



Article Green Extraction of Volatile Fatty Acids from Fermented Wastewater Using Hydrophobic Deep Eutectic Solvents

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Abstract: Volatile fatty acids (VFAs) are carboxylic acids mainly produced via the fermentation of organic waste streams. Being industrial platform chemicals, sustainable, green and economical recovery of VFAs is necessary. Herein, hydrophobic deep eutectic solvents (HDES), "a new generation of water-immiscible designer solvents", were assessed for the recovery of VFAs via liquid–liquid extraction. The eutectic mixture of menthol-lauric acid exhibited the highest stability and hydrophobicity. The binary solubility of the pairs {VFA in water} and {VFA in HDES}—and the saturation of the HDES with water were measured. Furthermore, the influences of key parameters on the extraction efficiency were investigated. On multi-stage extraction, an efficiency of 88% was achieved in 4 stages, and the HDES was successfully regenerated using vacuum evaporation. The HDES performance was also compared to other reported relevant solvents. It was concluded that menthol-lauric acid HDES is a promising candidate for the green extraction of VFAs from fermented wastewater.

Keywords: volatile fatty acids recovery; platform chemicals; liquid–liquid extraction; hydrophobic deep eutectic solvents

1. Introduction

In recent years, sustainability and green engineering principles have been the ground for scientific research. They are also considered the main routes for a successful economy with fewer environmental penalties. The production of platform chemicals such as volatile fatty acids (VFAs) via bio-based processes is a hot topic in the area of sustainable and "green" research. VFAs are precursors for the production of a wide range of daily life needs such as bio-plastics, food, pharmaceuticals, cosmetics, textiles, and bio-energy to name a few. Therefore, the development of sustainable, environmentally friendly, and economically viable bio-based processes for the production and recovery of VFAs is highly demanded. Furthermore, since they are mainly obtained from the degradation of organic matter [1], VFAs' production would contribute to better utilization of organic waste streams.

VFAs production can be achieved biologically via fermentation from biomass and waste streams (e.g., wastewater) [1–3]. However, due to inhibition, process conditions, and the self-regulating nature of the fermentative micro-organisms, VFAs are produced at



Citation: Darwish, A.S.; Warrag, S.E.E.; Lemaoui, T.; Alseiari, M.K.; Hatab, F.A.; Rafay, R.; Alnashef, I.; Rodríguez, J.; Alamoodi, N. Green Extraction of Volatile Fatty Acids from Fermented Wastewater Using Hydrophobic Deep Eutectic Solvents. *Fermentation* **2021**, *7*, 226. https:// doi.org/10.3390/fermentation7040226

Academic Editor: Emmanuel Atta-Obeng

Received: 5 September 2021 Accepted: 9 October 2021 Published: 11 October 2021

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). low concentrations [4,5], particularly in undefined mixed culture fermentation [6]. Therefore, continuous separation of the VFAs from the fermentation broth could improve the productivity of the micro-organisms. However, the separation of VFAs from mixed culture fermentation effluent is challenging, mainly due to their low concentrations and the simultaneous production of different types of hydrocarbons (i.e., ethanol) also at low concentrations that could lead to the formation of complexes and azeotropes [7]. Even though traditional distillation "thermal separation" techniques are known for their high energy intensity and cost, they have been and are still the default technique for separating VFAs from the aqueous fermentation medium [8]. However, over the past decades, the incentives for designing environmentally friendly, energy-efficient, and cost-effective processes have steadily grown. Therefore, affinity separations such as liquid–liquid extraction [9–14], adsorption [15], and membrane filtration [16] are becoming attractive alternatives when technically feasible.

Liquid–liquid extraction (LLX) is an affinity separation method usually conducted at mild operating conditions and consequently less energy consumption, in which an affinity separating agent (i.e., solvent) is applied [17,18]. Due to the introduction of the separating agent, at least one secondary separation, "a recovery step", is needed to obtain the final separated species—"the VFAs"—in a pure form. In the recovery step, the separating agent is regenerated and can be recycled back to the primary separation unit. An efficient separating agent for the extraction of the VFAs from the aqueous fermentation medium must primarily exhibit high hydrophobicity, high capacity, high solute distribution ratio, high selectivity, easy recoverability, environmental friendliness, and low cost. Different organic solvents such as medium-chain fatty acids (MCFAs) [12], organophosphorus [11], terpenes and terpenoids [13], and aliphatic amines [19,20] have been studied. However, several drawbacks were reported such as low selectivity, solvent miscibility, solvent losses via evaporation, and difficult regeneration. To address these limitations, designer solvents, particularly, deep eutectic solvents (DESs) [21] have been proposed for the extraction of VFAs [13,14,22].

DESs are commonly described as a mixture of two or more compounds that form upon mixing a liquid phase with a melting point far below that of its constituents [23–25]. It is anticipated that the formation of the DES occurs via a combination of entropy of mixing, van der Waals interactions, and hydrogen bonding, where one compound is considered a hydrogen bond donor (HBD) and the other is a hydrogen bond acceptor (HBA). The leverages of DESs over conventional solvents have been widely reported in the literature, such as simple preparation, low volatility, wide liquid range, and tunability of properties [26–29]. Furthermore, DESs are often biodegradable and non-toxic to humans, but these two properties should not be taken for granted as they truly depend on the choice of the DES starting materials and their synergetic/mixing effects. In addition, as mentioned earlier, for the separation of VFAs from wastewater application, the solvent should be water-immiscible; therefore, a hydrophobic DES (HDES) is a necessity [22,30]. Given their environmentally benign nature, and the sustainability of their raw materials, together with all the advantages of LLX, which include low energy consumption and high recyclability, we believe that designing an efficient separation process for the extraction of VFAs via HDES, would fulfill the principle of "green" extraction that has been stated as "Green extraction is based on the discovery and design of extraction processes which will reduce energy consumption, allow the use of alternative solvents and renewable natural products, and ensure a safe and high-quality extract/product" [31].

In a proof-of-concept study (2015) by van Osch et al. [22], HDESs were successfully used to extract VFAs from water. The group used ionic-based HDESs for the extraction of the VFAs: acetic, propionic, and butyric acids from water. Remarkably, all the prepared HDESs exhibited higher extraction efficiencies than the benchmark solvent "trioctylamine". Later in 2019, two studies appeared in the literature, one by van den Bruinhorst et al. [14], in which they showed that mixtures of dihexylthiourea and trioctylphosphine oxide (TOPO) are good extractors for VFAs. Another study by Rodrigues-Llorente et al. [13] showed that

an HDES composed of octanoic acid and thymol gave a comparable or higher extraction efficiency than organic solvents used in the literature. It should be pointed out that the three studies [13,14,22] concluded that the longer the chain of a VFA, and consequently the higher hydrophobicity, the higher the extraction efficiency. Nonetheless, the studies available in the literature only discussed the single-stage extraction of the VFAs using HDESs, without investigating the influences of key operational parameters, multi-stage extraction, repetitive usage of solvent, or HDES regeneration.

