



Design of novel aqueous two-phase systems to be coupled in biological remediation processes

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ABSTRACT

The ability of a biodegradable Deep Eutectic Solvent (DES) reline to induce phase segregation in aqueous solutions of polymeric non-ionic surfactants (Tween 20 and Tween 80) has been demonstrated as a preliminary step to be employed for assisting a biological degradation process of a model contaminant. The binodal curves for systems composed of (surfactants + reline + Water) have been determined at several temperatures, and the experimental data have been correlated using well-known equations. The equilibrium data have been analysed in the light of the operating temperature and the hydrophobicity of surfactants. Tie-lines of these systems have been determined, together with useful parameters such as tie-line length and tie-line slopes. Othmer-Tobias and Bancroft equations have been applied to provide an adequate description of the equilibrium data. Finally, the application of this new approach for the remediation of aqueous polluted effluent has proven to be highly efficient, with extraction percentages exceeding 99% for two model persistent contaminants (Acid Black 48 and Crystal Violet). Consequently, this ATPS is proposed as an extra-stage to enhance the remediation percentages achieved through biological methods. Furthermore, the process has been simulated using commercial software.

1. Introduction

Through history, a wide range of separation techniques has been proposed for vapour, liquid, solid and multiphase mixtures. These techniques have reaching up to 100 different operations, employing thermal, chemical, mechanical treatments, or membranes-based processes [1]. Among the possible options, Aqueous two-phase systems (ATPS) stand out due to several advantages, including their potential environmental friendliness, simplicity, ease of scalability and competitiveness [2]. ATPS are formed by mixing two hydrophilic compounds that, at certain concentrations in aqueous solutions, become mutually immiscible. Typically, these hydrophilic compounds consist of a salt and a polymer [3]. However, the investigation of alternative options has been driven by drawbacks such as the high viscosity or toxicity associated with some phase-forming agents [2]. As a result, novel approaches have emerged, including the combination of ionic liquids and salts [4], ionic liquids and polymers [5], or polymer–polymer systems [6].

Ionic liquids offer advantages such as negligible vapour pressure and tunability, where the latter meaning the ability to be intentionally

designed for a specific task. Consequently, ATPS carried out with these compounds are benefitted from these features. However, the toxicity and biodegradability of commercially available ionic liquids have been raised as concern in different research works, as recently discussed by Stolte and coworkers [7]. Therefore, the selection of a suitable neoteric solvent is equally crucial to attain a truly environmentally friendly.

The discovery of reline, a Deep Eutectic Solvent (DES) composed of cholinium chloride and urea, at the beginning of the current century has opened up new opportunities in the development of economically and environmentally sustainable solvents [8]. These novel compounds can typically be synthesized by mixing a hydrogen bond acceptor, HBA, (e.g., quaternary ammonium salts), and a hydrogen bond donor, HBD, (e.g., alcohols, amino acids, carboxylic acids, etc.) at specific ratios.

On the one hand, the production of DES is comparably quicker, more straightforward, and cost-effective compared to that of ionic liquids. It typically involves mixing two components at moderate temperature, offering the advantage of carefully selection of the parent materials [10] for the creation of biodegradable DES. In this context, cholinium-based HBA stands out as an appealing choice due to their proven low cost and

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Table 1Parameters of Eq. (1) for: Surfactant (1) + reline (2) + H₂O (3) at several temperatures and 0.1 MPa.^a

T/K	a	b	c	σ
Tween 20 (1) + reline (2) + H ₂ O (3)				
298.2	100.0	-0.0770	4.7.10 ⁻⁶	1.85
313.2	100.0	-0.0963	6.0.10 ⁻⁶	1.61
323.2	100.1	-0.1057	8.4.10 ⁻⁶	1.45
333.2	100.1	-0.1284	9.7.10 ⁻⁶	1.93
Tween 80 (1) + reline (2) + H ₂ O (3)				
298.2	100.0	-0.0764	5.8.10 ⁻⁶	2.09
313.2	100.1	-0.0886	6.4.10 ⁻⁶	2.05
323.2	100.1	-0.1078	7.9.10 ⁻⁶	2.26
333.2	100.0	-0.1188	1.1.10 ⁻⁵	1.84

