to approximately 8 Peta (10^{15}) BTU [3]. This worldwide growth and demand put stress on fuel reserves, and raise elevating concerns on energy security. Thus, to alleviate the stresses on the conventional diesel and curb the increasing CO_2 emissions associated with it, there is a need to adopt biodiesel wherever possible. To counter anti-environment effects and maintain carbon neutrality in the diesel-oriented transportation industry, biodiesel is advocated. Switching to biodiesel would reduce emissions considerably. It also provides the necessary time to develop futuristic technologies that are much cleaner and purer quintessentially.

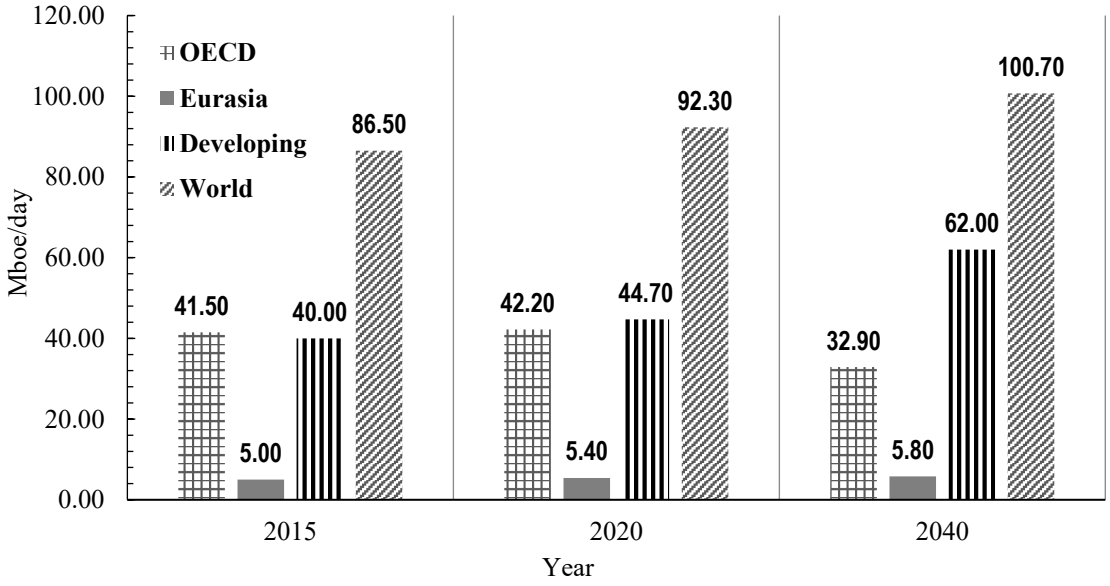


Figure. 1. Oil consumption and predicted demand for OECD countries, Eurasia, Developing countries and the world, in Million barrels of oil equivalent/day (Mboe/day) [4].

In biomass to fuel conversion, it is only via transesterification that biodiesel or fatty acid alkyl esters (FAAE) are produced. The typical preparation recipe is: 6:1 ratio of methanol to vegetable oil added with a catalyst at 45-50 °C with constant stirring for half an hour producing glycerin and desired ester layers, after gravity separation [5]. The catalyst employed can be acids, bases or lipases. Apart from being slower than base-catalyzed reactions, acid-catalyzed reactions also require large molar ratios of alcohol to oil. Presence of a higher percentage of FFAs in the feedstock is not suitable for the base catalysts since it leads to side reaction (saponification). Most commonly, a homogenous catalyst such as sulfuric acid is used for high FFAs feedstock and have serious limitations due to corrosion problems, catalyst removal post-reaction and high wastewater generation [6-8]. While heterogeneous catalysts can be easily removed, catalyst leaching, catalyst inhibition and lower reaction rate are problems. Lipase-catalysts provide milder reaction conditions, reusability and are insensitive to the presence of water in the feedstock. However, the high cost of lipases and slow reaction rate limit their application [9]. The conventional chemical-based methods employed in biodiesel production scheme starting from lipid/biomass extraction, pre-treatment, transesterification and purification steps have their limitations already reviewed by many researchers [10-16].

There has been a constant improvement in the biodiesel production area, providing the latest generations of chemicals and methods which promoting biodiesel production towards a lesser-polluting fuel economy. Investigators use various analysis methods to gain a better insight into projected production processes/schemes and provide better environmental/commercial outlook for all possible results [17-24]. Chemicals used in biodiesel production have also been replaced by green or sustainable alternatives [25] wherever possible, subject to economic viability. Particularly, ionic liquids (ILs) have been thoroughly investigated [26] as catalysts/ cosolvents for the biodiesel production as they have low vapour pressure, good thermal stability and can

be recycled. These properties make them easily tunable which has attracted focus in various phases of biodiesel production. Scientific research has been done with ILs acting as co-solvents in biodiesel preparations [27-33], as catalysts in esterification/ transesterification [34-36], for extraction of biomass [37, 38], for extracting FFAs [39, 40] and for glycerol removal [41]. Significant works have been published on the use of ILs for the biodiesel production [42, 43], but the industrial application of these methods has not come into practice yet. This is attributed to higher costs of ILs, their higher viscosities, difficult recovery and concerns over toxicity [44] and biodegradability [45]. Interestingly, one way to cope with the problems experienced in the application of ILs, without losing much of inherent qualities, is to substitute deep eutectic solvents (DESs), made up of individual components of organic origins.

Though ILs and DESs have similar physical properties, chemically they are quite different. While ILs, usually consists of discrete anions of organic/inorganic origins and organic heterocyclic cations, DESs are eutectic mixtures of acids and bases containing a variety of anionic/cationic entities. Figure. 2 illustrates the summary of the comparison between ILs and DESs. Putting aside their effectiveness as a catalyst or solvent, of ILs as compared to DESs in the whole biodiesel production scheme; based on the 12 principles of green chemistry [46], ILs perform inferior to DESs. This is especially when considering principles 1 (waste prevention), 2 (atom economy), 3 (less hazardous chemical synthesis), 5 (safer solvents and auxiliaries), 8 (reduce derivatives) and 10 (design for degradation). Thus limiting the application of ILs.

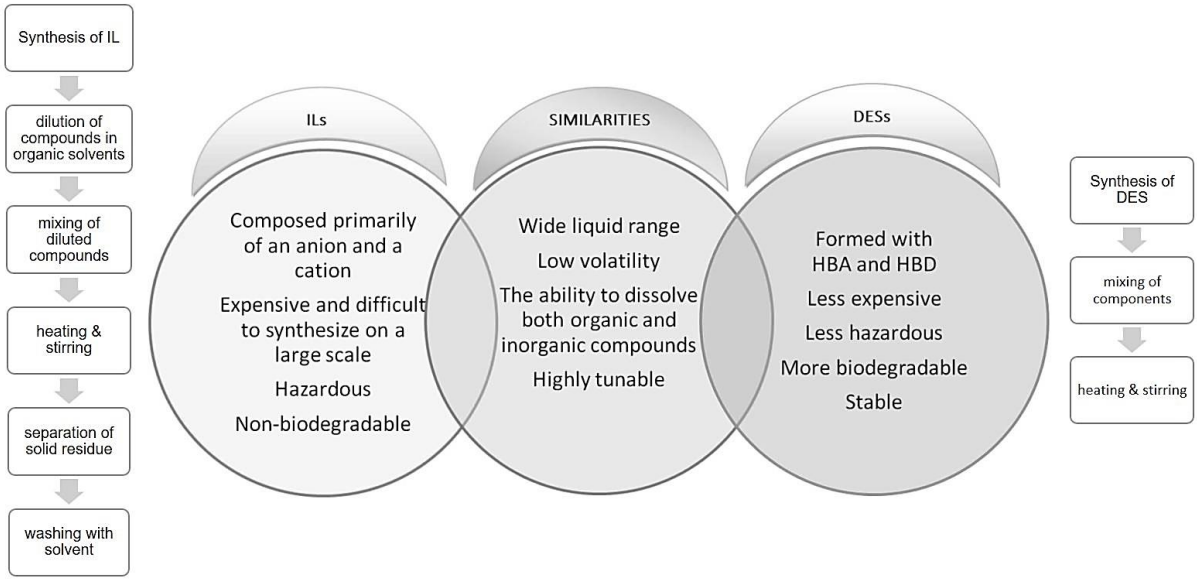


Figure. 2. An insight into similarities and differences of ionic liquids and deep eutectic solvents, adapted from [47].

Abbott first showed the applicability of these low-cost eutectics in their research in 2003 [48] followed by publications in 2004 [49], and 2006 [50].

General preparation of deep eutectic solvents aims at producing a homogenous mixture, which usually is achieved by stirring a hydrogen bond acceptor (HBA) with a hydrogen bond donor (HBD). As generalized by Smith et al. [51], the general formula for DESs is –

$$\mathbf{Cation^{+}\ X^{-}\ y{*}Z}$$

A cation is usually the salt of any positively polyatomic ions of phosphorous, nitrogen or sulfur. X is generally a halide ion, Z is generally Lewis or Brønsted acid but can also be an amide or alcohol, and y is the number of Z molecules. The cationic part accompanied with its halide ion usually serves as HBA while accompanying Z molecule is HBD. As per Abbott et al. [52], the actual reason behind DESs having lower melting points than its constituents is a combination of:

1. lattice energies of constituents,

2. the extent of hydrogen bonding formed on interactions of the anion (X^-) – HBD (y*Z) and,
3. entropy changes when liquid is formed.

Review studies in 2010 [53], 2013 [43] and 2016 [42] have comprehensively discussed the developments of biodiesel production using ionic liquids and deep eutectic solvents. Mamtani et al. [54] conducted a thorough review on the glycerolysis of FFAs and suggested DESs-based homogenous catalysis for future development. However, these studies have been mostly dedicated to ionic liquids and thus, may have undermined the potential of DESs application in biodiesel production. Since then there have been many developments in various phases of the biodiesel production using DESs and thus a review is much needed. To date, no dedicated evaluation exists that critically examines DESs strengths and limitations in biodiesel production, making this review an original work. This study aims to fill this gap and has the application of deep eutectic solvents in various aspects of biodiesel production as its main consideration. The role of DESs in the extraction of biomass/ lipids and other valuable compounds, feedstock pre-treatment, ester production, extraction of impurities for improving the biodiesel quality is reviewed and discussed. The discussion also comprises recyclability and optimization studies.