Therefore, in this work, a comprehensive parametric study on the extraction of VFA using the HDES was conducted. The HDESs were initially screened for their storage stability and the stable ones were screened for their acetic acid extraction efficiency from the broth model. Then, the selected HDES was characterized by its physicochemical properties. Furthermore, a series of equilibrium extraction experiments were carried out to determine the influences of the process operating parameters on the extraction efficiency. These were: the stirring time, the initial concentration of acetic acid, and the solvent-to-feed ratio (S:F). Moreover, the multi-stage extraction efficiency of the HDES was determined for four stages. In addition, the HDES capacity was estimated and the HDES regeneration was successfully achieved. The extraction efficiencies were then determined for VFAs of different chain lengths, namely propionic acid, butyric acid, and valeric acid, and compared to their extraction efficiencies in a mixture of them including acetic acid. Finally, the HDES performance for the extraction of VFAs was compared to trioctylamine (TOA) as a benchmark solvent and to the relevant literature.

2. Materials and Methods

2.1. Materials

The chemicals used in this work are listed in Table 1. The table also shows their chemical structures, CAS numbers, purity (as stated by the supplier), and sources. All chemicals were used as received without further purification.

Chemical	CAS Number	Purity (wt%)	Source
Acetic acid	64-19-7	≥ 99.5	Surechem Products
Propionic acid	79-09-4	≥ 99.0	Acros Organics
Butyric acid	107-92-6	\geq 99.0	Sigma-Aldrich
Valeric acid	109-52-4	\geq 99.0	Sigma-Aldrich
Octanoic acid	124-07-2	≥ 99.0	Sigma-Aldrich
Decanoic acid	334-48-5	≥ 98.0	Sigma-Aldrich
Lauric acid	143-07-7	≥ 98.0	Sigma-Aldrich
DL-Menthol	89-78-1	≥ 99.0	Sigma-Aldrich
Tetraoctylammonium bromide	14866-33-2	≥ 98.0	Merck

Table 1. List of chemicals and their specifications.

2.2. Extraction Experiments

We prepared 25 g of dilute aqueous solutions of acetic acid, propionic acid, butyric acid, and valeric acid at concentrations of 3 wt%. The solutions were added to the solvent "HDES" at 2:1 solvent-to-feed mass ratio in 50 mL centrifugal tubes. The two-phase mixtures were stirred for 2 h at 1000 rpm using an Eppendorf ThermoMixer C (Hamburg, Germany) at a controlled temperature and pressure of 298.2 K and 1.01 bar, respectively. The samples were then centrifuged for 30 min at 3500 rpm using an Eppendorf Centrifuge 5804R (Hamburg, Germany) to ensure proper phase separation. Finally, a sample from the water-phase "the bottom phase" was taken using a needle syringe. The concentration of the VFAs in the water-phase was determined using high-performance liquid chromatography (HPLC) as described in the following section.

The concentration of VFAs in the water-phase was determined using an Agilent 1260 Infinity HPLC (Santa Clara, CA, USA) with the specifications shown in Table 2. Using the results from the HPLC, the extraction efficiency of the solvent was evaluated as follows [22]:

$$E(\%) = \frac{w_{i,VFA} - w_{f,VFA}}{w_{i,VFA}} * 100$$
(1)

where $w_{i,VFA}$ is the initial weight fraction of the *VFA* in the water solution and $w_{f,VFA}$ is the final weight fraction of the *VFA* in the water-phase after extraction with the HDES. The HPLC method was verified by analyzing mixtures of known composition. The largest obtained standard deviation between the known samples and the HPLC measured values was ± 0.2 wt%. All the measurements in this study were performed in duplicate, and the statistical uncertainty in the measurement was found to be ± 0.2 wt%.

Table 2. The high-performance liquid chromatography specifications.

	Specification
Equipment model	Agilent 1260 Infinity
Column	Agilent Hi-Plex H, 7.7 $ imes$ 300 mm, 8 μ m
Detector	UV/Vis, 210 nm
Pump	Isocratic
Injection volume	5 µL
Column temperature	55 °C
Eluent	$5 \text{ mM H}_2\text{SO}_4$
Flowrate	0.6 mL/min

2.3. Solvent Preparation and Selection

The selection of the HBAs (menthol and Tetraoctylammonium bromide (TOABr)) and the HBDs (Octanoic acid (OctaAc), Decanoic acid (DecAc), and Lauric acid (LaAc)) for the VFA recovery application was based on the use of renewable, biodegradable components with the ability to form a liquid DES at ambient temperature and extract the VFAs from an aqueous phase with minimized cross-contamination [32]. Hence, the different types of hydrophobic acids, menthol, and TOABr were selected as they have been previously reported in the literature for their ability to form HDESs and for their renewable and biodegradable nature [30]. Additionally, menthol-based DESs [33] and acid-based DESs [34] are known for their low viscosities in comparison to ionic-based DESs (e.g., quaternary ammonium based DESs) [23]. This is important since low viscosity solvents facilitate mass transport between the phases [32].

The constituents of each HDES were weighted in screw-capped glass bottles at their respective molar ratios using a Shimadzu balance AUX220 (Kyoto, Japan) with uncertainty in the measurement of ± 0.0002 g. The mixtures were heated to a temperature of 333.2 K and stirred at around 300 rpm in a temperature-controlled incubated shaker (IKA KS 4000, Staufen, Germany) i-control with temperature stability of ± 0.1 K until a clear and homogeneous liquid phase was formed. Table 3 lists the HDESs prepared with their constituents and corresponding molar ratios. The stability of the HDESs was visually assessed by storing at room temperature in a well-sealed glass bottle and monitoring the solidification of the HDES for one week. Thereafter, a single-stage extraction experiment was conducted for an acetic acid solution (3 wt%) at a S:F ratio of 1:1 using the stable HDESs to select the most suitable candidate for further experiments. Thereafter, the molar ratio of selected HDES' components was optimized by preparing the HDES at a molar ratio of 1.5:1, 2:1, 3:1, 4:1, and 5:1, and performing a single-stage extraction experiment for an acetic acid solution (3 wt%). The best performing HDES was then used for the subsequent experiments.