^aStandard deviation (σ) was calculated by means of Eq. (5)**Table 2**Parameters of Eq. (2) for: Surfactant (1) + reline (2) + H₂O (3) at several temperatures and 0.1 MPa.^a

T/K	a	b	c	d	σ
Tween 20 (1) + reline (2) + H ₂ O (3)					
298.2	86.28	5.1354	-2.1721	0.0072	1.66
313.2	79.74	8.2873	-2.9535	0.0130	1.74
323.2	75.37	10.880	-3.6203	0.0182	1.47
333.2	77.33	7.1778	-3.1911	0.0176	1.61
Tween 80 (1) + reline (2) + H ₂ O (3)					
298.2	87.56	7.0863	-2.8106	0.0120	2.57
313.2	89.44	4.5053	-2.5507	0.0120	2.72
323.2	89.51	3.3148	-2.6070	0.0139	2.44
333.2	73.31	10.622	-3.7342	0.0201	1.85

^aStandard deviation (σ) was calculated by means of Eq. (5)**Table 3**Parameters of Eq. (3) for: Surfactant (1) + reline (2) + H₂O (3) at several temperatures and 0.1 MPa.^a

T/K	a	b	c	d	σ
Tween 20 (1) + reline (2) + H ₂ O (3)					
298.2	4.8859	-0.3052	0.0495	-0.0007	2.26
313.2	4.6942	-0.2144	0.0361	-0.0008	2.00
323.2	4.6644	-0.2079	0.0354	-0.0009	1.72
333.2	4.3920	0.0000	-0.0082	-0.0006	2.25
Tween 80 (1) + reline (2) + H ₂ O (3)					
298.2	4.9077	-0.3364	0.0574	-0.0008	2.11
313.2	5.5568	-0.6856	0.1031	-0.0011	1.72
323.2	4.7300	-0.2369	0.0369	-0.0008	2.56
333.2	4.6544	-0.2600	0.0502	-0.0012	1.79

^aStandard deviation (σ) was calculated by means of Eq. (5)**Table 4**Parameters of Eq. (4) for: Surfactant (1) + reline (2) + H₂O (3) at several temperatures and 0.1 MPa.^a

T/K	a	b	c	d	e	σ
Tween 20 (1) + reline (2) + H ₂ O (3)						
298.2	148.9	-0.4165	2.7.10 ⁻⁵	0.1770	2.6032	2.05
313.2	148.1	-0.4879	7.5.10 ⁻⁵	0.1430	2.4314	1.89
323.2	148.9	-0.5144	9.1.10 ⁻⁵	0.1356	2.4541	1.58
333.2	148.8	-0.5850	9.4.10 ⁻⁵	0.1234	2.4905	1.63
Tween 80 (1) + reline (2) + H ₂ O (3)						
298.2	148.9	-0.4087	1.8.10 ⁻⁵	0.1917	2.7315	1.87
313.2	148.9	-0.4279	1.4.10 ⁻⁵	0.2043	2.8171	1.86
323.2	148.9	-0.4705	9.9.10 ⁻⁶	0.2009	2.9632	2.39
333.2	148.9	-0.5295	8.1.10 ⁻⁶	0.1756	3.1164	1.58

^aStandard deviation (σ) was calculated by means of Eq. (5)

minimal environmental impact [11]. Furthermore, DES production boasts other benefits, including nearly 100% atom economy in the preparation process and the availability of chemical precursors in bulk

quantities [9].

These characteristics have led to the proposal of these solvents for a range of applications, including metal treatment, organic synthesis, biomass processing, biocatalysis, and separation processes [12]. However, research efforts in the application of these solvents in APTS have been relatively limited, with fewer than 50 articles focusing on this topic in recent years [13,14].

More specifically, the use of DES as salting out agents in aqueous solutions of non-ionic surfactants was first reported by our research group [15]. This choice was motivated by the inherent advantages associated with surfactant-based systems, including their lower cost, reduced surface tension, rapid phase disengagement and the widespread availability of these compounds [16].