2 Lipids and other Valuable Compounds Extraction

In biodiesel production based on microalgae, oil/lipid extraction is an energy-intensive process, influencing the related algal-fuel economy. Conventional chemical processes like Soxhlet, organic solvent and supercritical (SC) fluid extraction have either long process times or high associated costs in comparison to passive techniques such as in-situ transesterification [55]. For extracting lipids from microalgae using ILs, polarizability and acidity of the hydrogen bond

were found more important influencing factors than the basicity of hydrogen bond [38]; with extraction efficiency being directly proportional to acidity and inversely proportional to polarizability. In comparison to conventional Bligh & Dyer extraction method, DESs pretreated (with microwave radiation) microalgal biomass followed by extractions using Dimethyl Carbonate (DMC) and supercritical (SC) CO_2, showed better selectivity and yield [56]. A similar study [57], established that DESs pretreatment of the microalgal biomass enhances the main extraction process significantly. Study results showed an appreciable recovery rate of algal lipids. DESs-based on ChCl with oxalic acid (1:2 molar ratio), or ethylene glycol or acetamide had approximately 80%, 67% and 75% recovery rates, respectively. The study confirms that DESs have the ability to rupture cell walls and increase extraction efficiency. Generally, DESs based extraction processes are limited by viscosity. However, Dai et al. [58] suggested a simple solution: liquefying the DESs (by adding water in suitable proportion) before application and creating a pseudo-ternary DESs system with water as the third component.

Currently, biodiesel facilities have limited product inventory in comparison to petroleum refineries. Bio-based feedstocks are rich in certain valuable compounds, the production of which would be beneficial for the whole biodiesel production facility. For instance, crude feedstocks such as palm oil, vegetable oil deodorizer distillates (VODDs) and other biomasses contain different forms of bioactive compounds like tocotrienols, tocopherols, vitamins and even a few valuable medicinal compounds. Nevertheless, their simple and cost-efficient extraction makes the biodiesel inventory broader and economically robust. Common organic solvents such as hexane, hexane/methanol mixture or chloroform/methanol mixtures used in the extraction of biomass are toxic and their usage has been declining steadily [59]. In this regard, deep eutectic solvents have offered a sustainable and environmentally friendly

alternative. Tune-ability of DESs is a key factor in designing them as solvents, as extraction is dependent on partition coefficients, polarity, thermal stability and viscosity among many other factors. Tocols from crude palm oil were successfully extracted using choline chloride: carboxylic acids DESs and malonic acid-based DESs performed best [60]. The investigators were able to recycle the used DESs with water: hexane (4:1, v/v) solution under gravitational settling, but with a significant drop in concentration in first recycle, attributed to inefficient recycling methods. The polarity of DESs is comparable to commonly used solvents such as water and methanol. Of these, ChCl-based DESs, polarity increases as a function of salt`s concentration [61]. Whereas for HBD part, the polarity decreases as phenols > organic acids > amino acids > polyalcohol [62]. ChCl: p-Cresol (1:2 molar ratio) DES was also used to specifically extract α, δ and γ- tocopherols from Soybean oil deodorizer distillates [63]. Π-Π bonds interaction between the phenolic-based DESs (o-Cresol, m-Cresol and p-Cresol) and tocols is suggested as a key factor in affecting the extraction. However, this Π-Π stacking effect was reduced when phenolic-based DESs had substituents at ortho and meta positions, due to steric hindrance in attractive, non-covalent interactions between aromatic rings of DESs and tocols [64]. Additionally, in the extraction of tocols from DESs phase, vortex assistance (agitation using vortex mixer) expedited the process and finished in 30 seconds, at 25°C. Complete mixing of the soybean oil and DESs thereby increasing the contact area between the two was achieved when vortex mixing was used, while ultrasonic-assisted mixing showed inferior mixing owing to the higher viscosity of the reactants.

Another novel extraction method involving methylated oil deodorizer distillates, from which α-tocopherol (representative of vitamin E) binds with tetra butyl ammonium chloride (TBAC) to form a DES were introduced by Qin et al., [65]. From phase-diagram, the eutectic point -50 °C was obtained when the mass fraction α-tocopherol of 0.20, which indicates a molar ratio of

6.2:1 for TBAC to α-tocopherol. TBAC-association solvent was effectively recovered using vacuum vaporization and, for six cycles, α-tocopherol extraction efficiency dropped from 92.95% for fresh to 91.58% for recycled TBAC. A possible explanation for the effectiveness behind TBAC as HBA could be attributed to its long carbon-chain, which makes it more hydrophobic [66]. This approach gives a perspective of broad workability of DES systems. The removal of γ-Oryzanol from rice bran-FAMEs as part of the ester purification process has been successfully done at laboratory scale [67, 68]. In that study, investigators used choline chloride and ethylene glycol (1:2 molar ratio) to form a DES. FAME concentration increased to 96.60% in 240 minutes of extraction process at 30°C with 1:4 (FAME: DES) molar ratio, satisfying EN14214 conditions.

Recently, Tang et al. [69], further developed the science of DESs assisted extraction by applying a biphasic DESs system in the extraction of hydrophobic and hydrophilic compounds in a one-pot system. A biphasic system consisting of hydrophilic DES phase and a hydrophobic DES phase showed better polarity-specific extraction of bioactive compounds; the recoveries of quercetin, artemisinin, chlorogenic acid, and anthocyanidins from Artemisia annua Leaves were 86.5%, 85.7%, 91.8% and 88.1%, respectively.

De Angelis et al. [70], in their patent (WO2020053118), reported a method for extracting bio-oil from microalgal biomass by using DESs, minimizing inorganic salts and phospholipids, as these cause deactivation of a hydrogenation catalyst in Ecofining process [71]. In this method, a mixture of algal mass (saline algae and NaCl) and DES, 1:1 and 5:1 in relation to the algal mass, is added in a glass reactor in suitable proportions. This mixture is agitated for 30 minutes at temperatures between 80°C to 100°C, after which residue is separated while the supernatant oily phase is sent to the second reactor. In the second reactor, water is added and the mixture is again agitated giving two distinct phases, top oily phases containing bio-oil (which can be

sent as-is for Ecofining process) and bottom phase containing DESs and NaCl (which can be recycled back). The ChCl based DES with, ethylene glycol (1:2 molar ratio), formic acid, oxalic acid and levulinic acid (all in 1:1 molar ratios), is used for extracting bio-oil. Nuclear Magnetic Resonance (NMR) spectroscopy of bio-oil obtained from second reactor, showed that for all DESs used: NaCl < 0.1%, phospholipids <0.1%, FFAs < 8%, triglycerides >92% and ketones content was 0; all quantities in moles. In comparison, when ionic liquid methylactylimidazolium chloride (1:2 in relation to algal mass) was used NMR analysis showed: increased NaCl (2.63%), FFAs (12%), phospholipids (1%) and reduced triglycerides content (87%); all quantities in moles. Thus it seen observed that this process ensures longer catalyst life and reduced cost for industrially processing of algal biomass in producing biodiesel.

Hence, it is observed that effective extraction of tocols using TBAC from oil distillates is a very important result as it shows the novel binding scheme of one component of DES directly with the target compound and this saves input cost. Application of the biphasic system shows the potential of one-pot simultaneous extraction and separation of bioactive compounds and opens up scope for further development. Similarly, the polarity, viscosity and aromaticity of the DES structure are responsible for the extraction of bio-active compounds.

3 Feedstock Pretreatment

Certain quality standards are set for a feedstock before it can go to the transesterification reaction. This is essential to maintain efficient production as the conventional transesterification unit is sensitive to inputs. Even if sensitivity is less, standardization helps in maintaining continual production at most optimal inputs. Water and FFAs are among the major unwanted impurities in feedstocks. Non-edible oils such as Polanga seed oil, Mahua seed oil,

used cooking oil (UCO), yellow and brown grease, Rubber seed oil, Tobacco seed oil, Sludge palm oil, vegetable oil fatty distillate etc., have similar impurities requiring pretreatment before transesterification [72]. These alternative feedstocks have high FFA content [73] and they require a pre-treatment process to reduce the FFAs content below 0.5 % [74] before being fed into the transesterification process. Figure. 3 depicts the different deacidification routes reported in the literature and their possible consequences. Neutralization is simply alkaline catalyst based transesterification, which results in the loss of neutral oils due to saponification side-reaction. Also, wastewater generated after intensive biodiesel washing requires expensive treatment before the effluent can be discharged as mandated by local wastewater restrictions [75, 76].

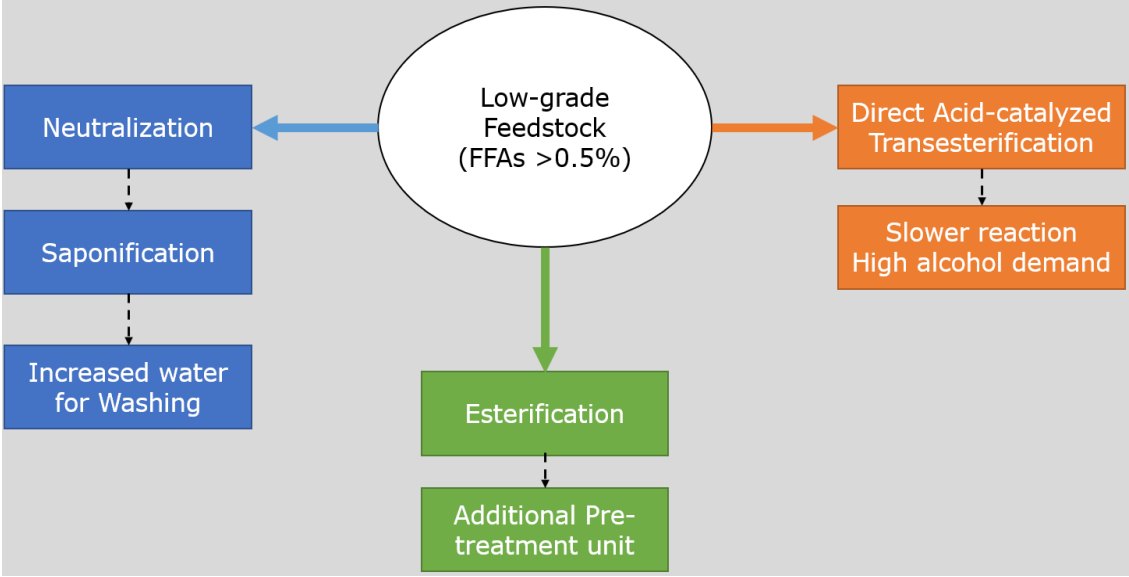


Figure. 3. Deacidification routes for low-grade feedstock. (colour)

Neutralization and direct transesterification only differ in the catalyst used and both avoid the requirement for the pre-treatment unit. In the case of FFA esterification, methanol, being cheaper and reactive, is frequently used [77]. Another way to deacidify or remove FFAs from the feedstocks via the esterification reaction is to use crude glycerol as the alcohol moiety instead of methanol or any other monohydric alcohol. High-temperature glycerolysis or

302 conventional catalyst-based glycerolysis requires excessive energy input making whole in-situ
303 glycerol valorization an uneconomical scheme. The best performing catalyst systems such as
304 metal derivatives, ionic liquids, heteropolyacids and heterogeneous catalysts like Amberlyst-
305 15, mesoporous silica and hydrotalcite, mostly do not satisfy principles 1, 2, 3, 5, 8 and 10 of
306 the green chemistry [46]. A much greener option is enzymatic catalysts, but lipase sensitivity,
307 higher prices and slow reaction rate are hurdles in upscaling to an industrial level. Strong acids
308 and bases such as H_2SO_4 and NaOH in homogeneous catalysis increase water requirements in
309 downstream purification. In this regard, the novelty in using Phosphonium based deep eutectic
310 solvents in glycerolysis of oleic acid (100 % FFA) was fully demonstrated by Williamson et
311 al. [78]. They studied reaction kinetics to study catalyst loading effects from an economic
312 perspective in an esterification reaction. Significant reduction (~34%) in the activation energy
313 (E_A) is achieved when the catalyst concentration is increased. Figure. 4 shows the variation of
314 catalyst loading with E_A. This catalyst loading effect on reducing the activation energy, in DES-
315 catalyzed esterification was validated when another study [79], worked using a second-order
316 rate equation for their kinetic study.