Component 1	Component 2	Molar Ratio
Tetraoctylammonium bromide (TOABr)	Octanoic acid (OctAc)	2:1
Tetraoctylammonium bromide (TOABr)	Decanoic acid (DecAc)	2:1
Tetraoctylammonium bromide (TOABr)	Lauric acid	2:1
DL-Menthol (Men)	Octanoic acid (Oct Ac)	2:1
DL-Menthol	Decanoic acid	2:1
DL-Menthol (Men)	Lauric acid (LaAc)	2:1

Table 3. The components of the hydrophobic deep eutectic solvents and their molar ratios.

2.4. HDES Characterization

The water content of the selected HDES was measured using the Karl–Fischer titration method on a Aquamax KF Coulometric titrator (GR scientific, Halle, Germany). The degradation temperature (T_{deg}) was determined using a thermogravimetric analyzer (Netzsch STA 449 F Jupiter, Selb, Germany) at a heating rate of 10 K·min⁻¹ from 298.2 K to 823.2 K). The densities of neat and water-saturated HDESs were measured at the temperature range between 298.2 and 368.2 K using an Anton Paar (DMA 5000 M, Graz, Austria) with a statistical uncertainty of 0.00001 g/cm³. The viscosity of the HDES was measured at 298.2 K using HAAKE Rheo Stress 6000 rheometer (Thermo Scientific, Waltham, MA, USA) with a "Cup and Bob" geometry at a shear rate of 240 s⁻¹ for 120 s with a maximum statistical uncertainty of 8 mPa·s.

2.5. Solubility Test

The equilibrium cell method [35–37] was adopted to measure the solubilities of the pairs: (acetic acid (1) in water or HDES (2)), (propionic acid (1) in water or HDES (2)), (butyric acid (1) in water or HDES (2)), (valeric acid (1) in water or HDES (2)), and the saturation of HDES with water at 298.2 K and 1.01 bar. In this method, 4 g of the mixtures was prepared in 8 mL glass vials. The mixtures' components (1 and 2) were weighted at an equal mass ratio. The vials were then placed in an Eppendorf ThermoMixer C and stirred for 2 h at 1000 rpm and 298.2 K. The samples were kept overnight at a controlled temperature of 298.2 K to reach equilibrium. If phase separation was observed, a sample from the water-phase "bottom phase" was then taken for analysis using a needle syringe, without disturbing the phase's coexistence interface. The water-phase samples were analyzed using HPLC (Section 2.2)

For the HDES saturation with water, samples from the HDES-phase "top phase" and the water-phase were taken using the appropriate syringes. The water-phase was analyzed using Fourier-transform infrared spectroscopy (FT-IR, Perkin Elmer, Billerica, MA, USA, equipped with a universal attenuated total reflectance module) and the total organic carbon (TOC-L, Shimadzu, Kyoto, Japan) content, while the water content of the HDES-phase was quantified using Karl–Fischer titration method.

2.6. Parametric Extraction Experiments

In these experiments, the effect of the following parameters on the HDES extraction performance was investigated: the stirring time, initial concentration, and solvent-to-feed ratio. This was done by conducting a single-stage extraction experiment for 3 wt% acetic acid solution using the selected HDES (Section 3.2). However, one of these operating parameters was to be changed in each experiment while keeping the rest constant. Moreover, the extraction efficiency of 4-stage extraction was determined at 298.2 K and 1.01 bar. In those experiments a fresh HDES was added to the water-phase of the previous stage, starting from 3 wt% acetic acid solution. Next, the HDES extraction capacity was quantified for 4 consecutive extraction cycles. Whereby after every extraction cycle the HDES-phase was separated from the water-phase, and without any treatment, the "used" batch of HDES-phase was contacted with a fresh aqueous solution containing 3 wt% acetic acid.

Finally, the HDES was regenerated under vacuum by placing the HDES sample in a rotary evaporator (Rotavapor R-215, BUCHI, Flawil, Switzerland) for a duration of 24 h. The pressure of the sample was controlled at 20 mbar using a vacuum controller (Vacuum controller V-850, BUCHI, Flawil, Switzerland), while the temperature was kept at 303.2 K in a BUCHI heating bath B-491. The regenerated HDES was then used for the extraction of acetic acid from a 3 wt% acetic acid solution. This was done in order to assess its efficiency after regeneration.

3. Results and Discussion

3.1. HDES Selection

Three tetraoctylammonium bromide-based HDESs and three menthol-based HDESs (Table 3) were prepared as described in Section 2.3. The HDESs were stored in wellsealed glass bottles at room temperature for 1 week to assess their stability through phase consistency monitoring. At 72 h following the HDESs preparation, it was observed that the TOABr:LaAc had completely solidified and the TOABr:DecAc formed emulsion, while the TOABr:OctAc remained a homogenous liquid. After a week of storage, the TOABr:OctAc formed a precipitate, while TOABr:DecAc and TOABr:LaAc had partially and completely solidified, respectively (Figure 1). This could be attributed to the limited ability of the decanoic acid and lauric acid to form hydrogen bonds with the tetraoctylammonium bromide or that their HDESs melting points were near the room temperature [38]. Therefore, these 3 HDESs were eliminated from the study. On the other hand, all the 3 menthol-based HDESs remained stable liquids as shown in Figure 1. In the case of the menthol-based HDESs, their stability could be due to the ability of menthol to act as a hydrogen bond donor and acceptor and similarly the acids. The strength of the hydrogen bonding and the entropic effects that are associated with the DES formation are determinant factors for the stability; however, further molecular level studies should be done to determine the ruling effect.



Figure 1. 72 h and 1 week stability tests for the tetraoctylammonium bromide-based and menthol-based HDESs at 298.2 K and 1.01 bar.

To further screen the menthol-based HDESs, their extraction ability of acetic acid (3 wt% solution) at 298.2 K was tested. The results are shown in Figure 2. As can be seen, the longer the chain length of the HDES, the lower the extraction efficiency. This could be attributed to the decrease of polarity with the length of the chain. However, the differences in the extraction efficiencies are small and fall within the statistical uncertainty of the measurements. The selection of HDES was then based on the hydrophobicity of the HDES to minimize the solvent loss to the water-phase and the water uptake by the HDES. Both of these aspects are important for the integrity of the DES since minimum solvent loss means minimum water contamination as well as the ability to reuse the solvent and hence minimum amount of the required solvent in the extraction process, if the solvent is preserved during the regeneration process [39]. Florindo et al. [39] studied the leaching of HDESs composed of DL-Menthol and the considered acids, and found that no leaching of the acids to the water was observed. In another work by the same group, fatty acid-based HDESs showed that as the chain length of the HDES components increase, the saturation water content decreases. For example, Lauric acid: Decanoic acid HDES water content is 0.523% compared to Lauric acid: Octanoic acid water content of 1.353% [40]. Since the larger the carbon content in the HDES the higher the hydrophobicity, the Men:LaAc HDES was selected for this study.



