Food and Bioproducts Processing 143 (2024) 21–27

Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Lipid recovery from deep eutectic solvents by polar antisolvents

Calvin Lo [a], René H. Wijffels [a,b], Michel H.M. Eppink [a,*]

[a] Bioprocess Engineering, AlgaePARC, Wageningen University, PO Box 16, 6700 AA Wageningen, the Netherlands
[b] Nord University, Faculty of Biosciences and Aquaculture, N-8049 Bodø, Norway

ARTICLE INFO

Keywords:
Deep eutectic solvents (DES)
Polar antisolvents
Lipid recovery
Deep eutectic solvent (DES) regeneration

ABSTRACT

Deep eutectic solvents (DES) can be a green alternative extraction technology for microalgae lipids, acting simultaneously as a pre-treatment agent and solvent. However, due to the low vapor pressure of both DES and lipid solute, the recovery of lipids and solvent regeneration remains difficult. In this study, we developed a novel strategy to separate the dissolved sunflower and model algae oil from imidazole/hexanoic acid DES by using polar antisolvents (water, methanol, and ethanol). The polarity and the amount of antisolvent influenced the lipid solubility in DES. While the water was the strongest antisolvent, the alcohols were easier to evaporate, ensuring easy DES regeneration. By adding small amounts of water and methanol, more than 90% of the lipids were recovered in the form of high-purity oils (>90%). In the case of ethanol, a large amount of ethanol was required, which diluted the solvent-rich phase and solubilized more lipids in it. Based on three repeated cycles with the selected antisolvent methanol, > 90% of the eutectic solvent could be regenerated.

1. Introduction

Microalgae have been known as a sustainable feedstock for lipid production due to their advantages over oleaginous terrestrial plants. However, to obtain lipids from microalgae, energy-intensive cell disruption methods and unsustainable solvents are still often used (Halim et al., 2012; Kumar et al., 2017; Lee et al., 2017). Recently, deep eutectic solvents (DES), a "greener alternative" to ionic liquids, have been applied to improve the lipid extraction process (Alam et al., 2021; Cai et al., 2020; Cao and Su, 2021). Several water-soluble DES, such as combinations of choline chloride and oxalic acid or glycols, were reported to perforate microalgae cell wall, which could be enhanced by heating or microwave treatment. Then, another hydrophobic solvent (e.g., hexane) was able to penetrate the treated cell and dissolve the intracellular lipids (Lu et al., 2016; Pan et al., 2017; Tommasi et al., 2017). However, with this approach, an extra solvent would increase the process complexity and production cost. Hydrophobic DES holds the potential to both weaken the cell wall and dissolve the lipids simultaneously (Eppink et al., 2021). Recently, we demonstrated lipid extraction from undisrupted *Nannochloropsis oceanica* using imidazole-based hydrophobic DES (Lo et al., 2023). Oleic acid-based hydrophobic DES was also reported to be able to extract astaxanthin from intact *Haematococcus pluvialis* (Pitacco et al., 2022).

Similar to ionic liquids, DES suffers from low vapor pressure. This implicates the straightforward separation of lipid solutes from the solvents through evaporation and condensation, which is common for organic solvents, but does not work with DES (Ruesgas-Ramón et al., 2017; Tang and Row, 2020). One of the proposed strategies when extracting with DES is to include DES molecules in the product formulation, rendering the separation of target compounds from DES unnecessary. For instance, the use of edible and Generally Recognized As Safe (GRAS) terpene-based DES for astaxanthin extraction from microalga *H. pluvialis* and crustacean residual biomass for astaxanthin extraction (Pitacco et al., 2022; Rodrigues et al., 2020). However, this approach might not be suitable for all products due to the complication of product formulation (e.g., desired product concentration or undesired DES molecules) and the low economic value of certain products. Hence, an alternative strategy needs to be developed for lipid recovery and DES recycling to have a viable and sustainable process.

Our previous work screened semi-hydrophobic DES and developed a lipid recovery strategy by tuning the solvent polarity (Lo et al., 2021). Made of polar imidazole and nonpolar hexanoic acid, the DES dissolves water (hydrophilic) or lipids (hydrophobic). Hexanoic acid is a medium-chain carboxylic acid that can be produced fermentatively, whereas imidazole is a heteroaromatic compound that is readily biodegradable with low ecotoxicity (Cavalcante et al., 2017; European Chemicals Agency, 2022). The hydrophobicity of the mixture was dependent on the DES composition. At an imidazole molar ratio to

* Corresponding author.
E-mail address: michel.eppink@wur.nl (M.H.M. Eppink).

https://doi.org/10.1016/j.fbp.2023.10.003
Received 29 July 2023; Received in revised form 11 October 2023; Accepted 16 October 2023
Available online 18 October 2023

hexanoic acid greater than 3/7, the solvent became hydrophilic and rejected the dissolved lipids. However, this strategy is unsuitable for DES regeneration as imidazole binds strongly with hexanoic acid; thus, it would be difficult to separate the two constituents (Anouti et al., 2009). Furthermore, constantly adding a large amount of hexanoic acid to balance the DES molar ratio would not be economically favourable and unsustainable. Therefore, another approach to ensure the recyclability of the solvent is needed for regeneration.

Antisolvents have been widely used to separate the target product from polar DES. For example, the addition of water, alcohols, or acetone induced the precipitation of phenolic compounds, lignin, cellulose, and insoluble protein from DES (Chen and Wan, 2018; Das et al., 2016; Grudniewska et al., 2018; Grudniewska and Popłoński, 2020; Kumar et al., 2016; Maugeri and Domínguez De María, 2012; Procentese et al., 2015; Smink et al., 2020). Typically, the target solute has a low, if not negligible, solubility in the antisolvents, while the DES shows high miscibility with the antisolvent. Furthermore, the antisolvent can then be evaporated to regenerate the DES. In general, the product recovery is directly proportional to the amount of antisolvent. However, it is worth noting that the antisolvent amount directly correlates to the energy required for antisolvent removal and DES regeneration.