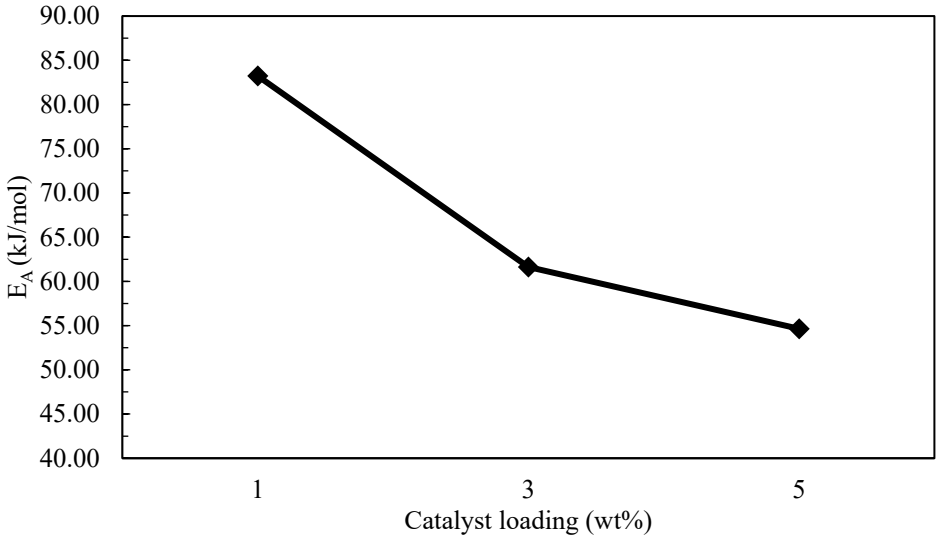


Figure. 4. Catalyst loading effect on activation energy in Phosphonium-based DES catalyzed esterification of Oleic acid, [78].

In the esterification of lauric acid with ethanol, Li et al. studied the kinetics by successfully integrating pseudo-homogeneous model, in case of BTMAC-PTSA [80] and CTAB-PTSA [80] as catalysts each with 1:3 molar ratio. Melting points and pH for BTMAC-PTSA and CTAB-PTSA were reported to be 58.5℃, 53℃ and 0.46, 1.65, respectively. In BTMAC-based catalyst, for the optimal conditions of 70 °C, 180-minutes reaction time, 3% w/w catalyst dosage and 1:1.5 lauric acid to ethanol molar ratio, conversion and activation energy (E_A) were reported as 90% and 49.04 kJ/mol, respectively. Whereas CTAB-based catalyst, gave 85% conversions, for the optimal conditions of 75°C, 5.0% w/w catalyst dosage and 1:2 lauric acid to ethanol molar ratio in 180-minutes of reaction time. The activation energy (E_A) reported was 48.78 kJ/mol, with recyclability up to six consecutive cycles while maintaining the conversion efficiency.

A comparison of the best three performing homogeneous catalysts is illustrated in Figure. 5. In comparison to DES-catalyzed reaction, the other two reactions have feedstock with lower initial acid values, 12 and 0.51 mg KOH/g for NaOH (cosolvent hexane) and Imidazolide Ionic

Liquid, 1-butyl-3methylimidazolium Imidazolide, respectively. In the particular case of the NaOH based catalysis, when FFA content increases, the reaction parameters such as temperature, pressure and time also increase. However, of these three, only the DES-based catalyst is environmentally benign and closely follows the principles of green chemistry.

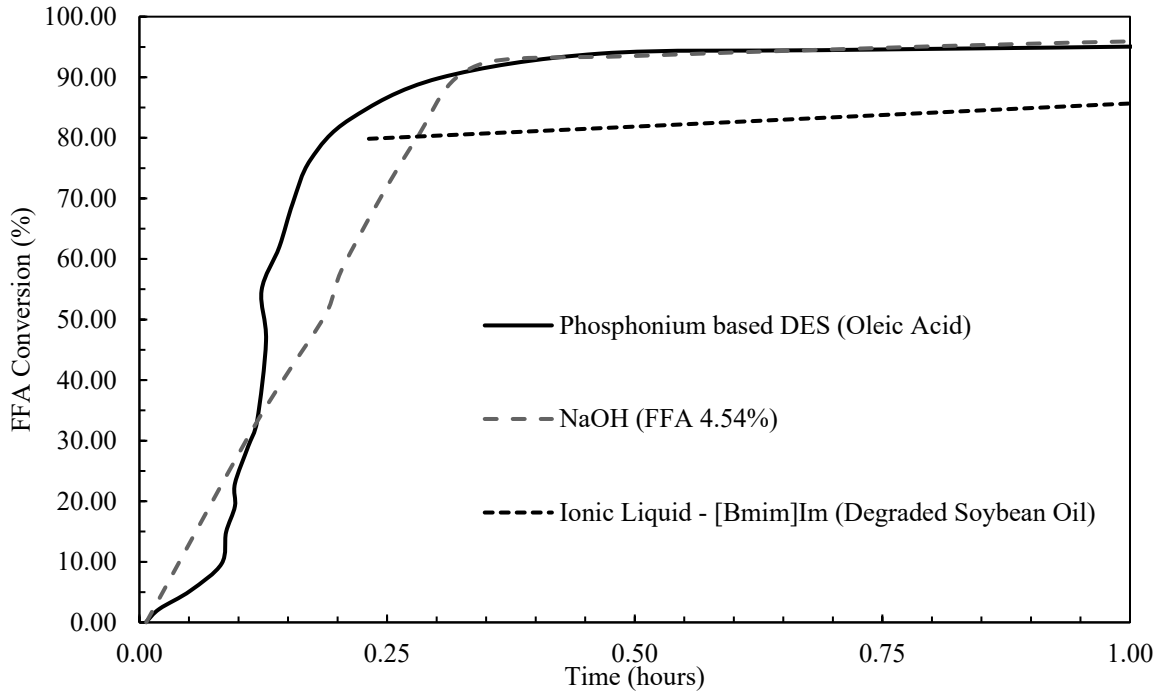


Figure. 5. Performance of few best homogenous catalysts in glycerolysis reaction, [78, 81].

Zhang [82], conducted a comprehensive study on DESs-based glycerolysis of lower quality oils in 2019. The investigator is first in applying DESs as a catalyst in the esterification of palm oil deodorizer distillate (a waste product) with glycerol. 90% conversion to glycerides for an initial FFA content of over 70% in oil, is achieved in optimum conditions, which are; oil to glycerol molar ratio 1:6, stirring at 600 rpm, N_2 purging at 80-100 ml/min, 160 ℃, 3 wt.% DES loading, and 30 min reaction time. Using Fourier-transform infrared spectroscopy (FT-IR) and Gas chromatography (GC), the investigator suggested second-order reversible reaction kinetics where, at 8% of catalyst dosage, the lowest activation energy of 58.80 kJ/mol is achieved. The

investigator also reported material and energy balances for a lab-scale system, concluding that around 94% of the total cost is spent only on material acquisition for this pre-treatment of lower quality feedstock. In addition, to the acidity of PTSA which is responsible for nucleophilic acyl substitution on the glycerol molecule, it is believed that the bulkier organic HBA is responsible for effective mass transfer, in the biphasic reaction system.

Details of reported usage of DESs as a catalyst in the esterification of low-grade feedstock are presented in Table 1. In the table, serial number (S.no.) 1 and 14, employ same Phosphonium based DES (ATPB: PTSA) for the esterification of low-grade crude palm oil (LGCPO). Although there is a slight difference in the acid value of two feedstocks used, the significant difference in optimal conditions tabulated might be due to alcohol moiety used. Polyhydric glycerol is more stable and less reactive than methanol, thus increasing activation energy for the reaction. Comparing S.no. 1 and S.no. 2, reduced consumption of alcohol is achieved but this results in a concurrent decrease in final FFA conversion. Similarly, in S.no.3, hydrogen bond acceptor (HBA) is changed to ChCl and almost no difference is seen in optimal conditions since the feedstocks have comparable acid values. A similar development was observed when the hydrogen bond donor (HBD) part was changed to camphor sulfonic acid (CSA), using a new DES (ChCl: CSA) for esterification of a mixture of two palm oils in S.no 11. No appreciable change is observed. On observing S.no. - 1, 2, 3 and 11, it is seen that even with switching the HBA and HBD part of DES, significant change in optimal conditions is not seen and we conclude that acidic strength of the HBD is more dominant in DES-based catalysis.

In entries S.no. 5-7, the effect of HBD part on the catalytic efficiency of DES is salient. In accordance with the acidic strength of HBD part: PTSA > OX > CA, the respective DESs have increased conversion rates. Similarly, in S.no. 8-10, the effect of alcohol moiety on the catalytic performance in esterification reaction is studied. Increased conversion rates in all the three

372 DESs show that, as alcohol (-C-) chain increases, so does the catalytic performance. This
373 implies that DESs are more efficient in catalyzing longer-chain molecules, thus showing how
374 flexible DESs are in homogenous reactions.

375

376

Table 1. Reported works on esterification of free fatty acid using DESs.