Figure 2. Effect of the HDES chain length on the extraction efficiency of acetic acid. (Conditions: T = 298.2 K, P = 1.01 bar, $w_{i,acid} = 3\%$, S:F = 2 mL:2 mL, stirring time = 2 h at 1000 rpm and centrifuge time = 30 min at 3500 rpm).

3.2. HDES Molar Ratio Optimization

The physicochemical properties and solvation capacity of DESs can be influenced by the choice of HBA, HBD, and their molar ratio. Thus, after selecting the HBA and HBD based on the criteria discussed previously, the effect of the molar ratio should be investigated to improve the extraction efficiency of the DES and to determine which part of the DES (the HBA or the HBD) is more responsible for the extraction. Here, the molar ratio of the Men:LaAc was optimized by conducting a single-stage extraction experiment for an acetic acid solution (3 wt%). The Men:LaAc was prepared at molar ratios of (1.5:1), (2:1), (3:1), (4:1), and (5:1). All the prepared DESs were liquid at 298.2 K. This is consistent with the solid–liquid phase diagram (S-L) for the Men:LaAc mixture reported by Matins' et al. [41]. As seen from Figure 3, the extraction of acetic acid increases as the molar fraction of lauric acid in the HDES mixture increases, and the highest efficiency was observed for 1.5:1 molar ratio with an extraction efficiency of 40.5%. This could be attributed to the increased polarity of the HDES as its acid fraction increased. Based on the results obtained, the Men:LaAc with a molar ratio of 1.5:1 was selected to be used for the rest of this study. The constituents' structures, molar fractions, and weight fractions of the selected HDES are listed in Table 4.



Figure 3. Extraction efficiency of acetic acid using Men:LaAc with different molar ratios, where X_{Ac} represents the mole fraction of the acetic acid. (Conditions: T = 298.2 K, P = 1.01 bar, $w_{i,acid}$ = 3%, S:F (w/w) = 2:1, stirring time = 2 h at 1000 rpm and centrifuge time = 30 min at 3500 rpm).

Table 4. The structures, molar fractions, and weight fractions of the constituents of the selected eutectic solvent.



The performance of the Men:LaAc (1.5:1) was further evaluated by calculating the distribution coefficient and the selectivity. The solute distribution coefficient (β) measures

the concentration of acetic acid in the HDES-phase with respect to its concentration in the water-phase. It can be calculated from the experimental data as follows:

$$\beta_{AA} = \frac{w_{AA,HDES}}{w_{AA,water}} \tag{2}$$

where $w_{AA,HDES}$ is the weight fraction of acetic acid in the HDES-phase, and $w_{AA,water}$ is the weight fraction of acetic acid in the water-phase. β was found to be 0.356 and since it is less than 1, that implies a higher affinity of acetic acid towards the water-phase, and more solvent is needed to reach the desired separation. However, it will be shown in the following sections that in a mixture of VFAs, the distribution coefficient of higher VFAs is greater than one, reflecting the affinity of the VFAs toward the HDES phase.

3.3. HDES Characterization

The water content of freshly prepared Men:LaAc (1.5:1) was found to be less than 0.09 wt% (less than 0.86 mol% on a molar basis); this can be considered as an indication of the HDESs high hydrophobicity and low hygroscopicity as it did not absorb moisture from the atmosphere during preparation. This result is lower than the water content of Men:LaAc (2:1) reported by Ribeiro et al. [42] of 0.276 wt% and higher than the water content of fatty-acid-based HDESs reported by Florindo et al. [40] of 0.019% indicating that an increase in the water content is associated with the increase in the menthol composition. The degradation thermograph of HDES is displayed in Figure 4. The thermal degradation temperature (T_{deg}) of the Men:LaAc (1.5:1) was found to be 485.7 K, which is an advantage in designing thermal-based regeneration processes of the HDES after extraction. The fresh HDESs density and viscosity were also measured at 298.2 K and 1.01 bar. The results are listed in Table 5. The viscosity of the HDES can be considered relatively "low" compared to many other HDESs such as ammonium-based hydrophobic deep eutectic solvents where the viscosity of the former is an order of magnitude lower [22,43].



Figure 4. Thermal degradation of Men:LaAc (1.5:1) measured at a heating rate of $10 \text{ K} \cdot \text{min}^{-1}$ from 298.2 K to 823.2 K.

Physical Property	Value
Density, ρ (g·cm ⁻³)	0.888 ± 0.002
Viscosity, (mPa)	24.98 ± 0.45
Degradation temperature, T _{deg} (K)	485.7
Melting point, T_m (K)	296.17 [41]
Water Content, w_{H2O} (ppm _{wt})	893 ± 101
Water Content, x_{H2O} (ppm _{mol})	8556 ± 391
	$+0.1 V_{-1}(-) + 0.041$

Table 5. Density, viscosity, thermal degradation temperature, and water content of freshly prepared Men:LaAc at a molar ratio of 1.5:1 measured at 298.2 K and 1.01 bar ^a.

^a Standard uncertainty in temperature and pressure $u(T) = \pm 0.1$ K, $u(p) = \pm 0.04$ bar.

The density–temperature dependence in the range of $298.2 \le T \le 368.2$ for freshly prepared and water-saturated Men:LaAc (1.5:1) has also been measured and is shown in Figure 5. The numerical density data are available in the Supplementary Materials (Table S1). As shown in Figure 5, the density of water-saturated Men:LaAc (1.5:1) is only slightly higher than that of the freshly prepared mixture; this suggests that HDES is hydrophobic as the water content of the saturated HDES is low. Over this temperature range, the density dependence with temperature is well described using linear regression by Equations (3) and (4) for the freshly prepared HDES and water-saturated HDES with R² values of 0.9989 and 0.9992, respectively.



Figure 5. Density of freshly prepared and water-saturated Men:LaAc (1.5:1) in the temperature range from 298.2 K to 368.2 K at 1.01 bar.

Freshly prepared HDES:

$$\rho_{(g \cdot cm^{-3})} = \left(-1.0439 \times 10^{-3}\right) \cdot T_{(K)} + 1.2001 \tag{3}$$

Water saturated HDES:

$$\rho_{(g \cdot cm^{-3})} = \left(-1.0514 \times 10^{-3}\right) \cdot T_{(K)} + 1.2057 \tag{4}$$

Furthermore, the critical properties of the HDES components, critical temperature $(T_{C,HDES})$, critical pressure $(P_{C,HDES})$, and critical volume $(V_{C,HDES})$, were estimated. The critical properties are a prerequisite for serval thermodynamic models. However, in the case of HDES these properties cannot be experimentally estimated as they will most probably decompose before reaching the critical state. Here, assuming the HDES is a pseudo-pure compound—this assumption will be validated in the next section—the critical properties

were estimated via the modified Lydersen–Joback–Reid group contribution approach [44]. The critical properties of the HDES were then determined by accounting for the molar ratio of the constituents via Lee–Kesler mixing rules, and the results are listed in Table 6.

