Journal of Cleaner Production 536 (2025) 147186

Contents lists available at ScienceDirect

Journal of Cleaner Production

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

Separation of alginate and recycling of deep eutectic solvents using temperature-responsive aqueous two-phase systems

Isa S.A. Hiemstra [a], Niels Lustig [a], Michel H.M. Eppink [b], René H. Wijffels [a,c], Antoinette Kazbar [a,*]

[a] Bioprocess Engineering, Wageningen University & Research, PO Box 16, Wageningen, Wageningen, 6700 AA, the Netherlands
[b] Bioprocess Engineering, Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, HZ Delft, 2629, the Netherlands
[c] Nord University, Faculty of Biosciences and Aquaculture, Bodo, N8049, Norway

ARTICLE INFO

Keywords:
Deep eutectic solvents
Recovery
Recyclable process
Aqueous two-phase system
Alginate

ABSTRACT

Deep eutectic solvents (DES) have emerged as green alternative extraction solvents. However, challenges in DES recovery and recycling limit their broader application. In this study, a novel thermo-separating aqueous two-phase system (ATPS) was developed for the continuous, cyclic extraction and separation of alginate from *Laminaria digitata* using DES and temperature-responsive copolymers. The system enables a novel approach by repeatedly reusing both the DES phase and the EOPO copolymer phase five times, thereby reducing waste generation and enhancing process sustainability. This research demonstrated that DES can be efficiently recycled for ten cycles using temperature-responsive ethylene oxide-propylene oxide (EOPO) copolymers, remaining stable yields. For the extraction and subsequent separation of alginate, three different DESs were evaluated, all of which demonstrated to extract and recover alginate. DESs ChCl:Ethylene glycol and ChCl:Urea exhibited a preference for EOPO1000 (66 and 75 % recovery, respectively), whereas Bet:Urea achieved the highest recovery with EOPO3900 (66 % recovery). Subsequent recycling of the recovered DES showed that DES could be recycled for ten cycles, maintaining stable extraction yields between 74 and 86 mg alginate/g DW and alginate recovery yields of 55–65 %. Furthermore, combined DES and EOPO recycling could be performed for up to five cycles while maintaining an alginate recovery yield between 50 and 65 mg/g DW. This thermo-separating ATPS presents a novel, circular and sustainable approach for DES recycling compared to the non-circular conventional alkaline extraction. This proposed method can be applied in a simple and effective manner to both recover and recycle DES.

1. Introduction

Many industrial processing and extraction methods currently depend on conventional organic solvents, such as methanol, ethanol and sulphuric acid, which pose environmental, health and safety risks and are often not recycled, generating chemical waste (Chemat et al., 2019; Patrice Didion et al., 2023). To address these issues, alternative and more sustainable processes are being investigated (Yang et al., 2025; Yi et al., 2019; L. Zhang et al., 2023), with particular focus on green solvents. One promising class of solvents are deep eutectic solvents (DESs). These solvents offer several advantages over traditional solvents, including lower toxicity, biodegradability, and the potential for recycling (Halder and Cordeiro, 2019; Khandelwal et al., 2016). DES are solvents composed of one or more hydrogen bond donors (HBD) and one or more hydrogen bond acceptors (HBA), formed upon a large depression in melting point when mixed at a certain ratio (Hansen et al., 2021; Ma et al., 2022).

One potential application of DES is the extraction of valuable marine constituents. For instance, alginate, a polysaccharide present in the cell wall of brown seaweed (Lee and Mooney, 2012), is widely employed as a thickening agent in food processing and in diverse biomedical applications (Abdul Khalil et al., 2018). Brown seaweeds contain approximately 20–35 % dry weight of the phycocolloid alginate (Belattmania et al., 2020). Alginate is composed of two uronic acids: β-D-mannuronic acid and α-L-guluronic acid (Fig. S1), with their composition and arrangement varying depending on the seaweed species, habitat, and seasonal

* Corresponding author.
E-mail address: antoinette.kazbar@wur.nl (A. Kazbar).

https://doi.org/10.1016/j.jclepro.2025.147186
Received 30 May 2025; Received in revised form 20 November 2025; Accepted 24 November 2025
Available online 30 November 2025

conditions (Mahmood Zia et al., 2017). Conventional processes for alginate extraction from seaweed rely on large amounts of harsh solvents and often yield limited efficiency (Dobrinčić et al., 2020; Saji et al., 2022). Hence, there is a strong need to develop novel and sustainable processes, such as the use of DES for alginate extraction and recovery. Recent studies have demonstrated that DES are effective for extracting polysaccharides from a variety of biomass sources, including seaweed (Hiemstra et al., 2024; Shang et al., 2021), molluscs (Qu et al., 2023), plants (Wu et al., 2021; L. Zhang and Wang, 2017) and fungi (W. Zhang et al., 2020). However, DESs are characterised by their low vapour pressure, which complicates both solvent recovery and product isolation. As a result, DES removal is typically performed through ethanol (Nie et al., 2020) or isopropanol (Das et al., 2016) precipitation, followed by multiple washing steps and vacuum evaporation. Wu et al. (2021) and Wang et al. (2022) employed ethanol precipitation combined with dialysis. Shang et al. (2021) used a brominated macroporous resin to load the DES phase, followed by washing with water. Although polysaccharides were successfully recovered, the DES was simultaneously washed out and therefore could not be reused.

To enhance process sustainability in industrial applications, DES recycling is essential. W. Zhang et al. (2020) recovered both polysaccharides and DES by repeatedly adding the DES to anhydrous ethanol for precipitation. The DES–ethanol precipitate was distilled via rotary evaporation, allowing the DES to be reused for up to five extraction cycles. While anti-solvent addition is a straightforward and selective approach, it poses challenges in process control due to potential loss of DES components. In addition, anti-solvent removal often requires long evaporation times (16–24 h) (Lo et al., 2024), and some DESs may themselves precipitate upon alcohol addition, further limiting their recyclability (Yu et al., 2024). Membrane-based separation processes, especially electrodialysis combined with ultrafiltration (Liang et al., 2019, 2023), have been used for DES recovery and product separation (Elizondo Sada et al., 2024). Liang et al. (2023) demonstrated the recycling of DES up to four times using ultrafiltration coupled with bipolar membrane electrodialysis. Although these techniques are easy to operate and cost-effective, they impose strict requirements on membrane performance due to the high viscosity of DES solutions, which increases the risk of membrane fouling (Moslehyani et al., 2018). Moreover, the recovery of concentrated extract is limited by concentration constraints inherent to the process (Yu et al., 2024).

In this context, aqueous two-phase systems (ATPS) present a promising and environmentally friendly alternative for recovering polysaccharides from DES and the potential recycling of the DES itself. ATPS is a liquid–liquid fractionation method that relies on the immiscibility of two aqueous solutions at specific concentrations (X. Zhang et al., 2025) and is widely used for gentle, efficient, and straightforward separation of biomolecules and cells (Xue et al., 2024). Several ATPS types have been explored, including polymer-polymer, polymer–salt, alcohol–salt, and thermo-separating polymer–salt systems (Iqbal et al., 2016; Pereira and Coutinho, 2019). Among these, thermo-separating ATPSs are particularly attractive due to their ability to induce phase separation and product recovery simply by altering the temperature (Leong et al., 2015; Musarurwa and Tavengwa, 2022). These systems have been applied in the purification of several biomolecules, including anthraquinones (Yao et al., 2025), saccharides and proteins (Lai et al., 2025) and vitamin B12 (Wan et al., 2018), lincomycin (Wang et al., 2021) and tea saponins (Wei et al., 2021). Thermo-separation is achieved by increasing the temperature to a point at which the copolymer becomes insoluble, resulting in phase separation. Ethylene oxide-propylene oxide (EOPO) is a thermo-sensitive copolymer that can induce phase separation upon temperature changes (Marimuthu et al., 2025). EOPO has been used for EOPO-salt ATPS separation methods to separate products based on temperature change, including peptides (Jiang et al., 2017), hyaluronic acid (Marimuthu et al., 2025), polysaccharides (Wang et al., 2016) and polyphenols (H. S. Ng et al., 2017). Limited studies have explored the use of thermo-separating aqueous two-phase systems (ATPS) for product recovery from DES. Gao et al. (2020) demonstrated that polysaccharides extracted from *Camellia oleifera* using DES could be recovered using a temperature-responsive ATPS, achieving recovery yields of up to 86 %. Similarly, Zhou et al. (2022) showed that polysaccharides from American ginseng could be extracted with DES and subsequently recovered through a thermo-separating ATPS. However, in both studies, while the polysaccharides were successfully recovered, the DES was not reused in subsequent extractions. Recovering both the bioactive compounds and the DES is essential for enhancing the sustainability of DES-based extraction processes by minimising solvent waste.