In these cases, Triton X-100 (alkylphenol polyethylenes) was employed, as it belongs to one of the most prevalent surfactant families. However, with the ban on its usage by the European Chemicals Agency effective since 2021 (REACH Annex XIV, [17]), there is a compelling need to explore more biocompatible alternatives. Among the available options, the Tween family (sorbitan esters) emerges as a promising candidate due to its established biodegradability and low toxicity [18]. Therefore, the primary focus of this work is to demonstrate the capability of the first synthesized DES cholinium chloride: urea (reline), belonging to type III [19], to act as segregation agent in aqueous solutions containing two of the most commonly used Tween surfactants (Tween 20 and Tween 80).

The suitability of these separation strategies for the removal of persistent model pollutants, such as dyes, is being investigated. Dyes are of particular interest due to the potential health and environmental risks they pose. They find extensive use across various industries, ranging from tanneries to pharma and cosmetics, with global production exceeding one million tons [20]. The complex chemical structures of dyes, often featuring aromatic moieties and different functional groups with electrons in π-orbitals, are responsible for the staining properties, making them ideal model pollutants.

For the present work, three acid and basic dyes commonly employed in textile, biological and paper applications were selected: Acid Black 48 (AB48), Methylene Blue (MB) and Crystal Violet (CV), respectively. Although several biological and physico-chemical methods have been employed to remove these dyes from aqueous effluents [21], several drawbacks have been encountered, like the low dye biodegradability [22] or the production of large quantity of sludges [23].

In this context, DES-based liquid-liquid extraction presents a promising potential as an additional stage to be integrated after bioremediation process. This approach holds the potential to improve the often limited degradation efficiencies observed with microorganisms [13].

In view of the above context, a comprehensive characterization of the binodal curves and tie-lines of the systems for systems containing reline and Tween surfactants (Tween 20 and Tween 80) at four temperatures (298.2, 313.2, 323.2, 333.2) K were carried out. Additionally, several empirical models, including Othmer Tobias and Bancroft equations [24,25] have been employed to effectively describe the experimental data. Once the systems were characterized, the potential suitability for enhancing the remediation levels achieved through biological treatment of model pollutants was assessed.

2. Experimental section

2.1. Chemicals

Cholinium chloride (CAS 67-48-1, mass fraction purity > 0.97), Tween 20 (CAS 9005-64-5; Hydrophilic lipophilic balance (HLB) 16.7; Critical Micellar Concentration (CMC) 0.060 μM; Purity > 0.98) and Tween 80 (CAS 9005-65-6; HLB 15.0; CMC 0.012 μM; Purity > 0.98) were provided by Sigma-Aldrich. Urea (CAS 57-13-6, mass fraction purity > 0.99) was acquired from Panreac. Dyes AB28 (CAS 1328-24-1),