It is then hypothesized in this study that polar antisolvents, such as water and alcohols, could promote the hydrophilicity of imidazole/hexanoic acid DES, consequently lowering the lipid solubility. Furthermore, if the antisolvents have relatively high vapor pressure, DES could also be easily regenerated by the antisolvent evaporation. To the best of our knowledge, the use of DES and antisolvents in the lipid extraction process is still limited.

This study is a proof of concept using antisolvents to recover dissolved lipids from DES and recover DES during regeneration. Commercial sunflower and algae oil were used as model lipids since they do not contain impurities from complex biomass that can interfere with DES. Water, methanol, and ethanol were added as antisolvents to separate the model oils from imidazole/hexanoic acid DES. The dissolved oils were used to mimic the lipid extract from microalgae. Afterwards, the DES was regenerated by evaporating the antisolvents and the best antisolvent methanol was re-used in three consecutive cycles. Based on the compositional analysis, the antisolvent performance and the loss throughout the process were evaluated.

2. Materials and methods

2.1. Materials

The materials which were used in this study were algae oil (Corbion Biotech, Inc., United States), ethanol (Merck Millipore, absolute), hexanoic acid (Sigma-Aldrich, ≥ 99%), imidazole (Sigma-Aldrich, ≥ 99%), methanol (Merck Millipore, pure), water (Milli-Q®, ultrapure), and sunflower oil (Jumbo Supermarkten BV, The Netherlands).

2.2. Preparation of the deep eutectic solvent

In this study, the combination imidazole/hexanoic acid (molar ratio 1:3) was used to represent the semi-hydrophobic DES. The DES was prepared by dissolving the pre-weighed imidazole flakes in hexanoic acid at room temperature until a clear homogeneous solution was obtained.

2.3. Lipid dissolution in DES and recovery using antisolvents

Sunflower and algae oil were used as model lipids in this study. To measure the effect of antisolvents, the lipid solubility was measured via cloud point determination. This was performed by a continuous dropwise (25 μL) addition of the oils to the DES which contained various concentration of water, methanol, and ethanol in glass tubes. After every drop, the system was vortexed to ensure homogeneity. The experiment was performed at room temperature and stopped as soon as the solution turned cloudy. The density of DES, algae and sunflower oil are 0.98, 0.90 and 0.92 g mL^{-1}, respectively, when measured gravimetrically at room temperature.

Besides that, the lipid recovery by addition of antisolvent was also performed. For this experiment, the initial DES (2.95 g) solution contained sunflower oil (38 wt%, 1.84 g) or algae oil (31 wt%, 1.36 g). Then, various amounts of antisolvents were added to the solutions at room temperature, which induced a phase split between the recovered lipid and the solvent-rich phase. The mixtures, which total mass depended on the amount of antisolvents, were centrifuged at 4000 RCF (Allegra X-30 Benchtop Centrifuge (Beckman Coulter)), before carefully separated and stored for further analysis.

2.4. Evaporation of antisolvents and DES regeneration

The antisolvents were evaporated from the solvent-rich phases using a rotary vacuum concentrator at 8 mbar, 35 °C (RVC 2–25 CDplus (Christ)). The low evaporation temperature was chosen to minimize the risk of undesired side reactions, such as esterification. Unless stated for kinetic study, the evaporation occurred ~16 h for the alcohols-containing mixtures and 24 h for water mixtures. For kinetic study, the total amount was based on 1 g of DES and differed with different antisolvents.

2.5. Gas chromatography analysis

All the analyses were performed using gas chromatography-flame ionization detector (GC-FID) system (Agilent Technologies) with H_2 as a carrier gas. The fatty acid analysis was performed based on the method described in our previous work (Lo et al., 2021). Known amount of the pure model lipids were also analyzed using the same method as a standard for lipid and total fatty acids conversion. For analyses of methanol, ethanol, imidazole, and hexanoic acid, the solvent-rich phases were diluted 10x in chloroform and run with DB-FFAP column (part #122–3232, Agilent Technologies) with a split-ratio of 20:1 and a split-flow of 45.27 mL/min. The oven temperature profile was 45–80 °C with 6.30 °C/min, and to 220 °C with 26.24 °C/min and held for 3 min.

2.6. Partition coefficient and separation efficiency

The partition coefficient K and separation efficiency of the lipid recovery were calculated by using these equations, respectively:

$$K = c_O / c_S$$

$$\text{Efficiency} = \frac{V_O \quad c_O}{V_O \quad c_O + V_S \quad c_S}$$

where V is the total amount of the phase [gram], c is the total fatty acids concentration measured in the oil- or solvent-rich phase [mg_{TFA} mg^{-1}], and subscript O and S denote the oil-rich and solvent-rich phase, respectively. Besides that, in this study, we refer to the purity of the recovered oil as c_O [mg_{TFA} mg_{oil}^{-1}] of that oil phase compared to c_O of the model oils.

3. Results and discussions

Initially, we evaluated sunflower and algae oil solubility in the deep eutectic solvent containing different concentrations of water, methanol, and ethanol (Section 3.1). Next, the dissolved lipids were recovered from DES mixtures with varying amount of antisolvents (Section 3.2). Furthermore, the antisolvents were evaporated from the solvent mixtures to mimic the DES regeneration step (Section 3.3). Finally, three repeated cycles of sunflower oil dissolution, recovery and solvent regeneration were performed to analyze the mass composition and to

identify potential losses (Section 3.4). Additionally, a rational is given about the hexanoic acid back-extraction as a small amount of hexanoic acid remains in the lipid fraction and can be back-extracted with an additional methanol wash step (Section 3.5).

3.1. Reduced solubility of lipids in the DES by antisolvents

To enable the lipid separation from the solvent, the addition of antisolvents should decrease the lipid solubility in the imidazole/hexanoic acid (1:3 molar ratio) DES. Both the concentration and the polarity of antisolvents influenced the solubility of sunflower and algae oil (Fig. 1). In general, lipids were less soluble at higher antisolvent concentrations regardless of the antisolvent. Water caused the most substantial solubility reduction, followed by methanol and ethanol. It is worth noting that water has limited solubility in the DES (~0.4 g g_{DES}^{-1}), whereas both alcohols are completely miscible with the solvent. Furthermore, it was known before that the algae oil has a lower solubility in DES than sunflower oil. It is due to the different degree of fatty acid saturation in the lipids, where algae oil contains > 90% oleic acid, C18:1, and sunflower oil is rich in linoleic acid, C18:2 (> 56%). The fatty acid composition of the model oils can be found in our previous study (Lo et al., 2021).