In the present study, we aimed to develop a novel and circular thermo-separating ATPS for the simultaneous recovery of functional alginate and recycling of both DES and EOPO after extraction from the brown seaweed *Laminaria digitata*. Various DESs and ethylene oxide–-propylene oxide (EOPO) copolymers were evaluated to determine the most effective combination for alginate recovery. A recycling study was conducted to assess the extraction efficiency and recovery yield of the reused DES. In addition, the feasibility of the combined recycling of EOPO and DES was investigated, allowing repeated reuse of both phases, thereby minimising waste generation. Overall, this study introduces an innovative and sustainable method for alginate recovery from DES while enabling effective solvent recycling, without significantly compromising extraction yields. These findings lay the groundwork for more environmentally friendly DES-based extraction processes, where both product and solvent can be efficiently recovered and DES can be reused.

2. Materials and methods

2.1. Materials

Brown seaweed *Laminaria digitata* was used for the extraction of alginate. Sun-dried *L. digitata* was obtained from Algaia in March 2023 (Fig. S2). Before use, the biomass was shredded, its dry weight was determined, and it was sieved using 710 μm and 90 μm mesh sizes. The fraction between 90 and 710 μm was collected and stored at 4 °C for extraction. For alginate extraction and isolation, three DESs were used, containing 15 wt% of water (Table 1, Table S1). As HBA, either choline chloride or betaine was used and as HBD, ethylene glycol or urea was used.

The DES constituents choline chloride (≥98 %), betaine (≥98 %), ethylene glycol (≥99 %), and urea (≥99.5 %) were purchased from Sigma-Aldrich (Merck). EOPO copolymers with Pluronic® 10 wt% PEG block-copolymers (PEG-PPG-PEG) with average molecular weights of 1000, 2500 and 3900 were purchased from Sigma-Aldrich (Merck).

2.2. DES preparation and characterisation

The DESs were prepared using the heating method (Abbott et al., 2004). The HBA and HBD were mixed in a molar ratio of 1:2 (Table 1) with 15 wt% of water, and this was put in a water bath at 80 °C for 1 h until a transparent solution was obtained. Subsequently, the formed DES was used for the extraction of alginate. The dynamic viscosity of each DES and EOPO was measured over a temperature range from 20 °C to

Table 1
Composition of DESs used in this study.

	HBA	HBD	Mole ratio	Abbreviation	Viscosity [a] (mPa*s)	Density [a] (g/cm^3)
DES 1	Choline chloride	Ethylene Glycol	1:2	ChCl:EG	17.11	1.105
DES 2	Choline chloride	Urea	1:2	ChCl:U	7.36	1.142
DES 3	Betaine	Urea	1:2	Bet:U	91.48	1.195

[a] Measured at 20 °C.

60 °C with 10 °C intervals using an Anton Paar rotational viscometer (Anton Paar ViscoQC 300 Rotational Viscometer) with measuring probe B-SC4-18. The density of the DES and EOPO was measured using an Anton Paar density and sound velocity meter (DSA 5000M) at 20 °C.

2.3. Alginate extraction and quantification

Three DESs were used to extract alginate from *L. digitata* (Table 1). A solid:liquid ratio of 1:40 was applied (Das et al., 2016). The extractions were performed at 80 °C for 1 h. During the extractions, the samples were regularly vortexed. As a control, alkaline extraction was performed. Acid water containing H_2SO_4 (pH 1.9) was added in a 1:1 (w/w) ratio to the seaweed, and this was set for 1 h at room temperature. Subsequently, Na_2SO_4 was added in a ratio of 0.0325:1 (w/w), and the extraction was performed for 1 h. DES and alkaline extractions were performed in triplicate. After extraction, the samples were centrifuged, and the supernatant was taken for alginate quantification. Quantification was performed using a method adapted from Cesaretti et al. (2003). Subsequently, the DES layer was separated from the supernatant and used for the first ATPS. The alginate extraction yield from *L. digitata* represents the quantity of alginate obtained using DES per gram of dry seaweed, and was determined using Equation (1):

$$\text{Extraction yield}\left(\frac{mg}{g\,DW}\right) = \frac{C_{extract} * V_{total}}{m_{biomass} * x_{DW}} \quad \text{(Eq. 1)}$$

where $C_{extract}$ is the concentration of the extract, $m_{biomass}$ is the weight of the initial biomass, x_{DW} is the weight fraction of the dry biomass and V_{total} is the total volume of the extract.

2.4. Alginate isolation

2.4.1. Aqueous two-phase separation between EOPO and DES

To all DESs, EOPO was added to obtain an EOPO wt% of either 30 %, 40 %, 50 %, 60 % or 70 % (Table 2). For each DES, EOPO copolymers with molecular weights of 1000, 2500, and 3900 were added to facilitate aqueous two-phase separation (Table 2). After EOPO addition, the solution was mixed thoroughly for 15 min and left for at least 1 h until phase separation was observed. The EOPO-containing layer was subsequently transferred to the second ATPS to recover alginate from the EOPO phase (Fig. 1).

2.4.2. Temperature-dependent aqueous two-phase separation

For the removal of alginate from the EOPO layer, a temperature-dependent aqueous two-phase separation was used (Fig. 1). To the isolated EOPO fraction, water was added in a 1:1 mass ratio. The solution was vortexed to mix the two layers. Once the EOPO was dissolved, the solution was heated at 60 °C for at least 2 h until phase separation occurred. From the observed phase separation, the position of the aqueous layer was determined. The alginate concentration was determined from the aqueous layer. The percentage of alginate recovery (%) was determined by comparing the initial alginate extracted to the alginate recovery yield obtained in the aqueous layer after the second ATPS. The alginate recovery yield represents the quantity of alginate obtained in the aqueous phase after the ATPS separations, and was calculated using Equation (2):

Table 2
Different variables tested per DES in the first ATPS.

	EOPO1000	EOPO2500	EOPO3900
EOPO (wt %)	30	30	30
	40	40	40
	50	50	50
	60	60	60
	70	70	70

$$\text{Recovery yield}\left(\frac{mg}{g\,DW}\right) = \frac{C_{aqueous} * V_{aqueous}}{m_{biomass} * x_{DW}} \quad \text{(Eq. 2)}$$

where $C_{aqueous}$ is the concentration of alginate in the aqueous phase, $m_{biomass}$ is the weight of the initial biomass, x_{DW} is the weight fraction of the dry biomass and $V_{aqueous}$ is the volume of the aqueous phase.

2.5. Alginate characterisation

The alginate fraction was characterised using Fourier Transform Infrared Spectroscopy (FT-IR) and compared to alginate extracted through alkaline treatment. Characterisation was conducted with a Nicolet Summit X ATR-FTIR Spectrometer (ThermoFisher Scientific) over a spectral range of 400–4000 cm^{-1}. The alginate extracts were isolated through a precipitation step using 1 % w/v calcium chloride dihydrate, followed by two successive washes with 80 % ethanol. The resulting precipitate was dried under a nitrogen flow and characterised using Fourier-transform infrared (FT-IR) spectroscopy.

The mannuronic to guluronic (M/G) ratio of the extracted alginate was determined through methanolysis followed by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described by Bojorges et al. (2023). The Dionex ICS-6000 system (ThermoFisher) with CarboPac 1 mm column was used. Alginate extracted using DES was compared with alginate obtained via conventional alkaline treatment. The extracted alginate was precipitated with 80 % ethanol containing 1 % NaCl, followed by washing twice with 80 % ethanol. Subsequently, the samples were resolubilised in Milli-Q water. Samples of 50 μL were used for the methanolysis.

2.6. DES recycling

For DES recycling, the optimal DES and EOPO conditions for alginate recovery were selected. The alginate extraction and subsequent alginate and DES separation were repeated up to ten times. In each cycle, both extraction and recovery yields were measured to evaluate the impact of DES reuse. After each extraction step, the alginate content was determined. After each cycle, the alginate recovery was assessed.

2.7. Combined DES and EOPO recycling

To assess the influence of recycling of both fractions, combined DES and EOPO recycling was performed. The same conditions were applied as for DES recycling (Section 2.6). Both fractions were recycled up to six times. Each cycle, the alginate extraction yield and recovery were assessed.

An overview of the extraction, two-step aqueous two-phase separation and solvent recycling is presented in Fig. 1. First, alginate is extracted using DES. In the initial aqueous two-phase system (ATPS1), EOPO is introduced, enabling alginate to partition into the EOPO-rich phase. After phase separation, the EOPO and DES phases are isolated, and the DES is recycled for subsequent extractions. The EOPO phase is then subjected to the second ATPS (ATPS2), where water is added and the temperature is increased to induce phase separation between EOPO and water. This facilitates the transfer of alginate into the aqueous phase, which is collected, while EOPO was recovered for reuse in the next ATPS1 cycle.