Table 6. Critical properties of the HDES.

T _{c,HDES}	P _{c,HDES}	V _{c,HDES}
(K)	(bar)	(mL·mol ^{−1})
767.5	21.79	649.3

3.4. Solubility Data

Table 7 lists the measured solubilities for systems of (VFA (1) in water (2)), (water (1) in HDES (2)), and (VFA (1) in HDES (2)) at 298.2 K and 1.01 bar. Moreover, Figure 6 shows the binary mixtures of (VFA (1) in water (2)). As it can be observed, systems of (acetic/propionic/butyric acid (1) in water (2)) exhibited no phase separation, while in the system of (valeric acid (1) in water (2)) phase separation was observed due to the partial solubility of the valeric acid in water. It can also be seen that the mixtures of (acetic/propionic/butyric acid (1) in water (2)) showed varying levels of turbidity in the following order: butyric > propionic > acetic acid; where the butyric acid mixture was highly turbid and acetic acid mixture showed no noticeable turbidity. The solubility of valeric acid in the water-phase was measured using HPLC and was found to be 4.0 wt% (or 0.73 mol%) in water, which is the lowest solubility among the VFAs. This trend is logical as increasing the chain length increases the acid hydrophobicity.

Table 7. Solubility of component (1) in solvent (2) measured at 298.2 K and 1.01 bar ^a.

Solute (1) in Solvent (2)	Solubility
{Acetic Acid (1) in Water (2)}	Fully miscible with no turbidity
{Propionic Acid (1) in Water (2)}	Fully miscible but slightly turbid
{Butyric Acid (1) in Water (2)}	Fully miscible but highly turbid
(Valoria Acid (1) in Water (2))	$w_1 = 4.01 \pm 0.01$ wt%
{valeric Acid (1) in vvaler (2)}	$x_1 = 0.73 \pm 0.01 \text{ mol}\%$
{Water (1) in Men:LaAc (2)}	w_1 = 2.10 \pm 0.01 wt%
HDES saturation with water	$x_1 = 17.16 \pm 0.10 \text{ mol}\%$
{Men:LaAc (1) in Water (2)}	Not soluble,
Migration of the HDES constituents to the	observed using FT-IR, and
water-phase	$\Delta TOC = 29.4 \pm 1.9 \text{ ppm}$
{Acetic Acid (1) in Men:LaA (2)}	Fully miscible with no turbidity
{Propionic Acid (1) in Men:LaAc (2)}	Fully miscible with no turbidity
{Butyric Acid (1) in Men:LaAc (2)}	Fully miscible with no turbidity
{Valeric Acid (1) in Men:LaAc (2)}	Fully miscible with no turbidity

^a Standard uncertainty in temperature and pressure $u(T) = \pm 0.1$, u(p) = 0.04 bar, the measurements were done in duplicates.

The HDES saturation with water was also measured as well as the HDES content in the water samples after extraction. It was found that the HDES-phase contained 2.1 wt% of water, which indicates the high hydrophobicity of the HDES. In addition, based on FT-IR analysis (Figure 7) none of the HDES constituents have migrated to the water-phase, which is illustrated by the identical spectra of the fresh broth model and the water-phase before and after contacting them with HDES. The latter finding was further validated via measuring the total organic carbon (TOC) of the water-phase. That was found to be Δ TOC = 29.4 ± 1.9 ppm; this value is considerably small, indicating that no HDES migration occurred to the water-phase. This also implies that the HDES stayed intact in one phase and it could be considered as a pseudo-pure compound.



Figure 6. Binary mixtures of (acetic/propionic/butyric/valeric acid (1) in water (2)) at 298.2 K and 1.01 bar.



Figure 7. FT-IR analysis. The measurements were conducted over a wavenumber range of $400-4000 \text{ cm}^{-1}$.

The binary mixtures of (acetic/propionic/butyric/valeric acid (1) in HDES (2)) showed a clear and homogeneous liquid with no phase separation or turbidity, which indicates the full solubility of the VFAs in the HDES. It is anticipated that a combination of electrostatic and hydrogen-bonding interactions contributed to the full solubility of the VFAs in the HDES. Based on these findings, it can be inferred that the selected HDES could be considered as a potential extractant for VFAs and merits further investigation of its extractive capabilities.

3.5. Parametric Study

It was found that using Men:LaAc (1.5:1) for the extraction of 3 wt% acetic acid with a S:F ratio of 2:1 at 298.2 K and 1.01 bar, stirred for 2 h at 1000 rpm, and then centrifuged for 30 min at 3500 rpm yielded an extraction efficiency of 40.5%, as shown in Figure 3. The effect of the operating parameters on the extraction efficiency is discussed in the following subsections.

3.5.1. Effect of Stirring Time

The effect of stirring time (contact time), is another important factor that directly influences the extraction of acetic acid, and thus, the stirring time was changed from 2 min up to 120 min as follows: 2, 5, 10, 20, 30, 45, 60, 90, and 120 min. Figure 8 illustrates the effect of the stirring time on the extraction efficiency. It can be observed from the figure that even in 2 min of stirring an extraction efficiency of 35.2% can be reached. This is presumably due to the low viscosity of the HDES and its high selectivity towards acetic acid. Afterward, a gradual increase in efficiency was observed between 5 and 30 min of stirring. The efficiency then became rather constant between 30 and 120 min, suggesting that 30 min is sufficient time to reach an equilibrium, which is similar to the findings of other LLX processes [45,46].



Figure 8. Effect of stirring time on the extraction efficiency of acetic acid. (Conditions: T = 298.2 K, P = 1.01 Bar, $w_{i,acid} = 3\%$, S:F = 2:1, stirring speed = 1000 rpm and centrifuge time = 30 min at 3500 rpm).

3.5.2. Effect of VFA Initial Concentration

The effect of the initial concentration of acetic acid on the extraction efficiency is shown in Figure 9. The initial concentration of acetic acid in water was varied as 5 wt%, 3 wt%, 1 wt%, 0.5 wt%, and 0.25 wt%. From Figure 9, it is clear that the extraction efficiency of acetic acid was not affected by the change of initial concentration of acetic acid at 298 K. This finding is encouraging, as fermented wastewater broths usually contain varying concentrations of VFAs.