3.2. Lipid recovery from DES extract using antisolvent

The pre-dissolved sunflower and algae oil (initial concentration: 38 and 34 wt%, respectively) were separated from the DES by adding various amounts of antisolvents. This led to oversaturation and eventually induced phase split between the oil-rich phase and the solvent mixture. The formation of phases was further accelerated by centrifugation. Fig. 2 provides the visual observation of mixtures treated with water and methanol. The increasing amount of water and methanol are ordered from left to right in the pictures. The location of oil-rich phase depends on the density of the formed phases (ρ of DES, algae and sunflower oil are 0.98, 0.90 and 0.92 g mL^{-1}, respectively). The water/DES mixtures were constantly denser than the oil-rich phase, whereas density of alcohol/DES mixture depends on the alcohols' concentration. Since the alcohols ($\rho \cong$ 0.79 g mL^{-1}) are much lighter than the lipids, the alcohol-rich mixtures are lighter than the oils.

Each phase was then analyzed to quantify the partition of the model oils at equilibrium, K (Fig. 3a). A high partition coefficient means that the lipid is less likely to be dissolved in the DES-rich phase, leading to a better separation. The partition coefficients linearly correlate to the amount of antisolvent added. Moreover, the polarity of antisolvent also determines the sensitivity of K towards the antisolvent amount (shown by the slope). For both model lipids, water achieved the highest K with the steepest slope, closely followed by methanol. As mentioned before, methanol is more soluble than water in DES. This might imply that higher K values could be achieved at higher methanol concentrations, whereas that from water reached the maximum considering the limited solubility of water in DES. Moreover, ethanol, as expected, did not give good partition since it did not induce enough polarity to reduce the lipid solubility in the solvent phase. Besides that, K values of algae oil are higher than that of sunflower oil. This implies that at the same antisolvent concentration, the solvent phase dissolved more sunflower oil than algae oil. This result is consistent with the antisolvent effect which was discussed above.

Furthermore, from the fatty acid analysis, the separation efficiencies in the lipid recovery step were calculated (Fig. 3b). The separation efficiency steeply increases with amount of water and methanol until reaching about ~90% recovery, then slowly increases until maximum was reached. As expected, water gave the highest recovery for both model oils (efficiency of 96%), while methanol reached the efficiency of ≥ 90%. Ethanol, however, performed poorly with the highest obtained efficiency of ~60% at relatively low concentration and K. The high solubility of lipids (triacylglycerol) in ethanol than in water and methanol caused higher concentration of oil in the solvent-rich phase (c_S). Also, unlike water and methanol, a much larger amount of ethanol was necessary to reduce the lipid solubility, significantly increasing V_S and consequently reducing the separation efficiency. This large difference in the performance of the alcohols was not expected since the solubility of tripalmitin (triacylgycerol of palmitic acid) in methanol and ethanol are in the same order of magnitude (0.01 and 0.03 mg/mL, respectively) (Zahler and Niggli, 1977). This result implies that other antisolvents with lower polarity, such as acetone, would not perform efficiently as antisolvent in this system.

Besides that, the purity of recovered oil was also evaluated (Fig. 4). Overall, the purity of the recovered oil was high, with the recovered algae oil being slightly higher than the sunflower oil. While methanol reached the highest purity, ~100%, water and ethanol yielded a lower purity, 80 – 90%. Note that the purity was based on the mass fraction of total fatty acids in that oil phase with reference to the mass fraction of total fatty acids in the pure oils [mg_{TFA} mg_{oil}^{-1}]. The contradicting nature of polar antisolvents and hydrophobic hexanoic acid influenced the distribution of hexanoic acid. Water, being more polar than methanol, caused extra leaching of hexanoic acid to the oil phase and ultimately lower the oil purity. However, in the case of ethanol, ethanol itself is also a contaminant in the oil phase. This might be due to the lower polarity of ethanol and its large presence in the overall system, causing distribution of ethanol over the phases. A quantitative analysis on the oil-rich phase

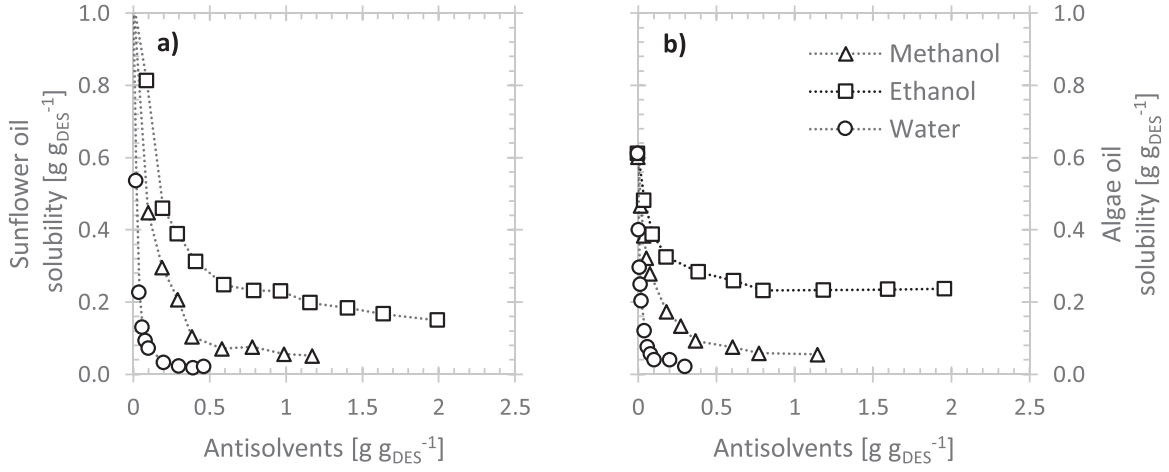

Fig. 1. Solubility of sunflower oil (a) and algae oil (b) in the imidazole/hexanoic acid (1:3) DES with various concentrations of water (circle), methanol (triangle), and ethanol (square).