S.no.	Deep eutectic solvents	Feedstock	Alcohol[1]	FFA[2] %	T°C	Molar[3]	Time (min)	Recyclability[4]	Ref.
1	Phosphonium-based DES (ATPB: PTSA)	LGCPO	C1	96	60	1:10	30	4 cycles	[83]
2	DEEAC-PTSA	LGCPO	C1	90	60	1:8	30	3 cycles	[84]
3	ChCl-PTSA	ACPO	C1	97	60	1:10	30	3 cycles	[85]
4	ChCl-PTSA	Acrylic acid	C4	90.2	85		240	3 cycles	[86]
5	BTMAC-PTSA			65.2	90		60		
6	BTMAC-CA	Acetic acid	C4	43.3	90		180		[87]
7	BTMAC-OX			61.3	90	1:1	180	4 cycles	
8	BTEAC-PTSA			90			180		
9	BTEAC-CA	Acetic acid	2-ethyl-hexanol	61	100		180		[79]
10	BTEAC-OX			71			180		
11	ChCl-CSA	ACPO + SPO	C1	96.5	60	1:10	40	2 cycles	[88]
12	Choline cholride.2$ZnCl_2$	C8-C18 acids	C8-C18	99	110	1:1	240-720	6 cycles	[89]
13	Imidazole-PTSA (1:2)	IBAc	IBOH	92.5	80	1:1.2	120	6 cycles	[90]
14	ATPB-PTSA (1:3)	Oleic acid	Gly	95	150	1:6	30	5 cycles	[78]
15	CTAB-PTSA (1:3)	Lauric acid	C2	85	75	1:2	180	6 cycles	[80]
16	TBAC-AA (1:2)	Palmitic acid	C1	94.3	60	1:10	60	-	[91]

1 – C1, 2, 3, 4…. refers to that straight-chained alcohol, for example, C1 – methanol; Gly – glycerol; IBOH- isobutanol
2 – Free fatty acid conversion percentage,
3 –Molar ratio of feedstock to alcohol,
4 – Number of cycles having similar efficiency as the (including) first cycle.

In the case of Zinc-chloride based DES, there is higher catalytic activity and no water removal is required for long-chain esters production. However, the intrinsic toxicity in this DES due to its metal composition makes it unsuitable in comparison to other DESs reported here. For a better outlook of the DESs reported, the structure of their constituents is presented in Figure. 6.

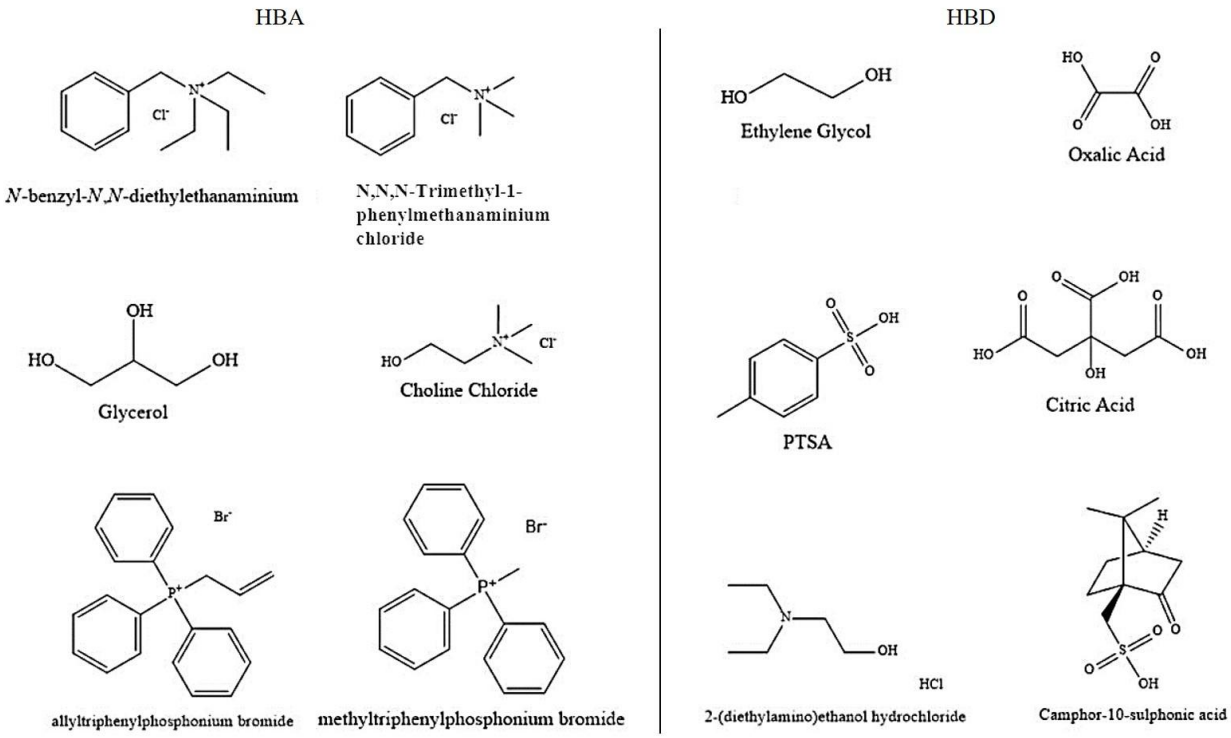


Figure. 6. Structure of HBAs and HBDs used to synthesize DESs in reported works.

Petračić et al. [92] explored DESs-assisted extraction of trace metals (Fe, Mg, Na, K, P, B, Ca) from non-edible low-grade waste feedstocks. Although both quality and origin of feedstock supposedly influence the pre-treatment process, polyol DESs based extractive deacidification enhanced the whole adsorption-extraction pre-treatment. Choline chloride based (ChCl: Gly) DES is best suited for the extraction of metals from waste animal feedstocks.

Mohd. et al. [93], in a patent (PI 2012700963) report a method to reduce FFAs content in a lower quality oil. The molar ratio of the alcohol to the crude plant-derived oil (preheated) is in a range of 1:1 to 20:1, in the presence of 0.25% to 3.5% (by weight) of deep eutectic solvent

catalyst. Gadilohar et al. [94], in their patent (IN201821032223), reported a strategy to reduce the free fatty acid content in highly acidic feedstocks using alkaline-DESs. Simple mixing of oil and DES is done, after which HBA part of DES is recovered and then it is recycled. These alkaline DESs consist of a salt (HBA) selected from the group consisting of potassium carbonate (K_2CO_3), sodium carbonate (Na_2CO_3), and ammonium carbonate ($(NH_4)_2CO_3$), while hydrogen donor part is selected from the group consisting of poly-hydroxy alcohol, an acid, an amine and a halide. Reduction of over 90% in Total Acidic number is reported in all the oils tested, simply and economically. However, for recycling the DEScatalyst, usage of mineral acid (such as HCl or H_2SO_4) and inorganic solvent (such as dichloromethane, chloroform, and carbon tetrachloride), makes the whole process less environment friendly.

Recently, novel usage of Amberlyst-15 with ChCl-based DES as a hybrid catalytic system to successfully produce ethyl lactate was reported [95]. ChCl: Gly (1:2) showed a significant reduction in activation energy and two fold increase in yield is seen when DES is added to Amberlyst-15 catalyzed reaction, whereas when only DES was used in the reaction, almost negligible catalytic ability. A similar study [96] was reported for methanolysis of oleic acid with Amberlyst-BD20 with ChCl: Gly DES, providing further evidence that the performance of the heterogeneous catalyst is improved when DES is used as cosolvent. The water absorbency ability of DES due to hydrogen bond effect enhanced the esterification reaction.

Thus, in the esterification of FFAs, it is seen that HBD part of DESs affects catalytic efficiency in proportion to its acidic strength, the more acidic having proven increased catalytic abilities. While heterogeneous catalysts are less efficient in esterifying when longer chain alcohols are involved, DESs are found to maintain their catalytic activity, indicating wider flexibility. DESs dosage is linearly inversely proportional to the activation energy. DESs are also efficient when pre-treatment involves trace metal removal from the low-grade feedstock. In-addition, in

heterogeneous catalytic systems, DESs can be applied as cosolvents to increase the reaction rate.

4 Transesterification

A triglyceride consists of tri-hydric alcohol (glycerol) with three FFA chains. The main aim of transesterification is to break the glycerol backbone and to produce fatty acid alkyl esters. For this, the desired alcohol (usually methanol) is added with triglycerides in a catalyzed reaction where the catalyst deprotonates the alcohol. Liu et al. [97] investigated metal and acidic ChCl based DESs as catalysts in the transesterification of soybean oil with methanol. With Zn-halide (Cl and Br) Zn: Halide in 1:2 molar ratio, the yield obtained was under 4% while with ChCl: PTSA (1:3) as a catalyst, the yield obtained was 92% when reaction conditions were 10% w/w catalyst, 2 hours, 100°C and 10:1 molar ratio of methanol to oil. When only PTSA was used as catalyst, approximately 80% yield was obtained. The addition of ChCl not only improved the yield for the reaction but also improved the by-product (glycerol) separation from the product. This is attributed to the catalyst molecule's bulking, which improves the mass transfer during the reaction while preserving the catalytic ability of PTSA. Manurung et al. [98] and Taslim et al. [99] employed ChCl: EG DES and ChCl: Gly DES as co-solvents in the base-catalyzed ethanolysis of palm oil. Product yield and ethyl ester content in a ChCl: EG DES (4 wt.%) based reaction was 81.72% and 99.3% respectively, while in the ChCl: Gly DES (2% conc.) case these were 83.67% and 99.7% respectively. Both studies showed that when DESs is used as co-solvent, better phase separation occurs and less water washing is required. In the base-catalyzed (KOH-1% wt. of oil) methanolysis of sesame oil (FFA = 0.2%), Kadapure et al. [100] studied the performance of three ChCl-based DESs. Overall, there was around a 12% increase in FAME yield when DES was used in comparison to conventional methanolysis. Out of citric acid, glycolic acid and ethylene glycol-based DESs, EG-based DES enhanced the yield up to 97.8%. The researchers also observed that, initially, the reaction is heterogeneous while in later

stages it becomes somewhat homogenous due to emulsification. On examining their results, it is seen that, the addition of DES to conventional methanolysis reduces chances of saponification reaction considerably. Overall, it can be seen that, the addition of DES as cosolvent works by restricting highly polar FFAs in the feedstock from reacting with alkaline catalyst and water molecules, so that saponification and hydrolysis can be limited. Moreover, post-reaction this DES ensures that FAMEs form a separate phase while unreacted methanol, glycerol retain in different phase. These effects may be attributed to the polarity of the DESs.