Figure 9. Effect of initial concentration of HA on the extraction efficiency of acetic acid. (Conditions: T = 298.2 K, P = 1.01 bar, S:F (w/w) = 2:1, stirring time = 2 h at 1000 rpm and centrifuge time = 30 min at 3500 rpm).

The pH of all the aqueous solutions before and after the extraction was calculated at 298.2 K as shown in Table 8. The pH calculations are shown in the Supplementary Materials. It can be observed that the pH of the water-phase increased after extraction while the concentration of the undissociated acetic acid [HA] decreased by approximately ~40%, and the concentration of the acetates was almost unchanged for all the studied initial concentrations of acetic acid. Since the decrease of the undissociated acetic acid [HA] equals the overall extraction efficiency (*E*%), this implies that only undissociated acetic acid [HA] was extracted and that the acetates [A⁻] remained in the water-phase. It also implies that presumably for a higher pH value where [A⁻] > [HA] and more towards an alkaline extraction medium, a substantial decrease in the extraction efficiency could be observed. However, it should be mentioned that these conclusions have to be further validated via conducting extraction experiments for a wider range of pH for the initial aqueous solutions and at different temperatures since the pH is sensitive to temperature variations too.

Initial Concentration	0.25%		0.50%		1%		3%		5%	
pH	3.	.1	2.	.9	2.	8	2.	.5	2.	4
[HA] [A ⁻] (M) ^a	0.041	0.001	0.084	0.001	0.168	0.002	0.506	0.003	0.844	0.004
Final Concentration (wt%)	0.1	5%	0.3	0%	0.6	3%	1.8	0%	39	%
pH	3.	.2	3.	.0	2.	9	2.	.6	2.	5
[HA] [A ⁻] (M) ^a	0.025	0.001	0.05	0.001	0.105	0.001	0.303	0.002	0.506	0.003

Table 8. The calculated pH for the aqueous solutions before and after the extraction at of 298.2 K. The [HA] and $[A^-]$ are the concentrations of the undissociated acetic acid and the acetate, respectively ^a.

^a The concentrations [HA] and [A⁻] are expressed in Molarity.

3.5.3. Effect of Solvent-to-Feed Ratio

When the S:F mass ratio was varied from 1:4 (20 wt% HDES) up to 4:1 (80 wt% HDES), Figure 10, the extraction efficiency of acetic acid substantially increased from 13.4% to 56.5%, which is a common trend in LLX [33,45]. This indicates that the acetic acid extraction efficiency is sensitive to variation in the solvent-to-feed ratio, which was expected due to its low solubility in the HDES in the presence of water as indicated by its low distribution

coefficient. Hence, more solvent is required to extract acetic acid more efficiently. This trend is expected to be less pronounced for higher VFAs (propionic, butyric and valeric) due to their hydrophobicity and their high distribution coefficients as will be shown in the next sections.



Figure 10. Effect of solvent-to-feed ratio on the extraction efficiency of acetic acid. (Conditions: T = 298.2 K, P = 1.01 bar, $w_{i,acid} = 3\%$ stirring time = 2 h at 1000 rpm and centrifuge time = 30 min at 3500 rpm).

3.6. Multi-Stage Extraction

As shown in Figure 10, higher extraction efficiencies can be obtained upon the increase of solvent-to-feed ratio. However, achieving high extraction efficiencies with a large amount of solvent is generally not economically sound. Hence, multi-stage extraction was considered. The multi-stage experiment was conducted by separating the HDES-rich phase from the aqueous phase after the LLX experiment, and then a 0.5 mL sample from the aqueous phase was taken for HPLC analysis. Afterward, a fresh batch of HDES was added to the previous stage's raffinate phase while maintaining a mass S:F of 2:1. The separation and analysis procedure was repeated three more times after the first extraction providing a total of four stages. Figure 11 presents the overall extraction efficiency and the acetic acid weight percentage throughout the four stages. It was found that the concentration of acetic acid in the water-phase was reduced to 0.36 wt% from an initial value of 3.00 wt% in only 4 stages with an overall extraction efficiency of 87.6%. It is worth noting that the overall efficiency achieved after the second-stage extraction, 64.9% (Figure 11), is higher than that obtained at a solvent-to-feed ratio of 4:1 that was 56.5% (Figure 10) given that the same amount of solvent was used in both cases. Hence it is preferable to perform the extraction process at a low solvent-to-feed ratio and a greater number of stages.

It is also observed that the extraction efficiency per stage was constant. Figure 12 shows the extraction efficiency calculated using Equation (1), with the only difference being that the initial weight fraction used was the weight fraction of the previous stage. It can be seen from Figure 12 that the extraction efficiency per stage was constant, which further confirms the findings of Figure 9 that the extraction efficiency was relatively independent of the initial concentration. Therefore, it is possible to theoretically forecast the number of stages required for certain extraction efficiency assuming that the behavior of the extraction process remains consistent at concentrations lower than 0.25 wt%. Since the end goal of this work was to recover the maximum possible amount of VFA from water, the number of

stages required to achieve an overall extraction efficiency of 99.0% can be calculated using Equation (5) as follows [45]:

$$E_{targeted} = 1 - \left(1 - E_{stage}\right)^{n} \tag{5}$$

where $E_{targeted}$ represents the targeted extraction efficiency, E_{stage} represents the constant single-stage extraction efficiency, and n represents the theoretical number of stages. Solving Equation (5) for n (using $E_{targeted} = 99\%$, and $E_{stage} = 40.5\%$); the theoretical number of extraction stages required to achieve 99.0% extraction efficiency of acetic acid is approximately $n \approx 9$ stages.



Figure 11. Overall extraction efficiency and the acetic acid concentration for multi-stage extraction using four stages of fresh HDES. (Conditions: T = 298.2 K, P = 1.01 bar, $w_{i,acid} = 3\% \text{ S:F} = 2:1$, stirring time = 2 h at 1000 rpm and centrifuge time = 30 min at 3500 rpm).



Figure 12. Extraction efficiency calculated for each extraction stage separately. (Conditions: T = 298.2 K, P = 1.01 bar, $w_{i,acid} = 3\% \text{ S:F} (w/w) = 2:1$, stirring time = 2 h at 1000 rpm and centrifuge time = 30 min at 3500 rpm).