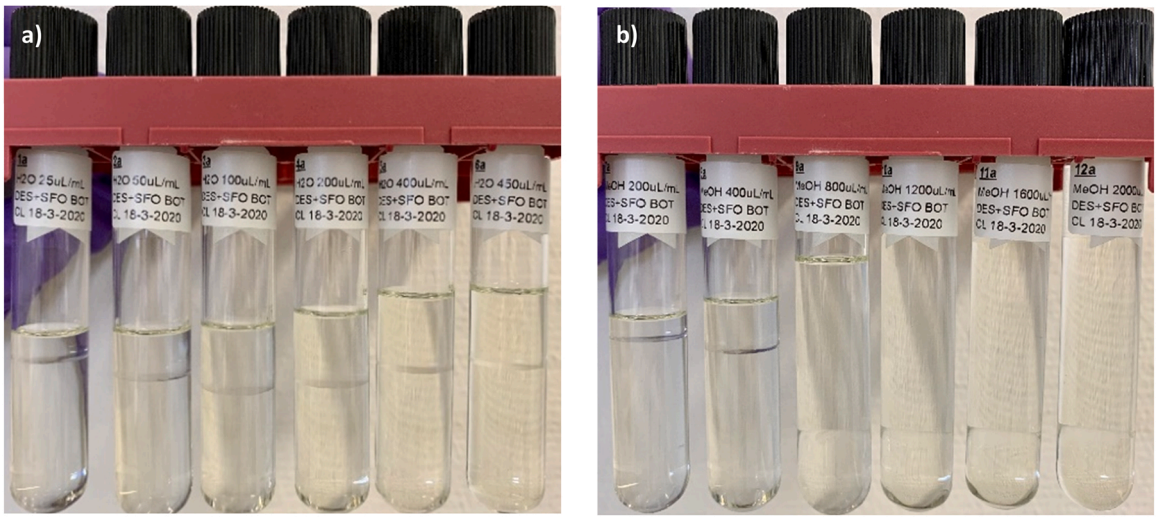

Fig. 2. Phase separation between the recovered oil and solvent mixtures by adding (a) water and (b) methanol, increasing concentrations ordered left to right. While water/DES mixtures were consistently denser than the lipids, the concentration of methanol determined the density of the solvent mixtures. Methanol-rich mixtures were lighter than the lipids, whereas the mixtures with low methanol concentrations were heavier.

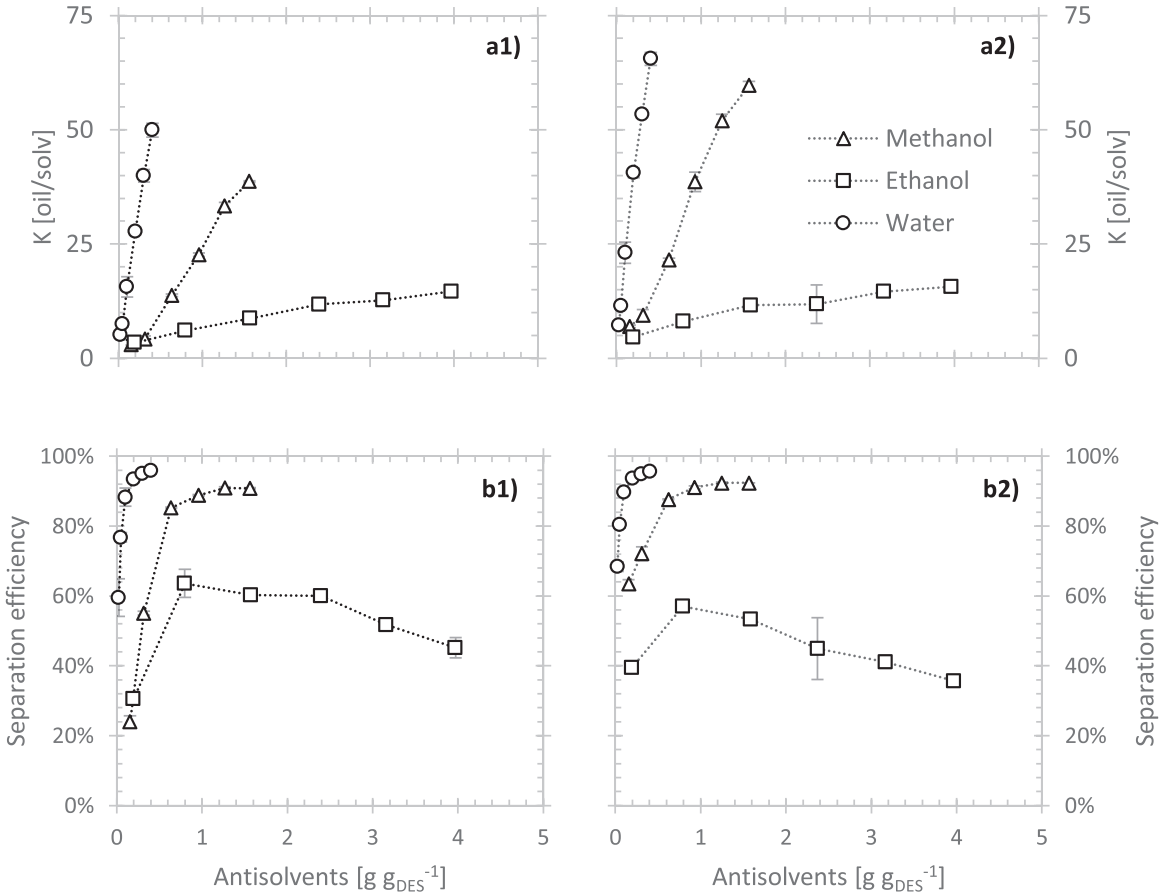

Fig. 3. a) Partition coefficient *K* and b) separation efficiency of sunflower oil (1) and algae oil (2) recovered by addition of water (circle), methanol (triangle), and ethanol (square).

can be found in Section 3.4.