In a thorough investigation of the base-catalyzed ethanolysis of sunflower oil using CaO, $CaCO_3$ and $Ca(OH)_2$ as illustrated in Figure. 7, Troter et al. [101] tested ChCl-based DESs as co-solvents. Ethylene glycol [37], Urea (Ur), 1,3-dimtheylurea (DMU), Thiourea (TU) and Glycerol (Gly) were used as HBD for preparation of DESs at 1:2 (ChCl: HBD) molar ratio.

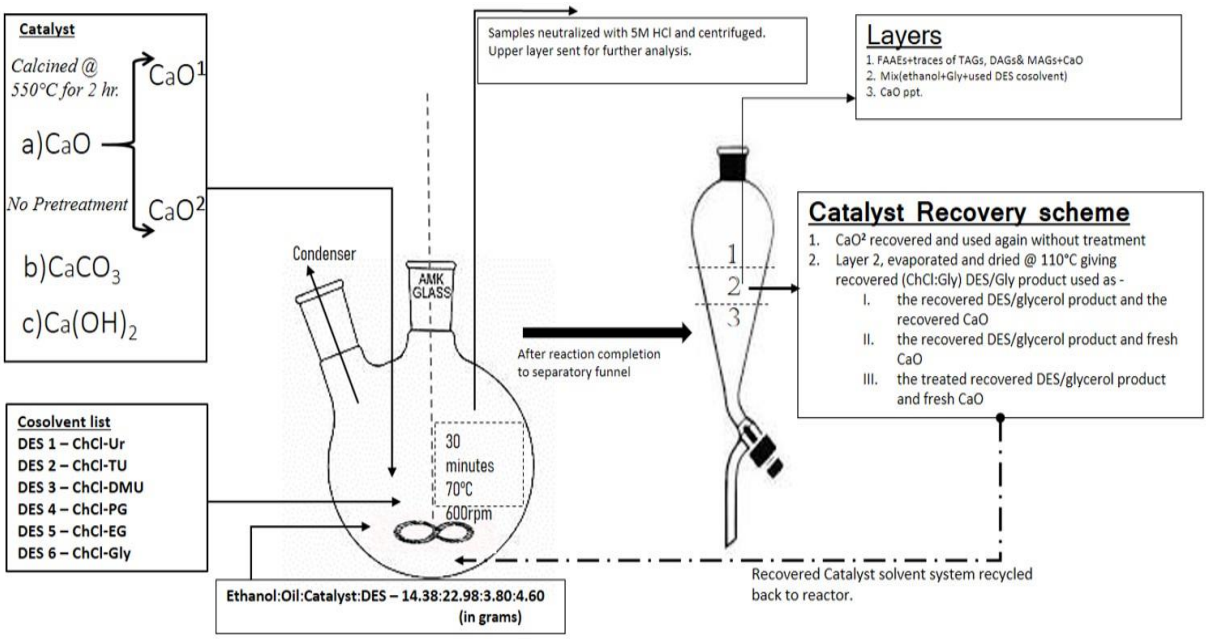


Figure. 7. Experimental setup for DESs assisted ethanolysis of degraded sunflower oil, adapted from [101].

The results indicated that $CaCO_3$ did not catalyze the reaction, whereas CaO and $Ca(OH)_2$ showed some catalytic activity. The catalytic activity correlated to the basic properties reported by the authors, i.e. the strength of basic sites in the respective calcium catalyst dictated their

performance, in the decreasing order as CaO > $Ca(OH)_2$ > $CaCO_3$. For calcium oxide, CaO^1 (calcined) showed four times more activity than CaO^2 (non-calcined) owing to the pretreatment (calcination) given to former whereas latter is used without any pretreatment. FAAE yield for CaO^1, CaO^2 and $Ca(OH)_2$ was around 96.5%, 98% and 30% in approximately 360, 420 and 480 minutes of reaction time, respectively. None of the DESs exhibited catalytic activity however when DESs were added as a co-solvent, they increased the catalytic activity of CaO^1 and CaO^2. In the solvent-based system, the efficiency of DESs in descending order is reported as: ChCl:EG > ChCl:PG > ChCl:Gly > ChCl:Ur > ChCl:DMU > ChCl:TU. Among polyol-based DESs, EG and PG are more efficient owing to their lesser viscosity. Also, ester concentration decreased in the final phases implying that, in glycerol-based DESs, reverse reactions are influenced by excess glycerol. For amide based DESs, the efficiency is related to their viscosity, less viscous ChC: Ur DES being more efficient. In reusability study, CaO^1 showed a reduction in catalytic activity ascribed to leaching, incomplete separation and inhibition by reactants. DES 1, 4, 5 and 6 all had similar reusability efficiencies in FAAE conversion for up to six cycles. Among the three catalyst recovery schemes shown in Figure. 7, the recovery scheme (2. (II)), in which the treated recovered ChCl: Gly DES was fed with fresh CaO^1, showed stable FAAE conversion efficiency, as illustrated in Figure. 8. CaO^1 and ChCl: Gly DES are better options from an economic and efficiency perspective. This study provides a notable result: by-product glycerol can be fed back into biodiesel production as co-solvent reducing the cost of production.

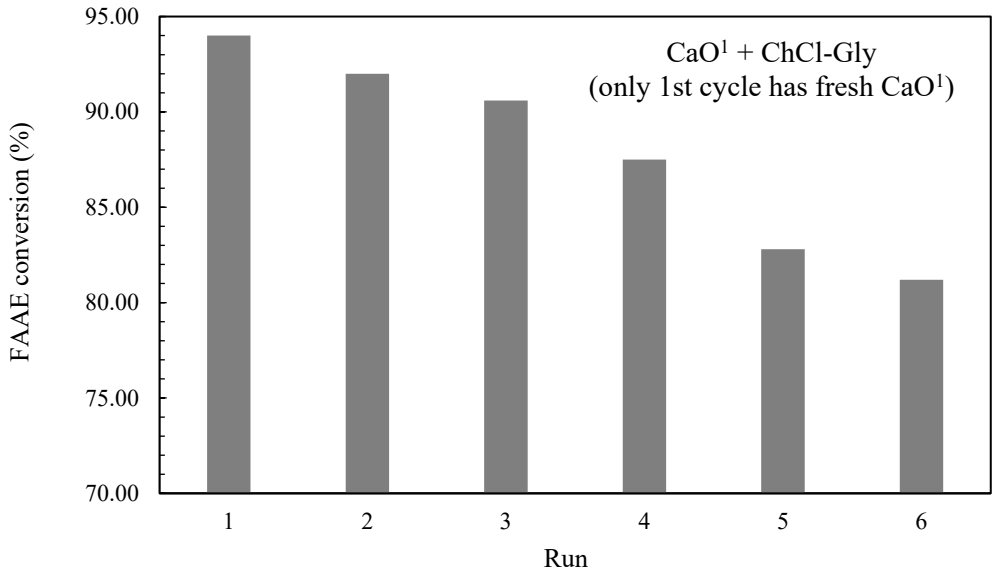


Figure. 8. FAAE conversion % in successive catalyst recycling runs, adapted from [101].

In lipase-catalyzed transesterification, Oh. et al. [102] studied seventeen ChCl-based DESs composed of one or two HBDs i.e., including ternary DESs. DESs consisting of ChCl, Urea, and Formamide (in a molar ratio of 1:1:1) gave highest lipase activity, whereas DES consisting of ChCl, Gly, and EG (in a molar ratio of 1:1:1) gave the highest lipase stability. Amides as HBDs were better in providing higher lipase activity while HBDs containing hydroxyl groups were better in making lipase more stable. They reported that lipase stability increases with an increasing polarity and acidity of DESs. Intermediate washings are done to avoid the glycerol build-up in the reaction in subsequent cycles, thus necessary to maintain the product yield.

Kleiner et al. [103] developed a two-step one-pot lipase-catalyzed transesterification process for a wide range of feedstock such as refined oils, acidic oils, used oils, greases and fatty acids. Reaction mixture consists of 1 kg of feedstock with 315 g ethanol, 1 ml KOH (1N), 13.75 g H_2O at 30°C with 250 rpm agitation for 24 hours. The reaction was started with the addition of 10 ml of Lipozyme TL 100L. Post 24 hours, 1 kg of solvent (DESs) and 10 ml of Lipozyme CALB L are added after the temperature is increased to 45°C. After more than 24 hours, the reaction stopped and gravity-based phase separation is done. Pre-purification ethyl ester contents (%) in ChCl: Gly DES, ChCl: Ur DES, Glycerol and no solvent-based

transesterification were 95.2, 87.8, 90.5 and 83.7 respectively. After purification, ethyl ester content for ChCl: Gly solvent system, was increased to satisfy EN14214. In this work, lipase recycling is reported to be complex, in addition to longer transesterification reaction times.

Similarly, ternary DESs are explored as alternative solvents for enzymatic transesterification by Merza et al. [104]. Waste cooking oil (WCO) is transesterified with a reaction mixture consisting of 0.5 ml WCO, 0.135 mL CH_3OH and 8% solvents (DESs and IL) at 45°C with 150 rpm in a 5 ml vial for 4 hours of reaction time. The reaction was started by adding 106.25 mg of Novozym® 435. ChCl: Gly DES was used as a ternary solvent by adding 4 wt.% H_2O which significantly reduced the viscosity and thus improving reaction rate. This is because viscosity directly affects enzyme mobility in the solvent. At this configuration, 50% of production yield is achieved and beyond this lipase loading, enzyme saturation takes place. However, increasing the water content in DES beyond 4% again resulted in a drop in conversion, which may be attributed to changing polarity due to the addition of -OH groups in the solvent system. In subsequent cycles, only crude biodiesel was removed using n-hexane extraction and after this glycerol removal was necessary to keep up the product yield. The product yield of 85% and 49% in comparison to 36% and 33% (in the absence of glycerol removal) is maintained in case of using solvents, DESs and ILs, respectively. Best glycerol removal is achieved by washing with 1-butanol. At the end of the reaction, the yield in ternary DES is 71.4% higher than 1-butyl-3methylimidazolium hexafluorophosphate. This study shows that ternary DES based on water is better in comparison to sole DES solvent due to a reduction in viscosity and glycerol accumulation being an issue, which requires extra washing. In the second run when the lipase-DESs system was catalyzing the reaction, the yields dropped in the next cycle. However, the addition of water to the DESs (making it a ternary system) increased the yield again making the system more resilient.