3. Results and discussion

3.1. Extraction and isolation of alginate

3.1.1. Extraction of alginate

Alginate was successfully extracted using each DESs (Fig. 2). Among them, ChCl:EG yielded the highest alginate content with an extraction yield of 100.3 ± 8.0 mg/g DW. ChCl:U resulted in the lowest yield,

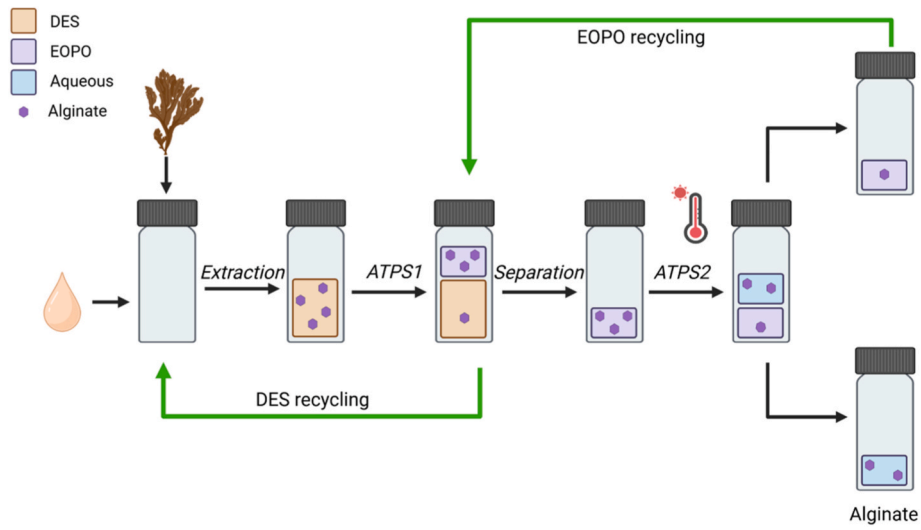

Fig. 1. Schematic overview of the alginate extraction and separation procedure. Alginate is first extracted using DES, after which EOPO is added in ATPS1 to transfer alginate into the EOPO-rich phase while the DES phase is recovered and recycled. In ATPS2, water addition and heating induce the separation of EOPO and water, enabling alginate to move into the aqueous phase. The aqueous phase is collected, and EOPO is reused in the next ATPS1 cycle.

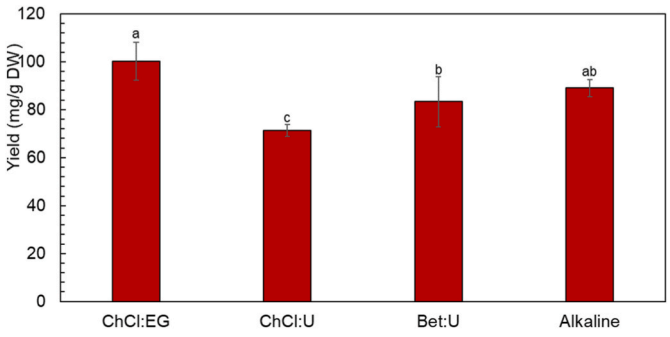

Fig. 2. Extraction of alginate from *L. digitata* with three DESs and alkaline extraction as control.

extracting 71.4 ± 2.4 mg/g DW, while Bet:U achieved an intermediate yield of 83.3 ± 10.5 mg/g DW. The conventional alkaline control method yielded 89.0 ± 3.6 mg/g DW, which was comparable to ChCl:EG and Bet:U. Although the extraction yields obtained via the alkaline method were similar to those of ChCl:EG and Bet:U, and higher than those of ChCl:U, this approach poses environmental concerns due to the harsh alkaline conditions and the use of sulphuric acid. Conventional alginate extraction relies on acidification with concentrated acids, including sulphuric acid, to remove divalent cations, thereby converting alginate salts into alginic acid. Subsequently, alkaline hydrolysis is applied for alginate extraction. This two-step process generates effluents that require neutralisation and waste management (Saji et al., 2022). Additionally, solvent regeneration is not feasible in the conventional method, further limiting its sustainability. The three DESs were subsequently utilised for alginate separation through the two-step ATPS separation.

3.1.2. Isolation of alginate

To assess the performance of the different DESs per EOPO, evaluating the recoveries of alginate per DES is essential (Table 2). A high extraction rate with a low recovery could still be less efficient. By combining alginate yield and recovery, the overall performance of the isolation method could be determined. For the first ATPS, three EOPOs were used in different ratios for each DES (Section 2.4.1). The phase separation of all combinations of DESs with EOPOs (Fig. S3) showed that the type of EOPO affects its behaviour towards the DESs. For all DESs, EOPO1000 and EOPO3900 appeared as the top layer in the system. For EOPO2500, no clear phase separation occurred for DES ChCl:EG and Bet:U (Fig. S3). Gao et al. (2020) used DES ChCl:EG, containing 30 wt% water, for the extraction and isolation of polysaccharides from *Camellia oleifera* Abel. The highest recovery was observed when using EOPO2500 with ChCl:EG (30 wt% water). The difference in water content between this DES and the DES used in our study probably affected phase separation differently, as increased water content reduces viscosity and density, thereby altering the physicochemical properties of the DES (R. Zhang et al., 2024). These changes can significantly impact the performance of the ATPS. In the case of EOPO2500, the intermediate molecular weight may result in an intermediate hydrophilic–hydrophobic character, providing insufficient polymer–solvent incompatibility to promote complete phase separation. Additionally, when combined with ChCl:EG and Bet:U containing 15 wt% water, EOPO2500 may lie closer to the binodal curve of the ATPS, making phase separation highly sensitive to DES composition and water content. This behaviour could explain the poor separation observed. Due to insufficient phase separation, EOPO2500 was excluded from further experiments.

In the consecutive thermo-separating ATPS step, the alginate recovery was assessed. For phase separation with EOPO1000, the EOPO was situated at the top layer because the density of this copolymer is 0.988 g/cm^3 (Table S2). In contrast, for EOPO3900, the EOPO was placed at the bottom layer since this copolymer has a density greater than water, measuring 1.056 g/cm^3 (Figs. S4 and S5 and Table S2).

DES ChCl:EG and ChCl:U displayed a preference for EOPO1000, while Bet:U recovered more alginate with EOPO3900 (Fig. 3). For ChCl:EG, the highest recovery reached was 66.0 ± 6.2 %, at 40 wt% of EOPO1000 (Fig. 3a). When taking into account the extraction yield, 66.2 mg/g alginate was recovered (Table 3). The recovery using EOPO1000 was approximately twice that observed with EOPO3900 for ChCl:EG (Fig. 3a). This difference could be due to the larger viscosity of EOPO3900 at 20 °C (Fig. S6), which may hinder efficient mass transfer and phase separation compared to EOPO1000. When applying DES ChCl:U, the highest alginate recovery was reached (75.4 %) at 50 wt% of EOPO1000 (Fig. 3b). This resulted in an alginate recovery yield of 53.8 mg/g DW (Table 3). ChCl:U resulted in the highest percentage of alginate recovery, which was the DES with the lowest viscosity (Fig. S7). The lower viscosity of ChCl:U compared to the other DES (Fig. S7) may have facilitated more efficient mass transfer into the copolymer phase (Liu et al., 2023; Yeow et al., 2024). For this DES, a clear influence of the EOPO concentration on alginate recovery was observed. The highest

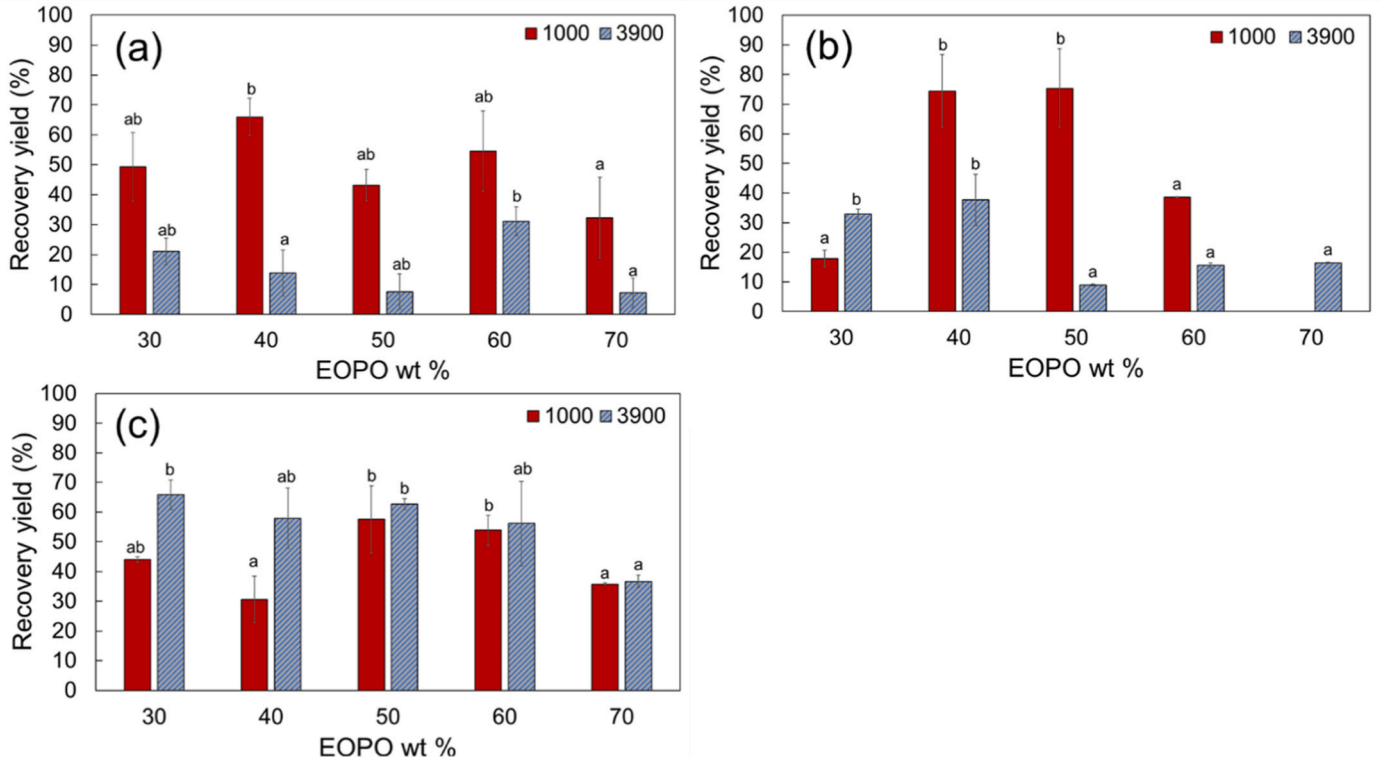

Fig. 3. Recovery yields of alginate for (a) ChCl:EG, (b) ChCl:U and (c) Bet:U using EOPO1000 and EOPO3900.