3.7. Extraction Capacity of HDES

The extraction capacity of the HDES determines the possibility to reuse the same solvent repeatedly without regeneration. Solvents with high extraction capacity are highly preferred industrially, and thus, the effect of repetitive usage of the HDES on the extraction efficiency of acetic acid was evaluated for four cycles by adding a fresh feed of the aqueous fermented broth model while reusing the same HDES from the previous cycle. As shown in Figure 13, the solvent, as expected, lost its capacity as the number of cycles increased; however, by the end of the fourth cycle the concentration of acetic acid in the HDES had increased from 0.6 to 1.17 wt%. This increase implies the ability of concentrating the VFAs in the HDES as they exist at low concentrations in the fermented water. It also suggests that for a continuous multistage countercurrent extraction, less solvent is required and higher VFAs recovery can be achieved despite the low distribution coefficient of acetic acid.





3.8. HDES Regeneration

The possibility for regeneration and recycling is an essential feature of any solvent, not only from an industrial point of view but also as an important characteristic of sustainable and "green" solvents. Hence, the vacuum evaporation technique was assessed for the regeneration of the HDES from the acetic acid and the water traces. A sample of the HDES-phase after extraction consisting of (0.6 wt% acetic acid + 2.1 wt% water + 97.3 wt% Men:LaAc), was placed in a vacuum rotary evaporator at a temperature of 303.2 K and pressure of 20 mbar and left overnight. The HDES was then analyzed for its water content where the concentration of water after regeneration was found to be 0.08 wt% which is almost identical to the initial water content of the HDES before saturation. This proves that water evaporated under the regeneration conditions. The recoverability of the HDES was also determined by comparing the weight of the HDES before and after regeneration. The recoverability of the HDES can be defined as follows [45]:

$$R(\%) = 1 - \frac{w_{i,HDES} - w_{f,HDES}}{w_{i,HDES}}$$
(6)

where $w_{i,HDES}$ is the initial weight of the HDES (before extraction), and $w_{f,HDES}$ is the final weight of the HDES (after regeneration). The recoverability of the HDES was determined to be 98.1% (i.e., loss of HDES weight was not more than 2 wt%). Due to the close match between the compositions of water and acetic acid in the HDES and the calculated weight loss, it is speculated that the HDES lost only its water content and the extracted acetic

acid. The high recoverability of the HDES under the regeneration conditions "303.2 K and 20 mbar" confirms the low volatility of the Men:LaAc HDES [45].

The regenerated HDES was then evaluated for its performance in a single-stage extraction experiment of the 3 wt% acetic acid aqueous solution. Figure 14 shows that the extraction performance of the regenerated HDES is slightly lower than that of the fresh HDES, implying that the HDES successfully recovered its full extraction capacity after regeneration. The above investigation demonstrates the possibility of regenerating the HDES using the vacuum flashing procedure. This finding is of great significance and encourages the application of the HDESs as green extractants.



Figure 14. Comparison of the extraction performance using fresh HDES and regenerated HDES. (Conditions: T = 298.2 K, P = 1.01 bar, $w_{i,acid}$ = 3%, S:F (w/w)= 2:1, stirring = 2 h at 1000 rpm and centrifuge = 30 min at 3500 rpm).

3.9. Extraction Efficiency of a Mixture of VFA

The extraction of acetic acid (1 wt%), propionic acid (1 wt%), butyric acid (1 wt%), and valeric acid (1 wt%) in 99 wt% distilled water was performed in four separate experiments and in a mixture consisting of (1 wt% of acetic acid, 1 wt% of propionic acid, 1 wt% of butyric acid, and 1 wt% of valeric acid in 96 wt% distilled water). The aqueous solutions were prepared as described in Section 2.2. The results in Figure 15 show that the pure component efficiency and the mixture efficiency of the VFAs are almost identical, suggesting that each acid occupies different void sites in the HDES-phase and does not compete with the others under the studied concentrations [36]. It can also be observed that the extraction efficiency was increasing with an increase in VFA chain length as follows: valeric acid (96.5%) > butyric acid (89.9%) > propionic acid (72.3%) > acetic acid (40.5%) with distribution coefficients of $\beta_{VA} = 13.46$, $\beta_{BA} = 4.36$, $\beta_{PA} = 1.3$, $\beta_{AA} = 0.36$, respectively. This trend was expected as they are consistent with the measured solubility data, which were shown in Table 7 and the implication that the HDES extracts the undissociated acid, where VFAs of longer chains show less dissociation in water. The observed trend also agrees with the findings in the literature [14,22].



Figure 15. Effect of VFA chain length and their mixing effects on the extraction efficiency. (Conditions: T = 298.2 K, P = 1.01 bar, $w_{i,acid} = 1\%$, S:F = 2:1, stirring time = 2 h at 1000 rpm and centrifuge time = 30 min at 3500 rpm).

3.10. Literature Comparison

Table 9 summarizes some of the previous studies in the literature on the liquid–liquid extraction of VFAs using terpenoids "geraniol, citral, and eugenol" [13], medium-chain fatty acids "hexanoic acid and octanoic acid" [12], HDESs [13,22], and kerosene-based solvents [11]. The extraction efficiency of the benchmark solvent trioctlyamine (TOA) [22] was also reported for comparison. In those studies [11–13,22], the extraction experiments were conducted at 298 K and at a S:F of 1:1 in volume. For the purpose of comparison, an HDES of Menthol:Lauric acid (2:1) was prepared and the extraction of acetic acid (1 wt%) at S:F (v/v) equal to 1:1 (v/v) was conducted.

From Table 9, it can be seen that the extraction efficiencies of the geraniol, citral, and eugenol are higher than the other solvents including the benchmark TOA [13,22]. This advantage is due to the green credentials of those three solvents; however, their regeneration might be challenging because of their volatilities. The same applies for medium-chain fatty acids that exhibit comparable results to the HDESs [12]. For that reason, HDESs appear to be more promising.

Regarding the ionic-based HDESs, their extraction efficiencies are higher than TOA, but very comparable to the other reported HDESs. The main downside is their storage stability as reported in Figure 1. On the other hand, the extraction efficiency of Trioctylphosphine oxide (20 wt%) in kerosene [11] was found to be the highest; however, this solvent is a petroleum-based solvent, which is not sustainable.

Comparing the solvents: menthol:octanoic acid (1:1) (E% = 15.7) [13], thymol: octanoic acid (1:2) (E% = 35.5) [13], menthol:lauric acid (2:1) (E% = 27.2), the thymol: octanoic acid exhibited the highest extraction efficiency for acetic acid. This is attributed to the higher acid content in the HDES. However, the use of HDES with higher carbon content such as menthol:lauric acid would increase its hydrophobicity and consequently reduce the cross-contamination of the two-phase. That would compromise the lower extraction efficiency. Another general observation from Table 9 is that for all the solvents, the extraction efficiency increases as the chain length of the VFA increases [22].