525 However, not all DESs are suitable for lipase-catalyzed transesterification. In the lipase-
526 catalyzed transesterification of vinyl laurate, Durand et al. [105], reported that certain DESs,
527 especially ones which were based on dicarboxylic acids and ethylene glycol as HBDs,
528 competed with the main reaction and produced side products. This also leads to DESs
529 destruction thus affecting the recyclability of DESs. Following the previous trend, they also
530 observed that ChCl with urea and glycerol to be better at selectivity and activity, as strong
531 hydrogen bonding in these DESs limits their side reaction capabilities.
532
533 Pan. et al. [106] investigated the applicability of ChCl: Acetic acid DES in the one-pot method
534 for micro-algal derived biodiesel production. Figure. 9 shows a schematic diagram of processes
535 with optimum parameters involved in this study and presents FAMEs yield from different
536 catalysis schemes. Ionic Liquid 1-ethyl-3-methylimmidazolium methyl sulphate, also
537 transesterified wet microalgae when assisted with microwave irradiation [107], showing a
538 similar instance of the one-pot extraction-transesterification process. In the case of (1%),
539 H_2SO_4 catalyzed hot water (120°C) extraction of algal lipids followed-by H_2SO_4 (1% w/w of
540 lipid) catalyzed methanolysis at 100°C for 60 minutes [108], impurities like chlorophyll must
541 be pre-treated to enhance reaction and lower catalyst dosage.

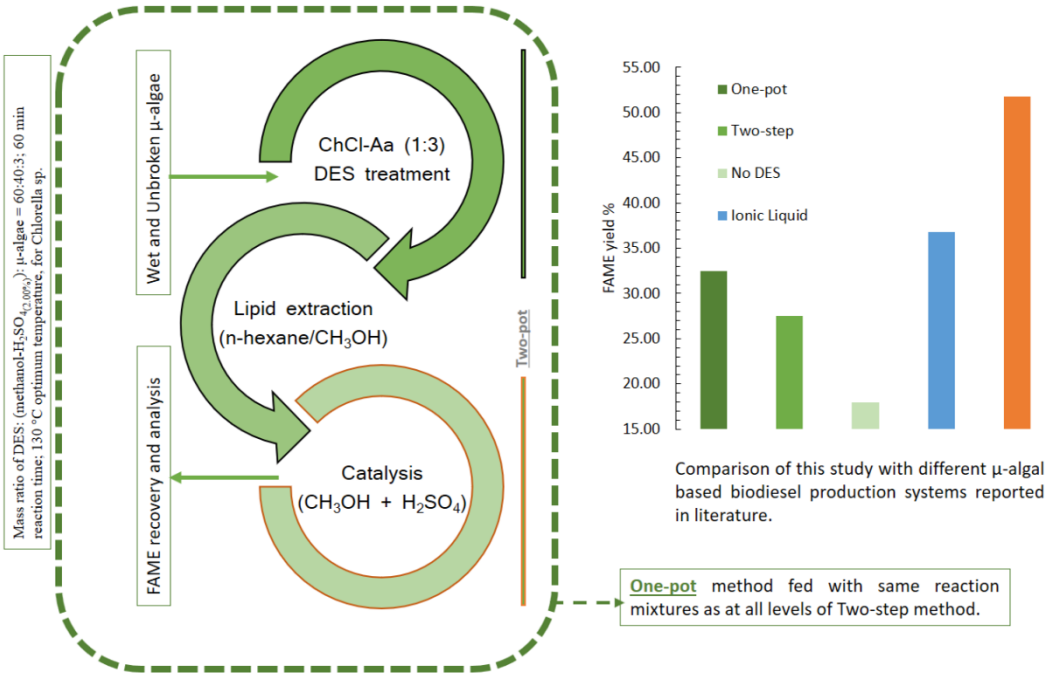


Figure. 9. DES-based micro (µ) algal biodiesel-production process outline and FAME yield comparison with ionic liquid and conventional acid system [106]. (colour)

Fang et al. [109], in their patent (CN103525562), made claims that, in a conventional base-catalyzed (NaOH) transesterification, a eutectic system can be induced by adding 2-10% of Choline Chloride salt. This choline salt makes a DES with by-product crude glycerol and induces a eutectic catalyst system. Typical reaction with soybean oil (0.034 mol) as feedstock, operates at 65°C for 4 hours with, NaOH (0.0075 mol), methanol (0.1875 mol) and 0.6 g of ChCl salt. As a result, post 2-6 hours of refluxing, easy separation (from separatory funnel) of biodiesel and other reaction products is achieved. Gorke et al. [110] in their patent (US20090117628) report enzymatic reactions (including transesterification) in a deep eutectic catalysis system. Most of the DESs consisted of ChCl salt and carboxylic acids.

In lipase-based transesterification, ternary-DESs act as reaction media or solvent and enhance the reaction. HBDs like amides and –OH, provide higher lipase activity and greater lipase stability, respectively. Polarity and acidic strength of DESs directly influence lipase-stability.

In comparison to ILs and sulfuric acid, ChCl-based DESs, when used as a pretreatment, provide superior recovery rate of algal lipids. It is established that DESs can break through the cell walls, enhancing extraction. Similarly, in progression, one-pot DESs-based (wet and unbroken) algal-biodiesel production on lab-scale is very promising, providing savings by eliminating drying and grinding. Yield through this method is comparable to conventional ionic liquids and acid-based production routes. Employing ChCl-polyol based DESs as a co-solvent during base-transesterification increases product yield and provides better phase separation.

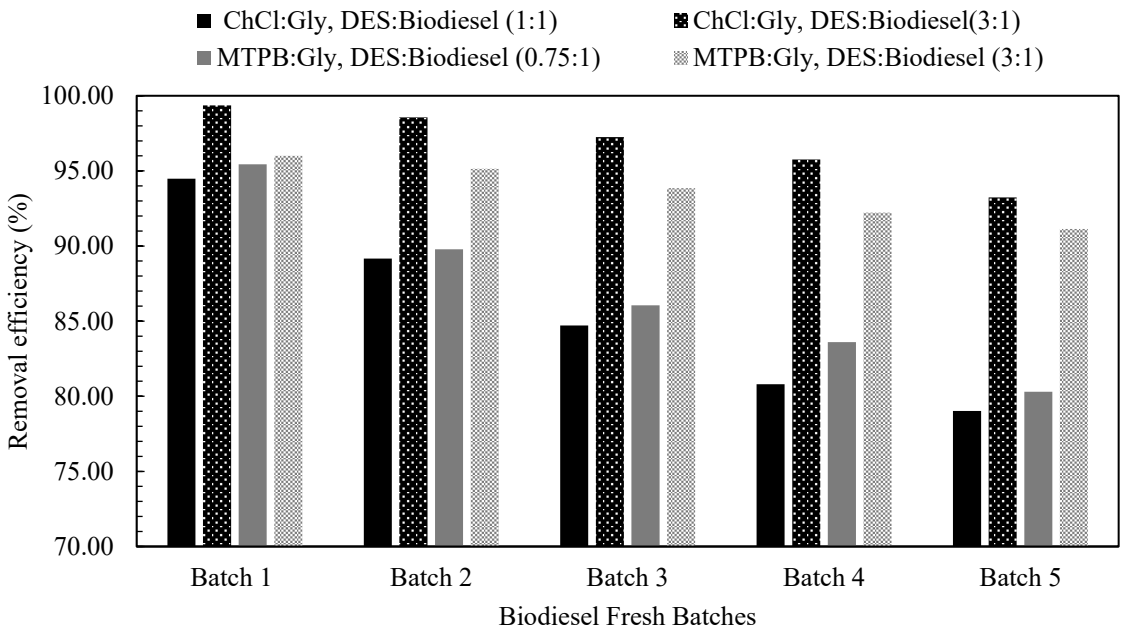
5 Ester Purification

Ester or crude biodiesel purification is a pre-requisite for enhancing the quality of the product to meet the desired specifications defined in the ASTM D6751 and EN14214 codes. These limits exist to counter the otherwise harmful effects of impurities in the finished biofuel product. Impurities are generally unreacted vegetable oil, reactant alcohol, dissolved catalyst, saponification products, free fatty acids and by-products. The glycerol portion still dissolved after the separation unit is free glycerol, poly-glycerides are collectively termed as bound glycerol and the summation of these two is total glycerol. Biodiesel is purified using various methods such as wet washing and dry washing or by membranes. In the purification of crude esters obtained from 2 step alkali-transesterification of waste frying oils: silica gel, 5% H_3PO_4 and hot distilled water gave 92.45%, 91.78% and 88.92%, respectively [111]. Purity levels in excess of 96.5%, when with (liquid-liquid extraction) inorganic solvents [112] such as hexane, 2-methyltetrahydrofuran, and cyclopentyl methyl ether (as an eluent in column chromatography), have been reported recently in the purification of algal-derived biodiesel. Longer decantation times in case of 2-methylhydrofuran and high cost of cyclopentyl ether, are the only limiting elements in upscaling. In their thorough review, Bateni et al, [113] gave an effective categorization for conventional separation processes involved in biodiesel purification as follows:

1. Equilibrium based – Distillation and Extraction (usually DESs work in this domain). Methanol or molecules containing HBDs can be efficiently dissolved in DES while FAMEs remain in a separate phase.
2. Affinity based – Adsorption and Ion-exchange
3. Reaction based – Membrane bioreactor and Reactive Distillation
4. Membrane-based – Membrane separation

Almost all commercial liquid-based purification methods use water as solvent (including wet washing), creating significant water demand (1 part crude biodiesel requires 10 parts water on a volume basis) [114]. This generates considerable wastewater streams. As an alternative, DES is a low-cost, environmentally benign option.

A comprehensive investigation of DESs-based residual catalyst and water removal strategy, from methyl palmitates after alkali-catalyzed transesterification reaction, is reported by Shahbaz et al. [115]. In their study, they prepared DESs based on ChCl and Methyl triphenyl phosphonium bromide (MTPB) as HBAs, with Gly, EG and Triethylene glycol (TEG) and 2,2,2 Trifluoroacetamide (TFA) as HBDs respectively. ChCl: Gly based DESs at all ratios of DES to biodiesel were efficient in KOH removal (99.87%), with removal directly influenced by increasing content of glycerol in the DES. Other ChCl based DESs were able to reduce the KOH content below the set limit of 5 mg/kg when higher molar ratios of DES to biodiesel were used. Similarly, in MTPB-based DESs, removal efficiency decreased as MTPB: Gly > MTPB: EG > MTPB: TEG, suggesting that trihydric alcohol has the best hydrogen binding ability to remove KOH. Figure. 10 shows how reusability of DESs based on glycerol is influenced by the molar ratio of DES to biodiesel feed in the reactor and more resilience is seen with higher molar ratios.



606

607 Figure. 10. Illustrating reusability of best performing DESs for 5 consecutive fresh biodiesel
608 batches, adapted from [115]. In the legend, values in brackets show the molar ratio of DES to
609 crude biodiesel.