The presence of the contaminants may decrease the quality of the recovered oil. The alcohols can easily be removed by evaporation. The presence of hexanoic acid in the oil is less desired since it would give rancid, unpleasant odor and increase the free fatty acid (FFA) content of the oil. High FFA lipids are associated with lower food quality (Osawa et al., 2007), health issues when consumed (Lee Kuek et al., 2020) and lead to saponification problem in the biodiesel synthesis (Đokić et al., 2012; Zhang et al., 2020). Therefore, lipid deodorization and deacidification, which are normal steps in vegetable oil processing, should be performed in the further downstream steps.

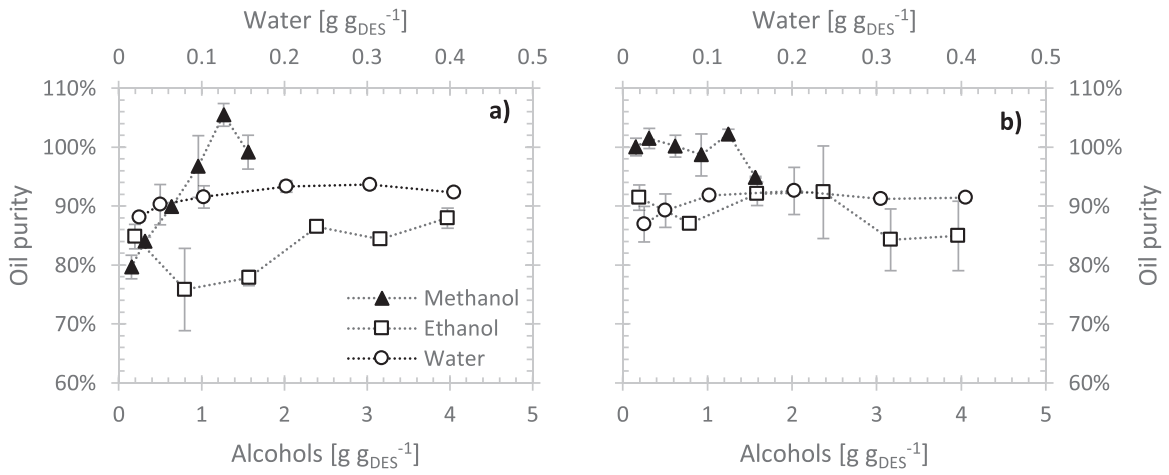

Fig. 4. Purity of recovered sunflower oil (left) and algae oil (right) with different antisolvents. Mind the different scale of water and alcohols concentrations.

3.3. DES regeneration via antisolvent evaporation

The ease of DES regeneration after the lipid separation step was evaluated based on the evaporation kinetics. In this experiment, we performed the evaporation of antisolvents at two different concentrations to mimic the possible mixtures after the recovery step at 8 mbar and 35 °C. This low temperature was chosen to avoid not only the evaporation of imidazole and hexanoic acid, but the undesired esterification of both hexanoic acid and the alcohols as well. This was also confirmed by gas chromatography analysis.

Fig. 5 shows the evaporation rate of water, methanol, and ethanol in the DES solutions. The evaporation rate of the antisolvents follow the vapor pressure of the pure compounds, i.e., methanol and ethanol have much higher vapor pressures than water (276, 135, and 42 mbar at 35 °C, respectively) (Dortmund Databank, 2020). Additionally, the low water concentration in the solution and the possibility of strong interactions between water molecules and DES components could further hamper the vapor pressure of water, resulting in problematic regeneration. Therefore, to ensure the easy regeneration step, the use of methanol or ethanol is favorable. Furthermore, unlike the case with water, complete removal of the alcohols is not necessary since the lipid solubility is less sensitive towards the presence of the alcohols than water (Fig. 1).

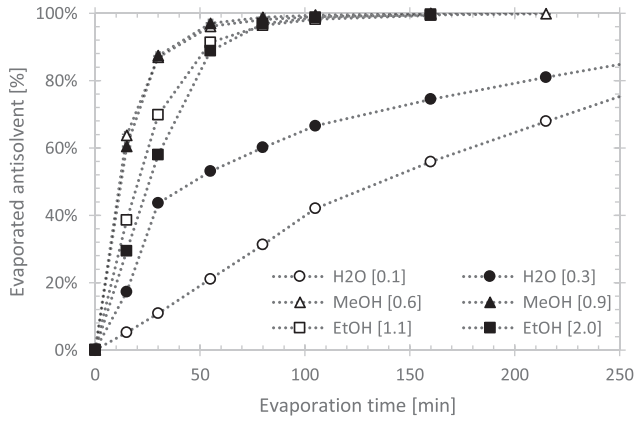

Fig. 5. Evaporation kinetics of water (circle), methanol (triangle), and ethanol (square) when mixed with imidazole/hexanoic acid (1:3) in low (open) and high (closed) concentration. The exact concentrations of antisolvents (in g g_{DES}^{-1}) are written inside brackets. 95% of water was removed after 600 min.

3.4. Recycle of the deep eutectic solvent

The final objective of this study was to investigate the feasibility of recycling the DES. In this experiment, we performed three repeated cycles of lipid dissolution, recovery, and solvent regeneration using sunflower oil (Fig. 6a) and the antisolvent methanol. This oil was chosen to simulate the worst-case scenario with lower recovery since the DES has a higher affinity to sunflower oil than algae oil. In each cycle, the extract was maintained to contain 38 wt% of oil in the DES. Then, the lipid recovery was performed by adding methanol to reach 0.96 g g_{DES}^{-1}. This concentration was chosen for its high separation efficiency with the least methanol amount. The mass composition of different phase throughout the entire process can be seen in Table 1. Throughout the cycles, no significant change in performance was observed. For example, the remaining unrecovered sunflower oil in the solvent-rich phase was consistent at ~5%. This small presence of sunflower oil in the solvent-rich phase did not hamper the reusability of DES to extract lipids.