Table 3
Alginate extraction yield, optimal recovery (%) and alginate recovery yield per DES.

	ChCl:EG	ChCl:U	Bet:U
Extraction yield (mg/g DW)	100.3 ± 8.0	71.4 ± 2.5	83.3 ± 10.5
Alginate recovery (%)	66.0 ± 6.2	75.4 ± 12.3	66.0 ± 5.0
Alginate recovery yield (mg/g DW)	66.2 ± 8.2	53.8 ± 9.0	54.8 ± 8.1

recovery yields were achieved at an EOPO1000 to DES ratio of approximately 1:1. Reducing the ratio to 30 wt% or increasing it to 70 wt% caused the recovery to drop to 17 % or, in the latter case, resulted in no detectable alginate recovery. Increasing the EOPO concentration up to 50 wt% likely enhances the contact area between alginate and EOPO (Fig. 3b), promoting the preferential partitioning of alginate into the EOPO-rich phase. However, further increasing the EOPO concentration to 60 or 70 wt% led to a decrease in alginate recovery. This decline may be attributed to the volume exclusion effect that becomes significant at high EOPO concentrations. The entropic effect is predominant at high EOPO concentrations, thereby hindering solute partitioning (Kepka et al., 2004; Leong et al., 2017). At elevated polymer contents, the EOPO-rich phase becomes increasingly crowded, reducing the available free volume for alginate. This crowding increases the entropic penalty associated with alginate entering the polymer-rich phase. As a result, alginate is thermodynamically disfavoured from partitioning into the EOPO phase, leading to reduced recovery. Such behaviour is well-described in polymer–salt and polymer–polymer ATPS (Iqbal et al., 2016), where high polymer concentrations limit solute accessibility due to steric constraints and reduced solvent activity.

Additionally, higher EOPO content increases the system's viscosity, which can reduce mass transfer efficiency and impair recovery yields (Kuznetsova et al., 2015).

For ChCl:EG and ChCl:U, the average molecular weight of the EOPO copolymer was found to significantly influence the alginate recovery yield. EOPO1000, which has a lower molecular weight than EOPO3900, possesses shorter polymer chain lengths. These shorter chains may provide more free volume within the solution (H. S. Ng et al., 2017), thereby enhancing the accommodation and solubility of alginate in the EOPO phase. As a result, up to eight times more alginate was recovered using EOPO1000 compared to EOPO3900 for ChCl:U at 50 wt%. Bet:U had the highest recovery (66 %) at 30 wt% of EOPO3900 (Fig. 3c), indicating that an alginate recovery yield of 54.8 mg/g DW was obtained (Table 3). In contrast to the behaviour observed for ChCl:EG and ChCl:U systems, both EOPO copolymers exhibited comparable recovery efficiencies when Bet:U was used. This could be due to the increased viscosity of this DES at 20 °C compared to ChCl:EG and ChCl:U (Fig. S7). The hydrophobicity of the polymer increases when the molecular weight increases, due to the increase in hydrophobic area compared to hydrophilic groups (H. S. Ng et al., 2012). Therefore, an EOPO with a higher molecular weight requires lower concentrations to form an ATPS, due to the increased hydrophobicity (Leong et al., 2015; Leong et al., 2017). This will, in turn, lead to enhanced phase separation. This trend correlates with the results obtained from Bet:U, where the highest recovery of alginate was obtained using the lowest weight percentage of EOPO3900. For EOPO1000 with the same DES, the highest recovery was obtained at 50 wt%.

No literature is available on the effect of the molecular weight of the EOPO on alginate separation. However, for the protein bromelain, previous studies have shown that increasing the molecular weight of EOPO leads to a higher partitioning coefficient (Rabelo et al., 2004). Similarly, lower separation of the enzyme guanylyltransferase was observed when EOPO with a lower molecular weight was used (H. S. Ng et al., 2012). For polyphenol extraction, a reversed trend was observed. A shorter chain length of EOPO resulted in a lower polyphenol yield (H. S. Ng et al., 2017). For alginate, notable differences in recovery were observed between EOPO1000 and EOPO3900 across the three DES systems. This suggests that the hydrogen bond acceptor (HBA) in the DES may influence alginate isolation. Specifically, Bet:U yielded higher alginate recoveries with EOPO3900, whereas ChCl:EG and ChCl:U showed greater recoveries when EOPO1000 was used. This indicates

that the selection of the DES constituents and thus the DES extract composition largely influences the recovery and the optimal choice of EOPO.

Interestingly, the DES with the highest extraction yield (ChCl:EG) gave lower alginate recovery, while the lowest-yielding DES (ChCl:U) achieved the highest recovery (Table 3). This indicates that recovery efficiency depends not solely on extraction yield but on alginate partitioning between the DES and copolymer phases. An optimal equilibrium between these phases appears to enhance recovery. Despite differences in efficiency, final alginate yields were similar (55–66 mg/g DW), highlighting the importance of this phase equilibrium in governing overall recovery performance.

Since the final alginate yields were similar (Table 3), Bet:U was selected for characterisation and subsequent DES recycling as the lowest amount of EOPO could be applied compared to ChCl:EG and ChCl:U (30 wt% for Bet:U compared to 40 and 50 wt% for ChCl:EG and ChCl:U, respectively).

3.2. Alginate characterisation

The alginate recovered from the DES Bet:U was characterised using FTIR and analysed for its mannuronic to guluronic acid (M/G) ratio. The M/G ratio is a critical parameter in alginate characterisation, as it influences the polysaccharide's physical and functional properties (Bojorges et al., 2023). Alginates with a higher M/G ratio typically form more permeable and flexible gel matrices, whereas those with a lower M/G ratio tend to produce mechanically stronger gels. This is due to the higher affinity of guluronic acid (G) blocks for calcium ions compared to mannuronic acid (M) blocks, resulting in stronger cross-linking and firmer gel structures (Ramos et al., 2018). The M/G ratio for alginate extracted with Bet:U was 1.96 ± 0.02. Compared to alkaline extraction, this ratio was 1.49 ± 0.02 (Table S3). These values are comparable to the reported values (1.44–1.82) for *L. digitata* (Saji et al., 2022; Sharma et al., 2023), indicating that the alginate is flexible and can form soft and elastic gels.

The FTIR spectra of alginate extracted through DES and alkaline treatment displayed similar patterns (Fig. 4). Both extracts showed characteristic peaks at 1035 cm^{-1} and 1080 cm^{-1}, as well as at 1600 cm^{-1} and 1400 cm^{-1}. The distinct peaks at approximately 1080 cm^{-1} and 1035 cm^{-1} are associated with mannuronic and guluronic acid units, respectively (Leal et al., 2008; Nesic et al., 2023). The bands at 1600 cm^{-1} and 1400 cm^{-1} are associated with asymmetric and symmetric stretching modes of carboxylate groups (Kuzmanović et al., 2017; Nesic et al., 2023; Rashedy et al., 2021). Additionally, a broad band at 3500 cm^{-1} and a weak band at 2900 cm^{-1} were observed, correlating to hydrogen-bonded O-H and C-H stretching vibrations, respectively (Kuzmanović et al., 2017). The appearance of these characteristic absorption bands demonstrates that the DES-EOPO system effectively extracts alginate and produces a spectral signature consistent with that of conventionally extracted alginate.

Based on FTIR and MG data, it was observed that alginate extracted and recovered with Bet:U in a temperature-responsive ATPS is not affected differently compared to the current alkaline extraction. Similar functional groups and similar M/G ratios were defined. Therefore, DES-based alginate extraction and subsequent recovery with a temperature-responsive EOPO could be a suitable approach that maintains alginate characteristics.