610 In addition to KOH, all of these DESs were also able to successfully reduce water content from
611 901 mg/kg to below 500 mg/kg (the set limit in EN14214), with ChCl-based DES being
612 efficient than MTPB-based DESs.

613 Shahbaz et al. [116] also employed these MTPB-based DESs for glycerol separation from palm
614 oil methyl esters. Glycerol removal efficiency is inversely proportional to glycerol content in
615 the DES, as such higher ratios of MTPB: glycerol are rendered ineffective in removal, which
616 is seen in the inadequacy of MTPB: Gly in removing glycerol except when a higher DES to
617 biodiesel ratio is used, further suggesting a cascading effect or forced extraction. MTPB: EG
618 DES and MTPB: TEG DES showed far better removal efficiency, removing all free glycerol
619 at an optimum molar ratio of 0.75:1 for DES to biodiesel. All DESs were able to reduce the
620 bound glycerol content (glycerides), resulting in managing set limits of total glycerol content
621 as per ASTM D6751.

622 Balaraman et al. [117], studied the effect of protic DESs (pDESs) in microwave-assisted
623 extractive transesterification of Karanja and chicken-waste oils. DESs are composed of

BTMAC as HBA and citric acid, lactic acid, malic acid and oxalic acid as HBDs (1:1 molar ratios). Methanol is added to oil samples (0.768 g) in an optimized ratio (1:4) with 8% (w/w) pDESs (0.384 g) incubated at 0.24 kW for 30 minutes. Oxalic acid-based DES showed best glycerol extraction and enhanced the reaction, around 97% and 89% ester yield in case of chicken-waste and Karanja oil respectively, owing to its lower density and higher acidic strength than others.

Homan et al. [118] studied the alcoholysis of soybean oil with longer carbon chain propyl and butyl alcohols instead of methyl alcohol since the better mass transfer is achieved with longer chain alcohols. They purified propyl and butyl esters using ChCl: Gly DES and ChCl: EG DES for extracting glycerol. Ethylene glycol-based DESs performed far better than glycerol ones owing to lower viscosity, especially when HBA to HBD molar ratios were 1:3 and 1:4. At DES to ester molar ratio of 3:1, complete removal of glycerol is obtained. Anti-solvent acetone can efficiently recover up to 70% ChCl from EG based DESs; this was then recycled for further purification batches. Helda et al. [119] used ChCl: EG DES for purifying FAMEs of highly acidic (FFA = 44.75%) rice bran oil. At 1:4 molar ratio of crude FAME to DES, the yield was 96.6% when purification time was 240 minutes at 30°C. Further validity of ChCl: EG DES`s ability to remove glycerol comes from similar reporting of using ChCl: EG DES. There were similar findings in the purification of esters of degraded sunflower oil [120], of waste animal fat [121] and for various other low-grade feedstock [122]. DES composed of ChCl and propylene glycol [37] in molar ratios of 1:2 and 1:3 has also been investigated in crude biodiesel purification [123, 124]. In all the studies, the extraction operation parameters vary, for the example reaction time of 90-270 minutes, agitation rate of 500-700 rpm and temperature of 25°C-50°C. However, it is noteworthy that reaction parameters are required to vary for different initial feedstock types.

Other than catalysis, the purification process is dependent on mass transfer rate; increasing that will make the process more efficient. Micro-extractors have been tested in lipase-catalyzed biodiesel production for efficient glycerol separation by Šalić et al. [125]. Šalić et al. also employed Y-shaped microchannel reactors and ChCl-based DESs, for the separation of glycerol from crude biodiesel [126], illustrated in Figure. 11.

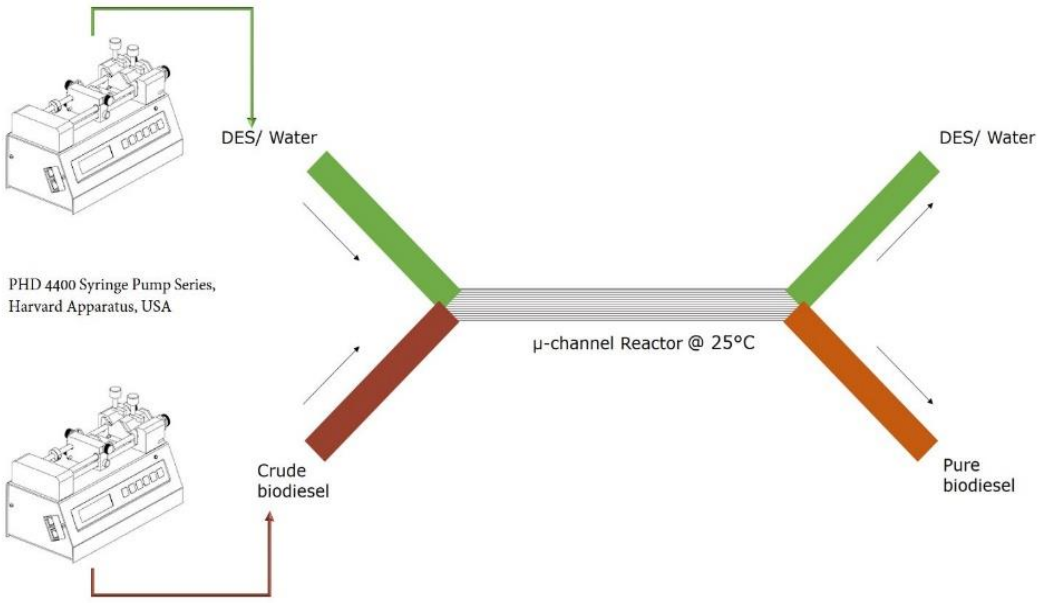


Figure. 11. Y-shaped micro-channel reactor-based glycerol separation. (colour)

After 48 hours of consecutive funnel separation, different separation methods such as filtration (0.20µm pore), centrifugation (14,000rpm) and water-washing in the micro-channel reactor, were applied to check the performance of DESs-based micro separation. As per the ASTM and EN standards, free glycerol content in the finished product should be less than 0.02%. ChCl: EG DES and ChCl:Gly: H_2O based aqueous DES proved to be very efficient in glycerol separation and also removed residual catalyst (KOH). Complete glycerol removal is achieved by ChCl: EG (1:2.5) DES in 180 seconds, ChCl: Gly (1:2.5) DES in 13.61 seconds and ChCl: Gly: H2O (1:1:0.5) in 15.81 seconds. Addition of water not only reduced the glycerol moles in the DESs which may be because of altered polarity due to addition of -OH groups of water; but also improved the volumetric flow ratios (biodiesel: DES) in the channel by decreasing the

viscosity of the DESs solution. It is seen that the width of the microchannel is inversely proportional to the residence time when similar extraction efficiency is maintained, i.e. when width (μm) was increased from 250 to 350 to 500, residence time increased from 15.81 to 26.21 to 39.21 seconds, respectively. DESs usage in purification significantly reduced water load in biodiesel production, as reported solvent usage (on a volume basis) was, 65% for DESs and 550% for water-based micro-extraction. **Error! Reference source not found.**

Farouq et al. [127], in their patent (MYPI 2011004493) reported that Choline Chloride salt is added to polyols or amides to form desired deep eutectic solvents. These are used for removal of residual catalyst from crude biodiesel when DES to biodiesel molar ratio is in between 0.75:1 to 0.75:3, and separating the mixture on settling.

Thus, ChCl-based DESs are very useful for purification of the ester phase, with hydrogen bonding capacity directly influencing alkaline catalyst and glycerol removal ability. It is also observed that tailor-made HBA salts can be used to retrieve glycerol from the FAMEs, such as MTPB. The viscosity of the DESs is also a critical property directly influencing the extraction process.

6 Problems with DESs

6.1 Environmental toxicity studies

Out of all possible DESs, only the metal-free configuration should be adopted in biodiesel production as metals have innate toxicity. With an increased interest in deep eutectic solvents, their environmental degradation and related toxicity have also gained importance. Choline chloride (ChCl)-based DESs have been discussed majorly in this review and thus a discussion on their environmental biodegradability and toxicity is necessary here. In this context, Table 2, lists the various HBAs, HBDs and the living organisms tested for their toxicity studies found in the literature. These studies reported in Table 2, are representative of most of the DESs

689 discussed in this study. In the MTPB based study (S.no 1), all the investigated DESs exhibit
690 inhibition on some bacteria, while in the case of the brine shrimp cytotoxicity of formed DESs
691 were higher than that of individual component`s aqueous solution. Whereas when an aqueous
692 solution of respective DESs was used cytotoxicity was reduced in comparison to non-aqueous
693 DESs, for all the three prepared MPTB-based DESs. Thus, it can be seen that the cytotoxicity
694 of prepared DESs was higher than their respective components. The plausible reason behind
695 increased cytotoxicity is unclear but attributed to the altered physical property of when DES is
696 formed, such as higher viscosity. In S.no 2, DESs showed inhibitory effects on E. Coli bacteria
697 and this effect was higher in the case of formed DESs than their respective components, and
698 this difference increased drastically with increasing concentration in the growth medium for
699 bacteria. This higher bacterial inhibition by formed DESs is attributed to hydrogen bonding
700 between the components, which delocalizes the charge, and chemicals with delocalized charges
701 are reported to be more toxic [128]. Among salt anions, ChCl-based DESs had a more
702 pronounced inhibitory effect than choline acetate (ChAc)-based DESs, when EG was used as
703 HBD. The effect of the two cholinium salts on H. sinensis was highly lethal reducing the
704 survival times from 12 hours (control point) to 3 hours (ChCl) and 6 hours (ChAc). Synthesized
705 DESs also exhibited toxicity towards the H. Sinensis and here also ChCl-based DESs were
706 more toxic than ChAc-based DESs. However, the toxicity effect was lowered by forming
707 DESs. This reduction is attributed to hydrogen bonding which reduces the dissociation of salt
708 in the aqueous solution. When A. sattivum was investigated, among salts ChCl worst effect on
709 the root growth, while for HBDs, the order of toxicity was decreasing as Gly > EG >Acetamide
710 (A) > Urea (U). Again, incorporation of these components into respective DESs showed
711 reduced toxicity for all the DESs. Biodegradability study of these DESs showed that only two
712 DESs, ChCl: U and ChCl: A were readily biodegradable while other DESs had similar
713 degradability as that of control IL, 1-Butyl-3-methylimidazolium tetrafluoroborate. The results

were found in agreement with another study [129] reporting biodegradability of ChCl-based DESs where HBDs used were Glucose (Guc), Gly and OX, all four of these individual components are reported to be readily biodegradable as well.