Furthermore, a loss of hexanoic acid was observed after each step, reflected by the increased molar ratio of imidazole to hexanoic acid (Table 1). It started from 0.336 towards 0.408 $mol_{Imi.}$ mol_{Hex}^{-1}, which is equivalent to (1:3) and (2:5) molar composition, respectively. The losses occurred mainly during the lipid recovery (phase split) step, at which a substantial fraction of hexanoic acid migrated to the oil-rich phase. Whereas during the evaporation step, a little amount of hexanoic acid evaporated despite the low vapor pressure, which could be due to the prolonged evaporation time (16 h instead of 3 h based on Fig. 5). The rising trend of imidazole molar ratio indicates the declining DES hydrophobicity, hence less capacity of lipid dissolution (Lo et al., 2021). To overcome this shortness, fresh hexanoic acid needs to be fed as make-up for the loss. Furthermore, the loss can be minimized by shortening the evaporation time and optimizing the condition (e.g., temperature swing) during the lipid recovery step to reduce the migration of hexanoic acid further. Despite the loss, > 90% deep eutectic solvent was regenerated after each step based on the mass balance (Fig. 6b). In ideally closed system, where all methanol vapor is condensed and reused to induce phase-splitting, evaporated hexanoic acid would not be lost and enter the system together with the antisolvent.

3.5. Hexanoic acid back-extraction

As mentioned before, the presence of hexanoic acid in the recovered oil phase is undesired; it would significantly hamper the product value and increase the need for solvent loss makeup. In this study, hexanoic acid had a higher affinity towards methanol than sunflower oil, shown by its concentration in each phase (Table 1). Thus, methanol washing on the recovered oil is proposed to be the back-extraction step of hexanoic

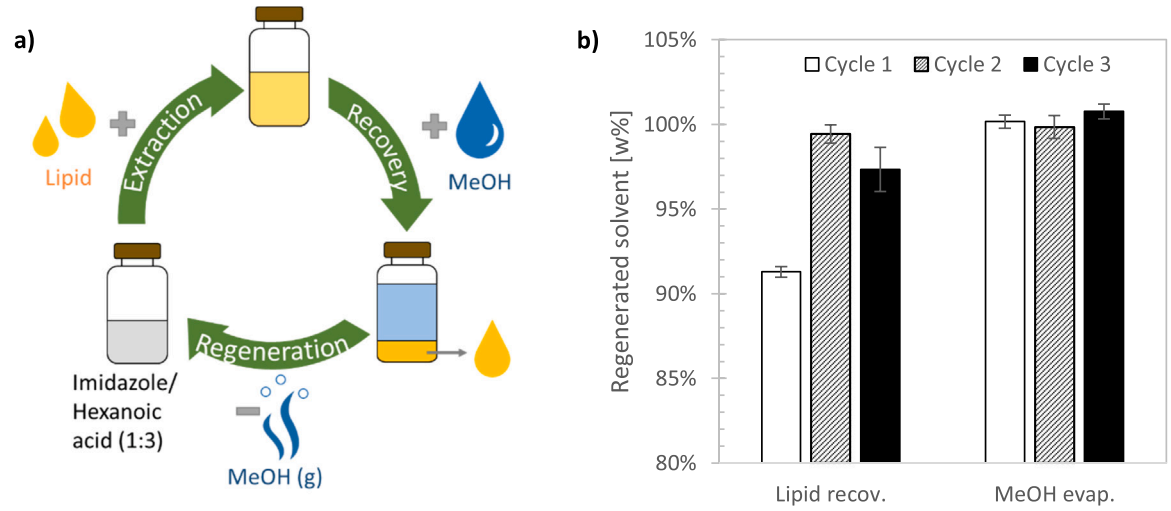

Fig. 6. a) Schematic process of the extraction cycle using imidazole/hexanoic acid DES, b) Fraction of DES regenerated in each process step in three cycles (cycle 1 – white, cycle 2 – striped, cycle 3 – black).

Table 1
Relative composition of each phase (recovered oil, solvent-rich phase, and regenerated DES) throughout the three extraction cycles.

Run	Phase	Hexanoic acid [w%]	Imidazole [w%]	Methanol [w%]	Sunflower oil [w%]	$mol_{Imi.}/mol_{Hex}$
0	DES + Oil	52.1 ± 0.7	10.3 ± 0.1	0.0 ± 0.0	38.9 ± 1.7	0.336
1	Recov. oil	5.5 ± 0.1	0.2 ± 0.0	3.4 ± 0.2	93.7 ± 2.8	0.071
	Solvent-rich	41.0 ± 0.4	8.4 ± 0.1	47.6 ± 0.5	5.4 ± 0.1	0.350
	Regen. DES	75.7 ± 0.3	15.7 ± 0.2	0.0 ± 0.0	9.7 ± 0.2	0.353
2	Recov. oil	7.3 ± 0.0	0.5 ± 0.0	5.4 ± 0.1	93.1 ± 2.7	0.121
	Solvent-rich	40.3 ± 0.0	8.7 ± 0.0	49.4 ± 2.6	4.8 ± 0.3	0.369
	Regen. DES	75.3 ± 0.9	16.4 ± 0.1	0.0 ± 0.0	9.5 ± 0.3	0.372
3	Recov. oil	6.1 ± 0.2	0.4 ± 0.1	6.2 ± 1.2	100.9 ± 0.2	0.114
	Solvent-rich	37.6 ± 0.4	8.8 ± 0.2	46.8 ± 0.0	5.4 ± 0.7	0.401
	Regen. DES	66.7 ± 0.6	15.9 ± 0.0	0.0 ± 0.9	11.1 ± 1.0	0.408

acid.

Here we show the simulation and rationale of the proposed methanol washing based on liquid-liquid extraction (LLE). Assuming 1) immiscibility between oil and methanol, and 2) constant volume of methanol and oil phase, the mass balance of hexanoic acid would be:

$$V_O \quad c_{O,F}^{hex} = V_O \quad c_O^{hex} + V_{Met} \quad c_{Met}^{hex}$$

where V_O and V_{Met} are the volumes of the recovered oil and methanol phase, $c_{O,F}^{hex}$ is the initial concentration of hexanoic acid in the recovered oil (feed), and c_O^{hex} and c_{Met}^{hex} are the concentrations of hexanoic acid in the oil and methanol phase after washing respectively.