3.3. Recyclability of recovered DES

Previous studies have reported DES recovery using temperature-responsive copolymers (Gao et al., 2020; Zhou et al., 2022). However, whether the recovered DES can be effectively reused as an extraction solvent has not yet been investigated. Therefore, Bet:U was utilised for extraction, recovered, and subsequently recycled for additional alginate extraction cycles. The optimal recovery conditions at 30 wt% EOPO3900 (Section 3.2.1) were used. Thus, it was investigated whether the alginate extractability or recovery capability would be impacted by reusing the DES within this system. For DES recycling, the process of alginate extraction followed by its separation from DES was repeated over ten consecutive cycles. After the first ATPS, the DES was reused for the next extraction cycle (Section 2.6). After ten extraction cycles with Bet:U, the alginate extraction yields remained stable between 74 and 86 mg alginate/g DW (Fig. 5). After cycle seven, a slight decrease in extraction yield was observed. The accumulation of other constituents in the DES (M. H. Ng et al., 2024; Qiu and Aita, 2013), present in low amounts, such as proteins, could have influenced the alginate yield. These additional solutes may compete with alginate for dissolution, alter the hydrogen-bonding environment, or increase the viscosity of the DES, thereby reducing the solvent's effective capacity to extract alginate. The results indicate that the extraction yield is not majorly influenced when reusing the DES up to ten times. The recovery of alginate when reusing the DES was found to remain stable between 55 and 65 % (56.0 ± 0.9 and 41.2 ± 5 mg/g recovery yield) (Fig. 5).

To the best of our knowledge, no studies have been performed yet on the recycling of DES after product recovery through EOPO addition. However, several studies have reported the recycling of DES through different approaches. For recycling DES that was used for pre-treating plant biomass, a highly used approach is anti-solvent addition (Chen et al., 2018; Ling et al., 2020). For this approach, DES could be recycled

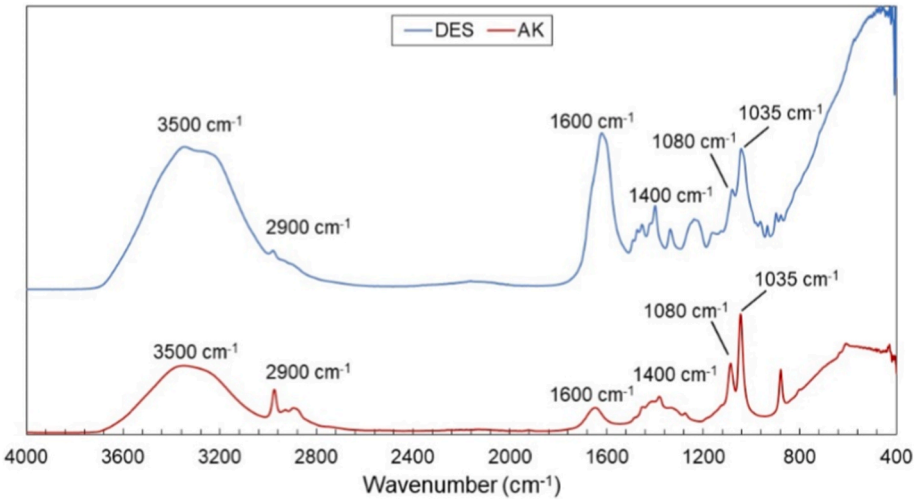

Fig. 4. FTIR spectra of alginate extracted with DES (Bet:U) and with the conventional alkaline (AK) extraction.

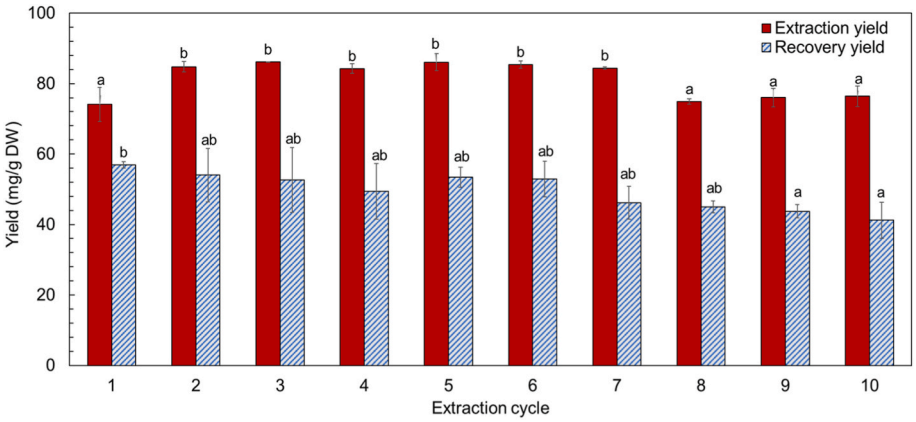

Fig. 5. Alginate extraction and recovery yield during DES recycling.

between 2 and 4 times. Jeong et al. (2015) recycled a DES composed of glycerol, L-proline, and sucrose (9:4:1) three times after performing extractions of ginsenoside from Korean ginseng. Recycling involved diluting the DES threefold with water, loading onto hydrophilic-lipophilic balance cartridges, followed by rinsing and vacuum drying. Xie et al. (2021) employed DES for the pretreatment of lignocellulosic biomass and recovered it by removing the water from the DES through rotary evaporation. This approach enabled the DES to be reused for up to five pretreatment cycles. For the extraction of ferulic acid from oil palm empty fruit bunch fibre, M. H. Ng et al. (2024) applied antisolvent addition to precipitate ferulic acid, followed by filtration. The DES was subsequently recycled successfully up to five times.

Compared to the other recycling strategies, the proposed ATPS-based approach enables a greater number of recycling cycles within the integrated extraction–separation system. Since both alginate yield and recovery remain stable, this indicates that DES can be recycled within a temperature-responsive ATPS for continuous extraction of alginate from brown seaweed.

To further improve the circularity and sustainability of the process, the potential of recycling EOPO together with DES was investigated, as this has not been assessed previously in literature. Both DES and EOPO fractions were recycled after each cycle for six cycles (Fig. 6, Table S4). As can be observed, the alginate extraction yield decreased from cycles 1 to 3 from 80.3 ± 10.5 to 64.2 ± 9.0 mg/g, followed by an increase from cycle 4. However, when considering the amount of alginate in the final aqueous phase, the recovery remained rather stable between 54.8 ± 7.8 and 46.6 ± 6.5 mg/g from cycles 1 to 5 (Fig. 6). Since the amount of recovered alginate increased while the extraction yield decreased, this could be due to the accumulation of alginate in the EOPO fraction. When the EOPO gets more saturated, the alginate in the DES phase increases (starting from cycle 3), as alginate cannot be fully

transferred to the EOPO in the first ATPS. Even though more alginate is present in DES, recovered alginate decreased to 25.1 ± 7.7 mg/g in cycle 6.

3.4. Overall extraction and recovery process

Two approaches can be employed for the temperature-responsive ATPS recovery process: (1) recycling only DES, and (2) recycling both the DES and the EOPO fraction. In the first scenario, up to ten reuse cycles are achievable. However, this strategy results in continuous EOPO consumption, potentially generating chemical waste. In contrast, simultaneous recycling of both DES and EOPO is feasible for up to five cycles, during which alginate extraction yields fluctuate between 60 and 100 mg/g DW, and the alginate recovery yields remain stable at approximately 50–65 mg/g DW (Fig. 7). The ability to recycle both phases is supported by the stable transfer of alginate from the DES to the EOPO phase over multiple cycles, indicating that the ATPS1 partitioning step remains effective under simultaneous recycling conditions. However, when only the DES fraction is recycled, the recovery yield remains constant despite repeated cycles, suggesting that alginate progressively accumulates in the EOPO phase. This accumulation likely impairs alginate partitioning efficiency, altering the EOPO phase properties, such as viscosity, polymer–solute interactions, or available partitioning capacity, ultimately reducing the number of effective EOPO reuse cycles.

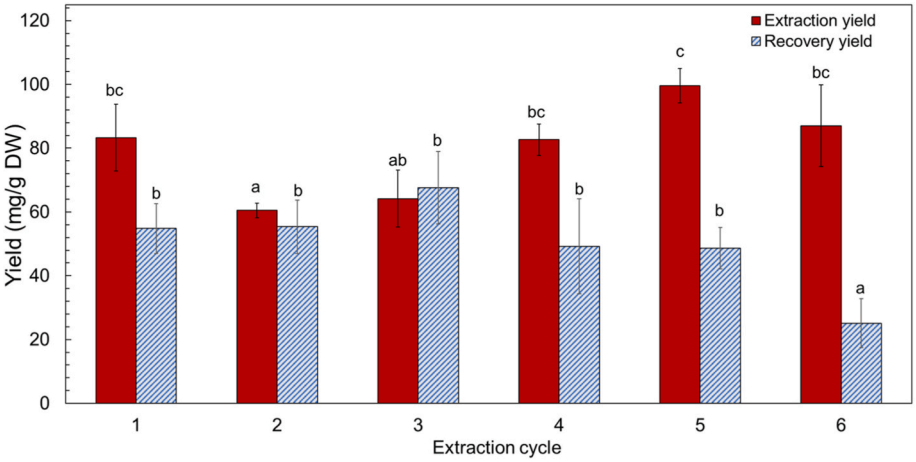

Fig. 6. Alginate extraction and recovery yield during simultaneous DES and EOPO recycling.