Similarly, in S.no 3, apart from ChCl; Gly, ChCl: Fructose (Fruc) and ChCl: Guc DESs all other DESs showed some anti-fungal activity. Of this $ZnCl_2$, PTSA and Malonic acid (MA) showed the highest inhibition for every fungal strain, individually as well as in DESs; the activity decreasing as $ZnCl_2$ > MA > PTSA. Incorporation into DESs for all the HBDs reduced the inhibitory effect greatly except for $ZnCl_2$. This reduction is due to the shift in pH towards comfort range from previous acidic range. For the C. Carpio, all the individual components and their respective DESs were low toxic or harmless, except $ZnCl_2$ being lethal individually and in DES form, and MA being lethal individually only. Still, the incorporation of components increased the lethal concentration of $ZnCl_2$. It is to be noticed here that zinc is classified as heavy metal and is known for its acute toxicity.

Halder et al. [130] applied in silico-based modelling for predicting the toxicity properties of DESs and reported that the likelihood for toxicity carried over by different HBD groups in the DESs is in the decreasing order as organic/ inorganic acid > sugar and amides > sugar alcohols and n-alcohols.

The increasing and decreasing of the toxicity profile of DESs when individual components are incorporated into DESs observed here is dependent on the tested organisms, HBDs used and configuration of the test, as no similarity has been observed for the latter. As such, certainly, DESs can no longer be termed non-toxic just because their components are non-toxic. There is a need for standardization of toxicity and biodegradability studies and adoption of the same for better understanding of the reported results within the scientific community.

738 Table 2. Various HBAs and HBDs studied in literature for their toxicity.

S.no	HBA	HBD	HBA: HBD Molar ratio	Test organisms	Ref.
1	MTPB	Glycerol, Gly	1:3	Artemia salina (brine shrimp, an invertebrate)	
		Ethylene glycol, EG	1:3	Bacillus subtilis (Gram +ve bacteria)	
		Triethylene glycol, TG	1:3	Staphylococcus aureus (Gram +ve bacteria)	[131]
				Escherichia coli (Gram -ve bacteria)	
				Pseudomonas aeruginosa (Gram -ve bacteria)	
2	ChCl	Urea, U	HBA/HBD molar ratios (1:1, 1:2	Escherichia coli (a bacterium),	
	ChAc	Acetamide, A	and 2:1) - used in the Hydra	Allium sativum (garlic, a plant)	
		Ethylene glycol, EG	sinensis tests, while HBA/HBD	Hydra sinensis (an invertebrate)	[132]
		Glycerol, Gly	molar ratio of 1:1 was selected for use in the other tests.		
3	ChCl	Zinc chloride, $ZnCl_2$	1:2	Cyprinus carpio (a vertebrate), and	
		Urea, U	1:2	Four fungi strains (eukaryotes):	
		Glycerol, Gly	1:3	P.chrysosporium	
		Ethylene glycol, EG	1:3	A.niger	
		Diethylene glycol, DEG	1:2	L.tigrinus	[133]
		Triethylene glycol, TEG	1:3	C.cylindracea	
		Fructose, Fruc	2:1		
		Glucose, Guc	2:1		
		PTSA	1:3		
		Malonic acid, MA	1:1		

739

740 6.2 Degradation

741 In preparing DESs complete atom economy is preserved, i.e. all the reactant atoms are
742 converted into product atoms and also, no purification is required unlike for ILs. As a result,
743 numerous studies exist on the preparation and properties of DESs [134-137]. In this context,
744 Florindo et al. [138], conducted a comprehensive study on the effect of method of preparation
745 used on the properties of ChCl: Organic Acids based DESs, by adopting two synthesis/
746 preparation methods: heating and grinding methods. The method of preparation affects the
747 water content of the synthesized DES. In particular, for the case of the ChCl: Glutaric acid-
748 based DES, after high vacuum drying treatment the water content was 12% higher when
749 synthesized using the heating method as compared to the grinding method. Water content
750 directly influences the viscosity of the DESs synthesized and thus influencing mass transfer
751 capabilities of the DESs ultimately. Similarly, the pH of this ChCl: Glutaric acid-based DES
752 was also lower when the heating method was used, pH being between 0 to 1 and when the
753 grinding method was used, pH being between 2 to 4. This lowered pH facilitates the production
754 of impurities in the DESs, as was observed with ester formation observed between cholinium
755 cation and glutaric acid, as seen in ^{1}H NMR analysis; the peak of which was also observed but
756 not confirmed by Fransico et al. [139]. For all the DESs investigated by authors [138],
757 impurities in the DESs formed by heating method varied from 5% to 30%. Thus, the grinding
758 method is preferred. However, Rodriguez et al. [140] examined ChCl-Carboxylic acids DESs
759 for their thermal stability and durability. They reported that an internal esterification reaction
760 takes place between the acid part of DESs and –OH (in ChCl) regardless of the preparation
761 method adapted. It takes place over time, independent of heating cycles and adversely affects
762 the potential solvent applications of ChCl-Carboxy acid DESs.

So, from the above studies, it can be concluded that over time, internal esterification takes place between salt and acid components within DESs, irrespective of the preparatory method adopted. This internal esterification is temperature-sensitive, i.e. heating promotes dehydration, eventually accelerating this esterification, resulting in ester formation between carboxylic acid part and hydroxyl part of salt (ChCl), giving water as a by-product. Similarly, production of HCl which may be due to heating the components (ChCl and glutaric acid) is also a possibility, presence of which is proved by lower pH in the case of DESs prepared using the heating method. This lowered pH makes the media acidic thereby facilitating ester formation between DESs components resulting in salt carboxylate. DESs containing such impurities may not yield results as expected. Hence, the DESs should be prepared and applied on site, to prevent its degradations affecting the desired applicability.

6.3 Recovery and Reusability

It is necessary to reuse the catalyst or co/solvents employed in the reaction or extraction process, to economize the process. Many methods to regenerate the DESs component from the product phase. These methods include distillation, vacuum distillation, salt recrystallization with the help of an anti-solvent or by cooling. Reusability of ChCl: Gly (1:1) and MTPB: Gly (1:2) DES used in separation of KOH from FAMEs, were investigated by Shahbaz et al. [115]. After 2 hours of settling time, the purified FAMEs layer was separated and the used DES was utilized for the next round of KOH extraction from a fresh batch of FAMEs. For, DES: FAME (1:1) around 16% drop and DES: FAME (3:1) around 6% drop, in KOH removal efficiency of DESs mentioned, was observed at the end of 5 cycles. Applying a similar recycling technique, Hayyan et al. [83] reported an approximately 20% drop during 4^{th} recycling run in FAME conversion using ATPB; PTSA (1:3) DES. Applying the same DES in glycerolysis of oleic acid, Williamson et al. [78], reported a negligible drop in conversion for 5 cycles. Acetone has

been employed as an anti-solvent agent to recrystallize the ChCl salt from ChCl: EG DES mixture [118]. 70% of ChCl salt was recrystallized from EG-based DESs using vortex mixing, suggesting the scope of optimizing this process. However, no investigation into recovering the DESs from impurities was undertaken. Similarly, diethyl ether showed very good regeneration ability for ChCl salt, where only 16% loss from the initial mass of salt used, and 90% removal efficiency was observed after 5 regeneration cycles [141]. In both the above studies involving application anti-solvent, no structural degradation was observed. It is observed that almost all the regeneration or recycling studies, focused on recycling the salt, and the HBD component of DESs is not recycled. This should be looked into further having a cost reduction potential.

Furthermore, there is still scope for the regeneration of DESs-based on its eutectic properties i.e., by altering the molar ratio or changing the temperature of the solution to make changes in the immediate physical properties, such a phase change.

7 Conclusion and future trends

Based on this review, in order to maintain carbon neutrality of feedstock and chemicals, biodiesel production needs to switch to greener options. The latest restrictions are more stringent and limit carbon emissions into the environment. With respect to catalysts and co/solvents involved, the last decade has been focussed on ionic liquids and deep eutectic solvents for biodiesel production. However, what ionic liquids lack, deep eutectic solvents deliver. Their complete atom economy, easy preparation, relaxed operational setting, simple recovery and greenness are exemplary. Recently properties related to thermal stability, biodegradability and durability have been studied. Choline chloride, extensively investigated as a co-solvent, degrades with time, because of esterification between its components.

Degradation is independent of the preparatory method employed. This finding limits co-solvency applications of ChCl-based DESs. However, durability is studied for a year, whereas application in co-solvency application is rarely more than a day. Similarly, concerns related to toxicity and biodegradability of DESs may be worked out by employing better recovery and reusability practices, as well as proper disposal of DESs. Furthermore, toxicity studies based on commonly used salts and HBDs used in DESs preparation, have again put its "green solvents" label under scrutiny and this requires the development of a standardized approach/ methodology to provide a relatable dataset/ outlook for the scientific community.

Currently, in biodiesel facilities, crude glycerol is the only other marketable produce. Biodiesel production facilities must widen product inventory to increase profits and become commercially competitive. Retrieving important bio-active compounds from the feedstock/ oils should be considered in the overall biodiesel production scheme. In the future researchers may also work on investigations into; the in-situ recycling of the crude-glycerol towards:

1) esterification of FFAs catalyzed by DESs,

2) synthesis of DES co-solvent during base-catalyzed transesterification,

3) synthesis of DES solvent during the extraction of glycerol, and

4) synthesis of DESs for extraction of trace metals from the animal waste feedstock.

These investigations may provide a reduction in the overall production cost.

Application of DESs with Amberlyst-15 during esterification and CaO during ethanolysis enhance their processing abilities. Deep eutectic solvents generally employed in the purification stages are mostly polar and possess good hydrogen bonding capabilities. They can remove almost all impurities having hydrogen groups, such as residual catalyst, unreacted

glycerides, water, glycerol and residual alcohol. In the future, purification studies should also measure these impurities more precisely, as one possibility, in the event of satisfactory water removal from crude biodiesel, is the elimination of drying unit.

Even after purification, biodiesel requires additives depending on the climatic conditions and engine characteristics. There is still no reported DESs application research in the production of additives such as cetane improvers, cold flow improvers or stability enhancers. It is anticipated that DESs can make a significant contribution to this phase of biodiesel production as well.

Several patents have been found in the literature. This indicates that there is a considerable research activity in the field of Deep Eutectic Solvents in biodiesel production. However, there are essential aspects which have not been yet worked upon, such as in the production of biodiesel additives, and this holds future possibilities in this area. It is seen that, even when DESs are employed in lipid recovery or tocol extraction, the use of conventional solvents like hexane is still necessary. Hence, future studies should focus on studies to address these shortcomings. Achieving this will make possible a completely DESs-based biodiesel production scheme which will satisfy modern green chemistry requirements. The process can then be optimized for scalability to an industrial level.

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