The final concentration of hexanoic acid in methanol and oil phase would be determined by thermodynamic equilibrium, defined as distribution coefficient ($K_D = c_{Met}^{hex}/c_O^{hex}$). This K_D value could be approximated from hexanoic acid concentration in the solvent-rich phase and the recovered oil during phase splitting (Table 1). From the three cycles of phase partitioning, the average K_D value is 6.4, showing the higher affinity of hexanoic acid in methanol than in oil. This higher affinity in methanol, when combined with higher volume ratio of methanol/oil, could result in effective back-extraction. For instance, when $V_{Met}/V_O = 2$, the concentration of hexanoic acid in the final oil phase (c_O^{hex}) would only be < 10% of the initial feed concentration ($c_{O,F}^{hex}$). This would mean hexanoic acid content in the final oil product to be ~0.05% weight. Besides the higher volume ratio, multiple stages could be performed to also increase the hexanoic acid removal.

After the washing step, the methanol phase containing hexanoic acid can be fed into the evaporation step, to separate methanol and DES. This strategy would significantly reduce the loss of hexanoic acid, preventing the DES becoming less hydrophobic (due to the changing imidazole/hexanoic acid composition) and improving the process sustainability and recyclability. At the same time, the final lipid product would contain only trace of hexanoic acid, increasing its functionality and value.

4. Conclusion

The presence of the antisolvents water, methanol, and ethanol in imidazole/hexanoic acid (1:3) DES reduced the solubility of model lipids, including algae oil. The solubility reduction positively correlated with the antisolvent polarity and amount. The antisolvents were then applied to recover the dissolved sunflower and algae oil in the DES, resulting in high purity oils with > 90% recovery. Furthermore, the DES can be regenerated via antisolvent evaporation. It was observed that there was a small loss of hexanoic acid, which mainly occurred during the phase split. Nevertheless, with this approach, > 90% of the DES can be regenerated. This loss can be reduced by introducing an extra methanol washing on the recovered oil, which would also yield lipid with higher purity. The recyclability of DES with the antisolvent methanol demonstrated in this study further boosted the potential of DES as an alternative method for lipid extraction from microalgae.

Declaration of Competing Interest

The authors of this manuscript have no “No Conflict of Interest”.

Acknowledgements

This research is part of the MAGNIFICENT project, funded by the Bio-Based Industries Joint Undertaking under the European Union’s Horizon

2020 research and innovation program (grant agreement No. 745754).

References

Anouti, M., Jones, J., Boisset, A., Jacquemin, J., Caillon-Caravanier, M., Lemordant, D., 2009. Aggregation behavior in water of new imidazolium and pyrrolidinium alkycarboxylates protic ionic liquids. J. Colloid Interface Sci. 340, 104–111.

Alam, Asraful, Muhammad, M., Khan, G., Mofijur, M.N., Lv, M., Xiong, Y., Xu, J, W., 2021. Choline chloride-based deep eutectic solvents as green extractants for the isolation of phenolic compounds from biomass. J. Clean. Prod. 309, 127445 https://doi.org/10.1016/J.JCLEPRO.2021.127445.

Cai, C., Wang, Y., Yu, W., Wang, C., Li, F., Tan, Z., 2020. Temperature-responsive deep eutectic solvents as green and recyclable media for the efficient extraction of polysaccharides from *Ganoderma lucidum*. J. Clean. Prod. 274, 123047 https://doi.org/10.1016/J.JCLEPRO.2020.123047.

Cao, J., Su, E., 2021. Hydrophobic deep eutectic solvents: the new generation of green solvents for diversified and colorful applications in green chemistry. J. Clean. Prod. 314, 127965 https://doi.org/10.1016/J.JCLEPRO.2021.127965.

Cavalcante, W. de A., Leitão, R.C., Gehring, T.A., Angenent, L.T., Santaella, S.T., 2017. Anaerobic fermentation for n-caproic acid production: A review. Process Biochem 54, 106–119. https://doi.org/10.1016/J.PROCBIO.2016.12.024.

Chen, Z., Wan, C., 2018. Ultrafast fractionation of lignocellulosic biomass by microwave-assisted deep eutectic solvent pretreatment. Bioresour. Technol. 250, 532–537. https://doi.org/10.1016/j.biortech.2017.11.066.

Das, A.K., Sharma, M., Mondal, D., Prasad, K., 2016. Deep eutectic solvents as efficient solvent system for the extraction of K-carrageenan from *Kappaphycus alvarezii*. Carbohydr. Polym. 136, 930–935. https://doi.org/10.1016/j.carbpol.2015.09.114.

Đokić, M., Kesić, Ž., Krstić, J., Jovanović, D., Skala, D., 2012. Decrease of free fatty acid content in vegetable oil using silica supported ferric sulfate catalyst. Fuel 97, 595–602. https://doi.org/10.1016/j.fuel.2012.03.039.

Dortmund Databank, 2020. Saturated Vapor Pressure [WWW Document]. URL http://ddbonline.ddbst.com/AntoineCalculation/AntoineCalculationCGI.exe.

Eppink, M.H.M., Ventura, S.P.M., Coutinho, J.A.P., Wijffels, R.H., 2021. Multiproduct microalgae biorefineries mediated by ionic liquids. Trends Biotechnol. 39, 1131–1143. https://doi.org/10.1016/j.tibtech.2021.02.009.

European Chemicals Agency, 2022. Imidazole [WWW Document]. URL https://echa.europa.eu/registration-dossier/-/registered-dossier/2052/ (accessed 1.23.22).

Grudniewska, A., De Melo, E.M., Chan, A., Gniłka, R., Boratyński, F., Matharu, A.S., 2018. Enhanced protein extraction from oilseed cakes using glycerol-choline chloride deep eutectic solvents: a biorefinery approach. ACS Sustain. Chem. Eng. 6, 15791–15800. https://doi.org/10.1021/acssuschemeng.8b04359.

Grudniewska, A., Popłoński, J., 2020. Simple and green method for the extraction of xanthohumol from spent hops using deep eutectic solvents. Sep. Purif. Technol. 250, 117196 https://doi.org/10.1016/j.seppur.2020.117196.

Halim, R., Danquah, M.K., Webley, P.A., 2012. Extraction of oil from microalgae for biodiesel production: a review. Biotechnol. Adv. 30, 709–732. https://doi.org/10.1016/j.biotechadv.2012.01.001.