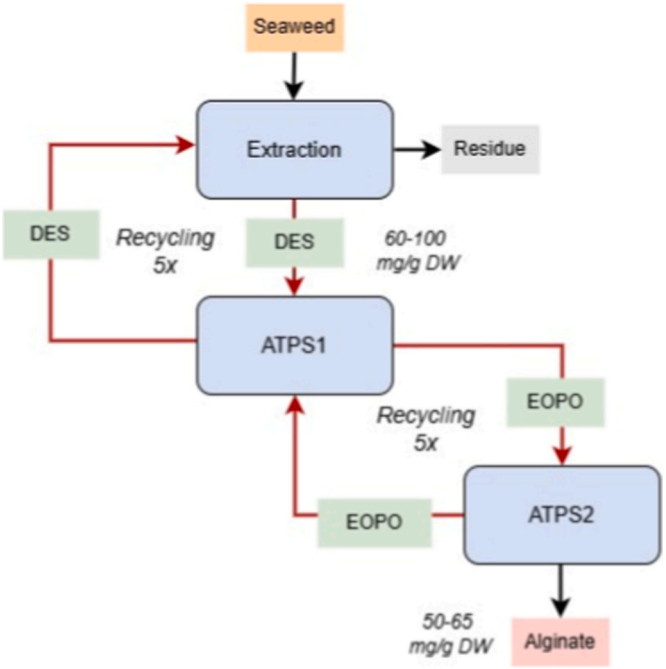

Fig. 7. Alginate extraction and recovery yields during simultaneous recycling of both DES and EOPO phases over five consecutive cycles. Extraction yields remain within 60–100 mg/g DW across cycles, while recovery yields are consistently maintained at approximately 50–65 mg/g DW.

Introducing a washing step to remove residual alginate from the EOPO fraction could mitigate this issue, potentially improving the transfer efficiency from DES to EOPO and enabling more recycling cycles.

Although recycling only the DES allows for greater reuse frequency, the dual-component recycling approach offers a more integrated and sustainable solution. By reducing chemical consumption and minimising waste, the combined recycling of DES and EOPO contributes to a more circular and environmentally friendly alginate extraction process (Fig. 7). Therefore, despite the reduced number of cycles, the second approach may be considered more favourable for long-term, sustainable operation.

Scaling up the DES–EOPO ATPS process may present challenges, particularly related to the high viscosity of DESs and the energy requirements for heating to induce phase separation. Elevated viscosity could hinder efficient mixing, mass transfer, and phase separation at larger volumes, while heating costs could increase operational expenses. Potential solutions include optimising DES composition to reduce viscosity, such as increasing the water content, provided that the impact on ATPS formation at the required EOPO concentration is systematically evaluated. Mechanical agitation or static mixers may further enhance phase contact, and alternative heating strategies such as localised or continuous heating could improve overall energy efficiency. This may further facilitate scalability while maintaining extraction performance. In addition, incorporating spectroscopic analyses (e.g. FTIR) on the used DES and EOPO fractions into future scale-up studies could help identify potential chemical or structural changes in the DES or EOPO phases, thereby supporting more informed optimisation at larger scales.

4. Conclusion

In this study, a thermo-separating aqueous two-phase system (ATPS) was developed for the continuous extraction and separation of alginate using DES as an alternative, circular extraction process compared to conventional alkaline extraction. This research demonstrated, for the first time, that DESs can be effectively recycled for at least ten cycles using a temperature-responsive ethylene oxide–propylene oxide (EOPO) copolymer. The three different DESs all successfully extracted and recovered alginate. Bet:U was selected for further recycling studies with EOPO3900 (54.8 ± 8.1 mg/g), as it required the lowest amount of copolymer for separation. The DES reusability study showed that the alginate extraction yield remained between 74 and 86 mg/g DW over ten cycles, with consistent recovery yields (55–65 %). Furthermore, the combined recycling of both the DES and EOPO was successfully performed for up to five cycles while maintaining stable recovery performance (between 54.8 ± 7.8 and 46.6 ± 6.5 mg/g).

This study presents a novel, efficient, and circular approach for DES recycling. The thermo-separating ATPS offers a suitable and straightforward method for the recovery and reuse of DES. Additionally, the simultaneous recycling of DES and EOPO could enhance process sustainability and significantly reduce solvent waste. The proposed system provides a viable, eco-friendly alternative for alginate extraction and isolation, while enabling effective regeneration of DES.

CRediT authorship contribution statement

Isa S.A. Hiemstra: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Niels Lustig:** Methodology, Investigation, Data curation. **Michel H.M. Eppink:** Validation, Supervision. **René H. Wijffels:** Validation, Supervision. **Antoinette Kazbar:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by the Dutch Research Council (NWO) (The Hague, NL) under the SeaSolv project (https://app.dimensions.ai/details/grant/grant.12924032). Grant number 19479.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2025.147186.

Data availability

Data will be made available on request.

References

Abbott, A.P., Boothby, D., Capper, G., Davies, D.L., Rasheed, R.K., 2004. Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids. J. Am. Chem. Soc. 126 (29), 9142–9147. https://doi.org/10.1021/ja048266j.

Abdul Khalil, H.P.S., Lai, T.K., Tye, Y.Y., Rizal, S., Chong, E.W.N., Yap, S.W., Hamzah, A. A., Nurul Fazita, M.R., Paridah, M.T., 2018. A review of extractions of seaweed hydrocolloids: properties and applications. Express Polym. Lett. 12 (4), 296–317. https://doi.org/10.3144/expresspolymlett.2018.27.

Belattmania, Z., Kaidi, S., Atouani, S. El, Katif, C., Bentiss, F., Jama, C., Reani, A., Sabour, B., Vasconcelos, V., 2020. Isolation and FTIR-ATR and 1H NMR characterization of alginates from the main alginophyte species of the atlantic coast of Morocco. Molecules 25 (18). https://doi.org/10.3390/molecules25184335.

Bojorges, H., López-Rubio, A., Fabra, M.J., Martínez-Abad, A., 2023. Estimation of alginate purity and M/G ratio by methanolysis coupled with anion exchange chromatography. Carbohydr. Polym. 321. https://doi.org/10.1016/j.carbpol.2023.121285.

Cesaretti, M., Luppi, E., Maccari, F., Volpi, N., 2003. A 96-well assay for uronic acid carbazole reaction. Carbohydr. Polym. 54 (1), 59–61. https://doi.org/10.1016/S0144-8617(03)00144-9.

Chemat, F., Vian, M.A., Ravi, H.K., Khadhraoui, B., Hilali, S., Perino, S., Tixier, A.S.F., 2019. Review of alternative solvents for green extraction of food and natural