Solvent	Acetic Acid	Propionic Acid	Buyuric Acid	Valeric Acid	Ref
Trioctylamine	18.6	45.9	73.5	_	[22]
Geraniol	55.9	74.9	85.6	92.8	[13]
Eugenol	40.9	63.2	70.4	91.8	[13]
Citral	45.7	64.2	70.0	76.0	[13]
Hexanoic acid	27.3	66.2	85.3	-	[12]
Octanoic acid	22.0	57.3	80.1	-	[12]
Decanoic acid:methyltrioctylammonium chloride (2:1)	38.0	70.5	89.8	-	[22]
Decanoic acid:tetraheptylammonium chloride (2:1)	32.0	76.5	91.5	-	[22]
Decanoic acid:tetraoctylammonium chloride (2:1)	25.0	52.7	81.3	-	[22]
Decanoic acid:methyltrioctylammonium bromide (2:1)	29.7	63.4	83.1	-	[22]
Decanoic acid:tetraoctylammonium bromide (2:1)	30.6	65.9	87.4	-	[22]
Menthol: octanoic acid (1:1)	15.7	60.4	82.0	91.3	[13]
Thymol: octanoic acid (1:2)	35.5	73.1	82.1	89.0	[13]
Menthol: lauric acid (2:1)	27.2	-	-	-	This work
Trioctylphosphine oxide (20 wt%) in kerosene	$\approx 60^{a}$	\approx 70 ^a	$\approx 85^{a}$	$\approx 95^{a}$	[11]

Table 9. Extraction efficiencies reported in the literature for various solvents that include the benchmark solvent "trioctylamine". Extraction conditions are at 298 K and at a solvent-to-feed ratio (S:F) of 1:1 in volume from a 1 wt% aqueous solution.

^a The efficiencies were approximated from graphical illustration.

4. Conclusions

In this work, the extraction of volatile fatty acids from a fermented wastewater model using hydrophobic deep eutectic solvents was studied. Six HDESs (tetraoctylammonium bromide: octanoic acid (1:2), tetraoctylammonium bromide: decanoic acid (1:2), tetraoctylammonium bromide: lauric acid (1:2), menthol: octanoic acid (1:2), menthol: decanoic acid (1:2), menthol: lauric acid (1:2)) were initially screened for their storage stability. Out of the six HDESs only the three menthol-based HDESs remained stable for a week after preparation, indicating that ionic-based HDESs have issues with their storability, which could increase their usage cost due to continuous need for reheating. The menthol: lauric acid (Men:LaAc) was selected for the extraction of the VFAs due to its stability and higher hydrophobicity. The Men:LaAc molar ratio was further optimized by conducting an extraction experiment of acetic acid from the broth model. It was found that a molar ratio of 1.5:1 gave the highest extraction efficiency for acetic acid, indicating that the extraction mechanism is mainly due to the addition of LaAc rather than menthol. Nonetheless, menthol still plays a key role in hydrophobicity and liquifying the DES.

The Men:LaAc (1.5:1) was then characterized by measuring its water content and its physicochemical properties: density, viscosity, and degradation temperature. Notably, the viscosity of the HDES was found to be considered relatively "low" compared to many other HDES. Thereafter, the critical variables were calculated and a solubility screening for the systems (VFA in water), (VFA in HDES), and the saturation of the HDES with water was conducted at a temperature of 298.2 K and a pressure of 1.01 bar. The VFAs used were: acetic acid, propionic acid, butyric acid, and valeric acid. It was evident that the longer the VFAs' chain length the higher the hydrophobicity, while all the VFAs were found to be fully soluble in the HDES. Moreover, remarkably, upon mixing the HDES with water, none of its constituents was detected in the water-phase, which would lower the costs from phase cross-contamination when using selected Men:LaAc HDES.

The influences of the stirring time on the extraction efficiency of acetic acid from the broth model were then investigated. It was found that a stirring time of 30 min was sufficient time to reach equilibrium. It was also observed that extraction efficiency was independent of the initial concentration of acetic acid, which is encouraging as fermented wastewater broths usually contain varying concentrations of VFAs. Regarding the multistage extraction, an extraction efficiency of 88% for acetic acid was reached in only four stages. Interestingly, the efficiency achieved after the second stage with a 2:1 S:F ratio was found to be higher than that obtained at a 4:1 S:F ratio, indicating that it is preferable to perform the extraction process at a low solvent-to-feed ratio and more stages to optimize the process.

It was also found that the HDES capacity increased during its application for 4 extraction cycles increasing the concentration of acetic acid in the HDES from 0.6 to 1.17 wt%. Furthermore, the HDES regeneration was successfully achieved via vacuum evaporation, which is favorable in terms of improving the sustainability of the process.

The extraction efficiencies were determined for the extraction of propionic acid, butyric acid, and valeric acid at a temperature of 298.2 K and a pressure of 1.01 bar. It was observed that the longer the VFA chain length, the higher the extraction efficiency. The extraction efficiencies were in the following order: valeric acid (96.5%) > butyric acid (89.9%) > propionic acid (72.3%) > acetic acid (40.5%). Moreover, it was found that the pure component efficiency and the mixture efficiency of the VFAs are almost identical, which is exceedingly useful as fermented broths often include a variety of acids in a mixture. Finally, the HDES performance for the extraction of VFAs was compared to trioctylamine (TOA) as a benchmark solvent and relevant literature. It could be concluded that the HDES presented in this work is a promising candidate in terms of green extraction of VFAs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10 .3390/fermentation7040226/s1, Table S1: Measured density values of the hydrophobic deep eutectic solvent, DL-menthol: lauric acid (1.5:1), at the temperature range of 298.2 K–368.2 K. The standard deviations were calculated from duplicate measurements, pH Calculation.

Author Contributions: Conceptualization, N.A.; methodology, S.E.E.W., A.S.D. and M.K.A.; validation, S.E.E.W. and A.S.D.; formal analysis, A.S.D.; data curation, A.S.D., T.L., F.A.H. and R.R.; writing—original draft preparation, A.S.D. and S.E.E.W.; writing—review and editing, S.E.E.W., N.A., I.A., J.R. and R.R.; visualization, A.S.D.; supervision, N.A.; project administration, J.R.; funding acquisition, N.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Khalifa University, grants number CIRA-2018-84 and CIRA-2018-23 and by Research and Innovation Center on CO₂ and H₂ (RICH, RC2-2019-007) at Khalifa University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in article.

Acknowledgments: The authors would like to thank our colleagues Ali A. Alnajjar and Ghaiath Almustafa from Khalifa University for their continuous and valuable help. The authors also acknowledge the support of the Center for Membrane and Advanced Water Technology (CMAT, RC2-2018-009) and the Research and Innovation Center on CO_2 and H_2 (RICH, RC2-2019-007) at Khalifa University.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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