Kumar, A.K., Parikh, B.S., Pravakar, M., 2016. Natural deep eutectic solvent mediated pretreatment of rice straw: bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue. Environ. Sci. Pollut. Res. 23, 9265–9275. https://doi.org/10.1007/s11356-015-4780-4.

Kumar, J., Garlapati, V.K., Dash, A., Scholz, P., Banerjee, R., 2017. Sustainable green solvents and techniques for lipid extraction from microalgae: A review. Algal Res. 21, 138–147. https://doi.org/10.1016/j.algal.2016.11.014.

Kuek, Lee, Ahmad Tarmizi, S., Abd Razak, A.H., Jinap, R.A., Norliza, S., Sanny, M, S., 2020. Contribution of lipid towards acrylamide formation during intermittent frying of French fries. Food Control 118, 107430. https://doi.org/10.1016/j.foodcont.2020.107430.

Lee, S.Y., Cho, J.M., Chang, Y.K., Oh, Y.-K.K., 2017. Cell disruption and lipid extraction for microalgal biorefineries: A review. Bioresour. Technol. 244, 1317–1328. https://doi.org/10.1016/j.biortech.2017.06.038.

Lo, C., Semerel, J., van den Berg, C., Wijffels, R.H., Eppink, M.H.M., 2021. Eutectic solvents with tuneable hydrophobicity: lipid dissolution and recovery. RSC Adv. 11, 8142–8149. https://doi.org/10.1039/D1RA00306B.

Lo, C., Wijffels, R.H., Eppink, M.H.M., 2023. Lipid extraction from fresh *Nannochloropsis oceanica* using semi-hydrophobic eutectic solvents. Algal Res 72, 103117. https://doi.org/10.1016/J.ALGAL.2023.103117.

Lu, W., Alam, M.A., Pan, Y., Wu, J., Wang, Z., Yuan, Z., 2016. A new approach of microalgal biomass pretreatment using deep eutectic solvents for enhanced lipid recovery for biodiesel production. Bioresour. Technol. 218, 123–128. https://doi.org/10.1016/j.biortech.2016.05.120.

Maugeri, Z., Domínguez De María, P., 2012. Novel choline-chloride-based deep-eutectic-solvents with renewable hydrogen bond donors: levulinic acid and sugar-based polyols. RSC Adv. 2, 421–425. https://doi.org/10.1039/c1ra00630d.

Osawa, C.C., Gonçalves, L.A.G., Ragazzi, S., 2007. Correlation between free fatty acids of vegetable oils evaluated by rapid tests and by the official method. J. Food Compos. Anal. 20, 523–528. https://doi.org/10.1016/j.jfca.2007.02.002.

Pan, Y., Alam, A., Wang, Z., Huang, D., Hu, K., Chen, H., Yuan, Z., 2017. One-step production of biodiesel from wet and unbroken microalgae biomass using deep eutectic solvent. Bioresour. Technol. 238, 157–163. https://doi.org/10.1016/j.biortech.2017.04.038.

Pitacco, W., Samorì, C., Pezzolesi, L., Gori, V., Grillo, A., Tiecco, M., Vagnoni, M., Galletti, P., 2022. Extraction of astaxanthin from *Haematococcus pluvialis* with hydrophobic deep eutectic solvents based on oleic acid. Food Chem. 379, 132156 https://doi.org/10.1016/J.FOODCHEM.2022.132156.

Procentese, A., Johnson, E., Orr, V., Campanile, A.G., Wood, J.A., Marzocchella, A., Rehmann, L., 2015. Deep eutectic solvent pretreatment and subsequent saccharification of corncob. Bioresour. Technol. 192, 31–36. https://doi.org/10.1016/j.biortech.2015.05.053.

Rodrigues, L.A., Pereira, C.V., Leonardo, I.C., Fernández, N., Gaspar, F.B., Silva, J.M., Reis, R.L., Duarte, A.R.C., Paiva, A., Matias, A.A., 2020. Terpene-based natural deep eutectic systems as efficient solvents to recover astaxanthin from brown crab shell residues. ACS Sustain. Chem. Eng. 8, 2246–2259. https://doi.org/10.1021/ACSSUSCHEMENG.9B06283/SUPPL_FILE/SC9B06283_SI_001.PDF.

Ruesgas-Ramón, M., Figueroa-Espinoza, M.C., Durand, E., 2017. Application of Deep Eutectic Solvents (DES) for phenolic compounds extraction: overview, challenges, and opportunities. J. Agric. Food Chem. 65, 3591–3601. https://doi.org/10.1021/acs.jafc.7b01054.

Smink, D., Kersten, S.R.A., Schuur, B., 2020. Recovery of lignin from deep eutectic solvents by liquid-liquid extraction. Sep. Purif. Technol. 235, 116127 https://doi.org/10.1016/j.seppur.2019.116127.

Tang, W., Row, K.H., 2020. Design and evaluation of polarity controlled and recyclable deep eutectic solvent based biphasic system for the polarity driven extraction and separation of compounds. J. Clean. Prod. 268, 122306 https://doi.org/10.1016/J.JCLEPRO.2020.122306.

Tommasi, E., Cravotto, G., Galletti, P., Grillo, G., Mazzotti, M., Sacchetti, G., Samorì, C., Tabasso, S., Tacchini, M., Tagliavini, E., 2017. Enhanced and selective lipid extraction from the microalga p. tricornutum by dimethyl carbonate and supercritical CO 2 atment. ACS Sustain. Chem. Eng. 5, 8316–8322. https://doi.org/10.1021/acssuschemeng.7b02074.

Zahler, P., Niggli, V., 1977. The Use of Organic Solvents in Membrane Research, in: Korn, E.D. (Ed.), Methods in Membrane Biology. Springer, Boston, MA, pp. 1–50. https://doi.org/10.1007/978–1-4684–2910-7_1.

Zhang, T., Shahbaz, K., Farid, M.M., 2020. Glycerolysis of free fatty acid in vegetable oil deodorizer distillate catalyzed by phosphonium-based deep eutectic solvent. Renew. Energy 160, 363–373. https://doi.org/10.1016/j.renene.2020.07.026.