products: Panorama, principles, applications and prospects. Molecules 24 (16). https://doi.org/10.3390/molecules24163007. MDPI AG.
Chen, Z., Reznicek, W.D., Wan, C., 2018. Deep eutectic solvent pretreatment enabling full utilization of switchgrass. Bioresour. Technol. 263, 40–48. https://doi.org/10.1016/j.biortech.2018.04.058.
Das, A.K., Sharma, M., Mondal, D., Prasad, K., 2016. Deep eutectic solvents as efficient solvent system for the extraction of κ-carrageenan from Kappaphycus alvarezii. Carbohydr. Polym. 136, 930–935. https://doi.org/10.1016/j.carbpol.2015.09.114.
Dobrinčić, A., Balbino, S., Zorić, Z., Pedisić, S., Kovačević, D.B., Garofulić, I.E., Dragović-Uzelac, V., 2020. Advanced technologies for the extraction of marine brown algal polysaccharides. Mar. Drugs 18 (Issue 3). https://doi.org/10.3390/md18030168. MDPI AG.
Elizondo Sada, O.M., Hiemstra, I.S.A., Chorhirankul, N., Eppink, M., Wijffels, R.H., Janssen, A.E.M., Kazbar, A., 2024. Pressure-driven membrane processes for the recovery and recycling of deep eutectic solvents: a seaweed biorefinery case study. Biotechnol. Rep. 43, e00849. https://doi.org/10.1016/j.btre.2024.e00849.
Gao, C., Cai, C., Liu, J., Wang, Y., Chen, Y., Wang, L., Tan, Z., 2020. Extraction and preliminary purification of polysaccharides from Camellia oleifera Abel. seed cake using a thermoseparating aqueous two-phase system based on EOPO copolymer and deep eutectic solvents. Food Chem. 313. https://doi.org/10.1016/j.foodchem.2020.126164.
Halder, A.K., Cordeiro, M.N.D.S., 2019. Probing the environmental toxicity of deep eutectic solvents and their components: an in silico modeling approach. ACS Sustain. Chem. Eng. 7 (12), 10649–10660. https://doi.org/10.1021/acssuschemeng.9b01306.
Hansen, B.B., Spittle, S., Chen, B., Poe, D., Zhang, Y., Klein, J.M., Horton, A., Adhikari, L., Zelovich, T., Doherty, B.W., Gurkan, B., Maginn, E.J., Ragauskas, A., Dadmun, M., Zawodzinski, T.A., Baker, G.A., Tuckerman, M.E., Savinell, R.F., Sangoro, J.R., 2021. Deep eutectic solvents: a review of fundamentals and applications. Chem. Rev. 121 (3), 1232–1285. https://doi.org/10.1021/acs.chemrev.0c00385. American Chemical Society.
Hiemstra, I.S.A., Meinema, J.T., Eppink, M.M.H., Wijffels, R.H., Kazbar, A., 2024. Choline chloride-based solvents as alternative media for alginate extraction from brown seaweed. LWT 214. https://doi.org/10.1016/j.lwt.2024.117174.
Iqbal, M., Tao, Y., Xie, S., Zhu, Y., Chen, D., Wang, X., Huang, L., Peng, D., Sattar, A., Shabbir, M.A.B., Hussain, H.I., Ahmed, S., Yuan, Z., 2016. Aqueous two-phase system (ATPS): an overview and advances in its applications. Biol. Proced. Online 18 (1), 1–18. https://doi.org/10.1186/s12575-016-0048-8. BioMed Central Ltd.
Jeong, K.M., Lee, M.S., Nam, M.W., Zhao, J., Jin, Y., Lee, D.K., Kwon, S.W., Jeong, J.H., Lee, J., 2015. Tailoring and recycling of deep eutectic solvents as sustainable and efficient extraction media. J. Chromatogr. A 1424, 10–17. https://doi.org/10.1016/j.chroma.2015.10.083.
Jiang, B., Zhang, X., Yuan, Y., Qu, Y., Feng, Z., 2017. Separation of antioxidant peptides from pepsin hydrolysate of whey protein isolate by ATPS of EOPO Co-polymer (UCON)/Phosphate. Sci. Rep. 7 (1). https://doi.org/10.1038/s41598-017-13507-9.
Kepka, C., Rhodin, J., Lemmens, R., Tjerneld, F., Gustavsson, P.E., 2004. Extraction of plasmid DNA from Escherichia coli cell lysate in a thermoseparating aqueous two-phase system. J. Chromatogr. A 1024 (1–2), 95–104. https://doi.org/10.1016/j.chroma.2003.10.028.
Khandelwal, S., Tailor, Y.K., Kumar, M., 2016. Deep eutectic solvents (DESs) as eco-friendly and sustainable solvent/catalyst systems in organic transformations. J. Mol. Liq. 215, 345–386. https://doi.org/10.1016/j.molliq.2015.12.015. Elsevier B.V.
Kuzmanović, M., Božanić, D.K., Milivojević, D., Ćulafić, D.M., Stanković, S., Ballesteros, C., Gonzalez-Benito, J., 2017. Sodium-alginate biopolymer as a template for the synthesis of nontoxic red emitting Mn2+-doped CdS nanoparticles. RSC Adv. 7 (84), 53422–53432. https://doi.org/10.1039/c7ra11011a.
Kuznetsova, I.M., Zaslavsky, B.Y., Breydo, L., Turoverov, K.K., Uversky, V.N., 2015. Beyond the excluded volume effects: mechanistic complexity of the crowded milieu. Molecules 20 (1), 1377–1409. https://doi.org/10.3390/molecules20011377. MDPI AG.
Lai, H., Yu, Q., Liu, Y., Liang, Z., Li, Y., Pan, J., Gao, J., 2025. Three-phase systems for simultaneous extraction and separation of bioactives from Haematococcus pluvialis: Role of thermo-switchable phase behavior. Sep. Purif. Technol. https://doi.org/10.1016/j.seppur.2025.43527. In press.
Leal, D., Matsuhiro, B., Rossi, M., Caruso, F., 2008. FT-IR spectra of alginic acid block fractions in three species of brown seaweeds. Carbohydr. Res. 343 (2), 308–316. https://doi.org/10.1016/j.carres.2007.10.016.
Lee, K.Y., Mooney, D.J., 2012. Alginate: properties and biomedical applications. Prog. Polym. Sci. 37 (1), 106–126. https://doi.org/10.1016/j.progpolymsci.2011.06.003. Elsevier Ltd.
Leong, Y.K., Lan, J.C.W., Loh, H.S., Ling, T.C., Ooi, C.W., Show, P.L., 2017a. Cloud-point extraction of green-polymers from Cupriavidus necator lysate using thermoseparating-based aqueous two-phase extraction. J. Biosci. Bioeng. 123 (3), 370–375. https://doi.org/10.1016/j.jbiosc.2016.09.007.
Leong, Y.K., Show, P.L., Lan, J.C.W., Loh, H.S., 2015. Thermoseparating aqueous two-phase system: recent trends and applications. Chem. Eng. Transact. 45, 1249–1254. https://doi.org/10.3303/CET1545209.
Leong, Y.K., Show, P.L., Lan, J.C.W., Loh, H.S., Yap, Y.J., Ling, T.C., 2017b. Extraction and purification of Polyhydroxyalkanoates (PHAs): application of Thermoseparating aqueous two-phase extraction. J. Polym. Res. 24 (10). https://doi.org/10.1007/s10965-017-1307-3.
Liang, X., Fu, Y., Chang, J., 2019. Effective separation, recovery and recycling of deep eutectic solvent after biomass fractionation with membrane-based methodology. Separ. Purif. Technol. 210, 409–416. https://doi.org/10.1016/j.seppur.2018.08.021.
Liang, X., Zhang, J., Huang, Z., Guo, Y., 2023. Sustainable recovery and recycling of natural deep eutectic solvent for biomass fractionation via industrial membrane-based technique. Ind. Crop. Prod. 194. https://doi.org/10.1016/j.indcrop.2023.116351.
Ling, Z., Guo, Z., Huang, C., Yao, L., Xu, F., 2020. Deconstruction of oriented crystalline cellulose by novel levulinic acid based deep eutectic solvents pretreatment for improved enzymatic accessibility. Bioresour. Technol. 305. https://doi.org/10.1016/j.biortech.2020.123025.
Liu, Y., Gao, L., Chen, L., Zhou, W., Wang, C., Ma, L., 2023. Exploring carbohydrate extraction from biomass using deep eutectic solvents: factors and mechanisms. iScience 26 (9). https://doi.org/10.1016/j.isci.2023.107671. Elsevier Inc.
Lo, C., Wijffels, R.H., Eppink, M.H.M., 2024. Lipid recovery from deep eutectic solvents by polar antisolvents. Food Bioprod. Process. 143, 21–27. https://doi.org/10.1016/j.fbp.2023.10.003.
Ma, H., Fu, P., Zhao, J., Lin, X., Wu, W., Yu, Z., Xia, C., Wang, Q., Gao, M., Zhou, J., 2022. Pretreatment of wheat straw lignocelluloses by deep eutectic solvent for lignin extraction. Molecules 27 (22), 7955. https://doi.org/10.3390/molecules27227955.
Mahmood Zia, K., Zuber, M., Ali, M., 2017. Algae Based Polymers, Blends, and Composites: Chemistry, Biotechnology and Materials Science.
Marimuthu, N., Chow, Y.H., Kee, P.E., Lan, J.C.W., Lai, Z.W., Yoon, L.W., Tam, Y.J., 2025. Recovery of hyaluronic acid using thermosensitive polymer-salt aqueous biphasic system with polymer recycling. J. Biosci. Bioeng. 140 (2), 79–89. https://doi.org/10.1016/j.jbiosc.2025.04.007.
Moslehyani, A., Ismail, A.F., Matsuura, T., Rahman, M.A., Goh, P.S., 2018. Recent progresses of ultrafiltration (UF) membranes and processes in water treatment. In: Membrane Separation Principles and Applications: from Material Selection to Mechanisms and Industrial Uses. Elsevier, pp. 85–110. https://doi.org/10.1016/B978-0-12-812815-2.00003-X.
Musarurwa, H., Tavengwa, N.T., 2022. Thermo-responsive polymers and advances in their applications in separation science. Microchem. J. 179. https://doi.org/10.1016/j.microc.2022.107554. Elsevier Inc.
Nesic, A., De Bonis, M.V., Dal Poggetto, G., Ruocco, G., Santagata, G., 2023. Microwave assisted extraction of raw alginate as a sustainable and cost-effective method to treat beach-accumulated Sargassum Algae. Polymers 15 (14). https://doi.org/10.3390/polym15142979.
Ng, H.S., Tan, C.P., Mokhtar, M.N., Ibrahim, S., Ariff, A., Ooi, C.W., Ling, T.C., 2012. Recovery of Bacillus cereus cyclodextrin glycosyltransferase and recycling of phase components in an aqueous two-phase system using thermo-separating polymer. Separ. Purif. Technol. 89, 9–15. https://doi.org/10.1016/j.seppur.2011.12.028.
Ng, H.S., Teoh, A.N., Lim, J.C.W., Tan, J.S., Wan, P.K., Yim, H.S., Show, P.L., Lan, J.C.W., 2017. Thermo-sensitive aqueous biphasic extraction of polyphenols from Camellia sinensis var. assamica leaves. J. Taiwan Inst. Chem. Eng. 79, 151–157. https://doi.org/10.1016/j.jtice.2017.02.018.
Ng, M.H., Nu'man, A.H., Hasliyanti, A., 2024. Recycling of deep eutectic solvent in the extraction of ferulic acid from oil palm empty fruit bunch. J. Separ. Sci. 47 (4). https://doi.org/10.1002/jssc.202300842.
Nie, J., Chen, D., Lu, Y., 2020. Deep eutectic solvents based ultrasonic extraction of polysaccharides from edible brown Seaweed Sargassum horneri. J. Mar. Sci. Eng. 8 (6). https://doi.org/10.3390/JMSE8060440.
Patrice Didion, Y., Gijsbert Tjalsma, T., Su, Z., Malankowska, M., Pinelo, M., 2023. What is next? The greener future of solid liquid extraction of biobased compounds: novel techniques and solvents overpower traditional ones. Separ. Purif. Technol. 320. https://doi.org/10.1016/j.seppur.2023.124147. Elsevier B.V.
Pereira, J.F.B., Coutinho, J.A.P., 2019. Aqueous two-phase systems. In: Liquid-Phase Extraction. Elsevier, pp. 157–182. https://doi.org/10.1016/B978-0-12-816911-7.00005-0.
Qiu, Z., Aita, G.M., 2013. Pretreatment of energy cane bagasse with recycled ionic liquid for enzymatic hydrolysis. Bioresour. Technol. 129, 532–537. https://doi.org/10.1016/j.biortech.2012.11.062.
Qu, H., Wu, Y., Luo, Z., Dong, Q., Yang, H., Dai, C., 2023. An efficient approach for extraction of polysaccharide from abalone (Haliotis Discus Hannai Ino) viscera by natural deep eutectic solvent. Int. J. Biol. Macromol. 244. https://doi.org/10.1016/j.ijbiomac.2023.125336.
Rabelo, A.P.B., Tambourgi, E.B., Pessoa, A., 2004. Bromelain partitioning in two-phase aqueous systems containing PEO-PPO-PEO block copolymers. J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 807 (1), 61–68. https://doi.org/10.1016/j.jchromb.2004.03.029.
Ramos, P.E., Silva, P., Alario, M.M., Pastrana, L.M., Teixeira, J.A., Cerqueira, M.A., Vicente, A.A., 2018. Effect of alginate molecular weight and M/G ratio in beads properties foreseeing the protection of probiotics. Food Hydrocoll. 77, 8–16. https://doi.org/10.1016/j.foodhyd.2017.08.031.
Rashedy, S.H., Abd El Hafez, M.S.M., Dar, M.A., Cotas, J., Pereira, L., 2021. Evaluation and characterization of alginate extracted from brown seaweed collected in the Red sea. Appl. Sci. 11 (14). https://doi.org/10.3390/app11146290.
Saji, S., Hebden, A., Goswami, P., Du, C., 2022. A brief review on the development of alginate extraction process and its sustainability. Sustainability (Switzerland) 14 (9). https://doi.org/10.3390/su14095181. MDPI.
Shang, X. chao, Chu, D., Zhang, J. xu, Zheng, Y. fen, Li, Y., 2021. Microwave-assisted extraction, partial purification and biological activity in vitro of polysaccharides from bladder-wrack (Fucus vesiculosus) by using deep eutectic solvents. Separ. Purif. Technol. 259. https://doi.org/10.1016/j.seppur.2020.118169.
Sharma, A., Kaur, I., Dheer, D., Nagpal, M., Kumar, P., Venkatesh, D.N., Puri, V., Singh, I., 2023. A propitious role of marine sourced polysaccharides: drug delivery and biomedical applications. Carbohydr. Polym. 308. https://doi.org/10.1016/j.carbpol.2022.120448. Elsevier Ltd.

Wan, J., Dong, W., Hou, D., Wang, Z., Cao, X., 2018. Polymerization of a new thermo-responsive copolymer with n-vinylcaprolactam and its application in recyclable aqueous two-phase systems with another thermo-responsive polymer. Biores. Bioprocess. 5 (1). https://doi.org/10.1186/s40643-018-0231-7.

Wang, Y., Hu, X., Han, J., Ni, L., Tang, X., Hu, Y., Chen, T., 2016. Integrated method of thermosensitive triblock copolymer-salt aqueous two phase extraction and dialysis membrane separation for purification of lycium barbarum polysaccharide. Food Chem. 194, 257–264. https://doi.org/10.1016/j.foodchem.2015.08.016.

Wang, Y., Xu, F., Cheng, J., Wu, X., Xu, J., Li, C., Li, W., Xie, N., Wang, Y., He, L., 2022. Natural deep eutectic solvent-assisted extraction, structural characterization, and immunomodulatory activity of polysaccharides from Paecilomyces hepiali. Molecules 27 (22). https://doi.org/10.3390/molecules27228020.

Wang, Y., Yang, T., Zeng, H., Wan, J., Cao, X., 2021. Study of lincomycin partition in a recyclable thermo-pH responsive aqueous two-phase system. Process Biochem. 109, 27–36. https://doi.org/10.1016/j.procbio.2021.06.019.

Wei, Y., Chen, X., Yang, T., Wan, J., Cao, X., 2021. Partition of tea Saponin with a novel recyclable Thermo-pH aqueous two-phase systems. Appl. Biochem. Biotechnol. 193 (10), 3062–3078. https://doi.org/10.1007/s12010-021-03583-z.

Wu, D.T., Feng, K.L., Huang, L., Gan, R.Y., Hu, Y.C., Zou, L., 2021. Deep eutectic solvent-assisted extraction, partially structural characterization, and bioactivities of acidic polysaccharides from lotus leaves. Foods 10 (10). https://doi.org/10.3390/foods10102330.

Xie, J., Chen, J., Cheng, Z., Zhu, S., Xu, J., 2021. Pretreatment of pine lignocelluloses by recyclable deep eutectic solvent for elevated enzymatic saccharification and lignin nanoparticles extraction. Carbohydr. Polym. 269. https://doi.org/10.1016/j.carbpol.2021.118321.

Xue, H., Wang, W., Wu, J., Xie, K., Ge, S., Tan, J., 2024. Ultrasound assisted aqueous two-phase extraction of polysaccharides from corn stigma: Process optimization, structure characterization, and immunomodulatory activity. Innov. Food Sci. Emerg. Technol. 91, 103531. https://doi.org/10.1016/j.ifset.2024.103531.

Yao, T., Feng, C., Hu, Y., Yang, S., 2025. Isolation and purification of aloe anthraquinones through recoverable triple-responsive aqueous two-phase system. Sep. Purif. Technol. 374, 133786. https://doi.org/10.1016/j.seppur.2025.133786.

Yeow, A.T.H., Hayyan, A., Hayyan, M., Usman Mohd Junaidi, M., Saleh, J., Jefrey Basirun, W., Roslan Mohd Nor, M., Al Abdulmonem, W., Zulhaziman, M., Mohamed Zuki, F., Diana Hamid, M., 2024. A comprehensive review on the physicochemical properties of deep eutectic solvents. In: Results in Chemistry, vol. 7. Elsevier B.V. https://doi.org/10.1016/j.rechem.2024.101378.

Yu, L., Li, Z., Huang, W., Ali, A., Chen, Y., Zhao, G., Yao, S., 2024. Recovery and post-treatment processes for ionic liquids and deep eutectic solvents. J. Mol. Liq. 402. https://doi.org/10.1016/j.molliq.2024.124767. Elsevier B.V.

Zhang, L., Wang, M., 2017. Optimization of deep eutectic solvent-based ultrasound-assisted extraction of polysaccharides from Dioscorea opposita Thunb. Int. J. Biol. Macromol. 95, 675–681. https://doi.org/10.1016/j.ijbiomac.2016.11.096.

Zhang, X., Han, M., Han, S., Zong, W., 2025. Aqueous two-phase system (ATPS): from basic science to applications. RSC Adv. 15, 9041–9054. https://doi.org/10.1039/D4RA08232J.

Zhang, R., Hong, M., Wu, J., Xiao, Y., Tong, J., 2024. Effect of water on the excess properties of eutectic solvents. J. Mol. Liq. 414. https://doi.org/10.1016/j.molliq.2024.126139.

Zhang, L., et al., 2023. Two-dimensional Na-Bentonite@MXene composite membrane with switchable wettability for selective oil/water separation. Sep. Purif. Technol. 306 (Part B), 122677. https://doi.org/10.1016/j.seppur.2022.2022341.

Zhang, W., Cheng, S., Zhai, X., Sun, J., Hu, X., Pei, H., Chen, G., 2020. Green and efficient extraction of polysaccharides from Poria cocos F.A. Wolf by deep eutectic solvent. Nat. Prod. Commun. 15 (2). https://doi.org/10.1177/1934578X19900708.

Zhou, R.R., Huang, J.H., He, D., Yi, Z.Y., Zhao, D., Liu, Z., Zhang, S.H., Huang, L.Q., 2022. Green and efficient extraction of polysaccharide and ginsenoside from American ginseng (Panax quinquefolius L.) by deep eutectic solvent extraction and aqueous two-phase System. Molecules 27 (10). https://doi.org/10.3390/molecules27103132.