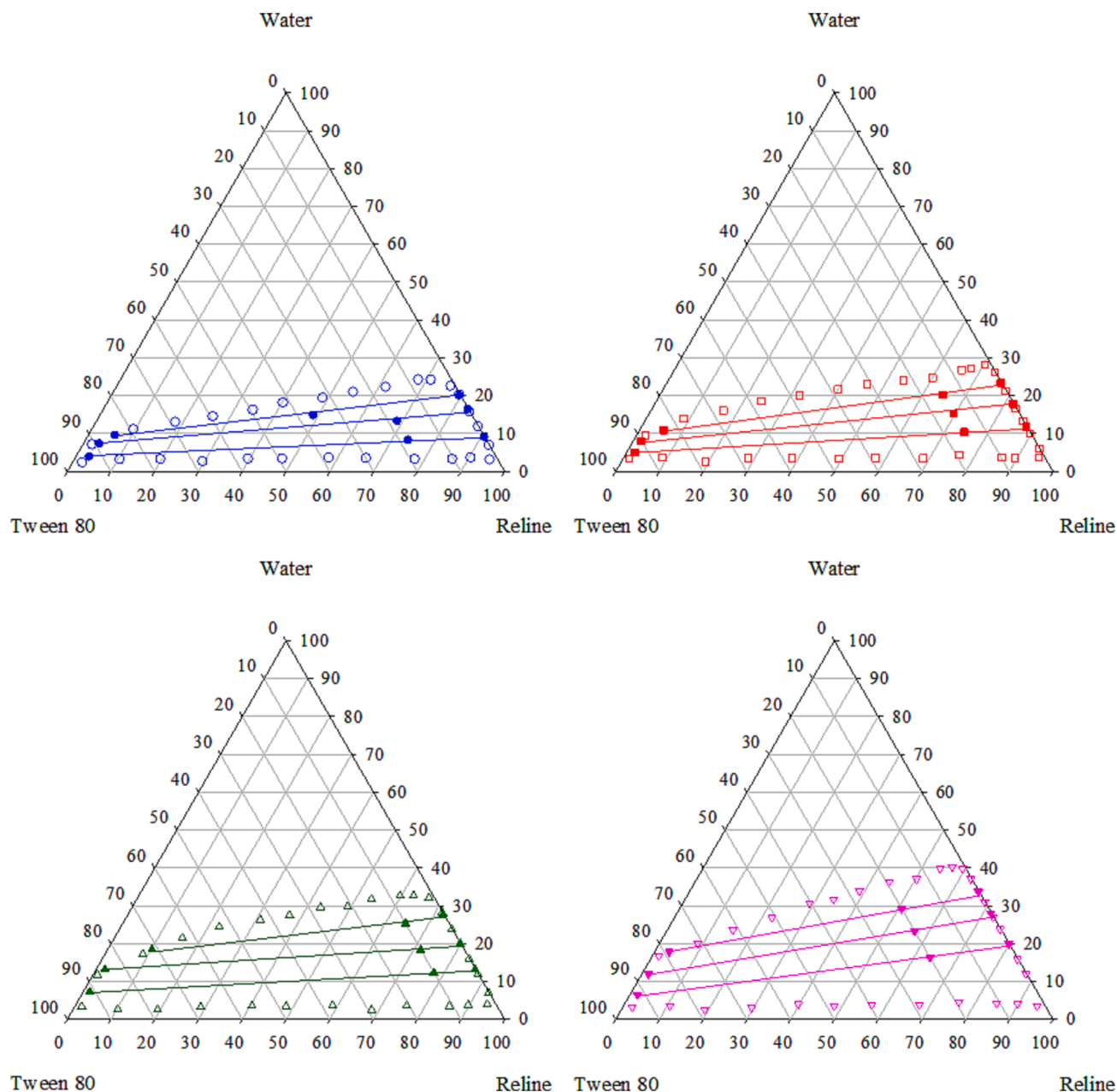


Fig. 1. Tie-lines for: (Tween 20 (1) + reline (2) + H₂O (3)) at 298.2 K (○), 313.2 K (□), 323.2 K (△), 333.2 K (▽) and 0.1 MPa. Experimental solubility data are represented by void symbols and experimental tie-line data are represented by full symbols.

MB (CAS 7220-79-3) and CV (CAS 548-62-9) were supplied by Sigma-Aldrich and their structures and main characteristics can be consulted in Table S1. The moisture and possible trace of solvents were removed by introducing the compounds in a vacuum line at moderate temperature (0.2 Pa and 323.2 K). The water content was determined to be lower than 0.05 (w/w) using Karl-Fischer titration. Double-distilled water was employed for all the experiments. The chemical structures of the surfactants and DES are provided in Fig. S1.

2.2. DES preparation

The preparation of the DES was carried out following the procedure previously described [15]. In brief, cholinium chloride and urea were mixed in a sealed glass tube at a molar ratio 1:2. The vial containing the

mixture was placed in a thermostatted bath set at 100 °C, with constant stirring until a homogeneous and transparent liquid (reline) was attained. The water content was measured through Karl-Fischer titration to ensure accuracy in determining the binodal curves and tie-lines.

2.3. Biological treatment

The bacterium *Anoxybacillus flavithermus* was obtained from a thermal spring in Galicia (Lobios, province of Ourense, Spain), as previously reported [26]. The microorganism was maintained in plates cultures composed of (g/L, in distilled water): 8 trypticase, 4 yeast extract, 3 NaCl, and 20 agar. pH was adjusted to 7.5 and the plates were incubated at 65 °C. Degradation medium was introduced in 250 mL Erlenmeyer flasks containing 50 mL of plate medium without agar including 0.07 g/

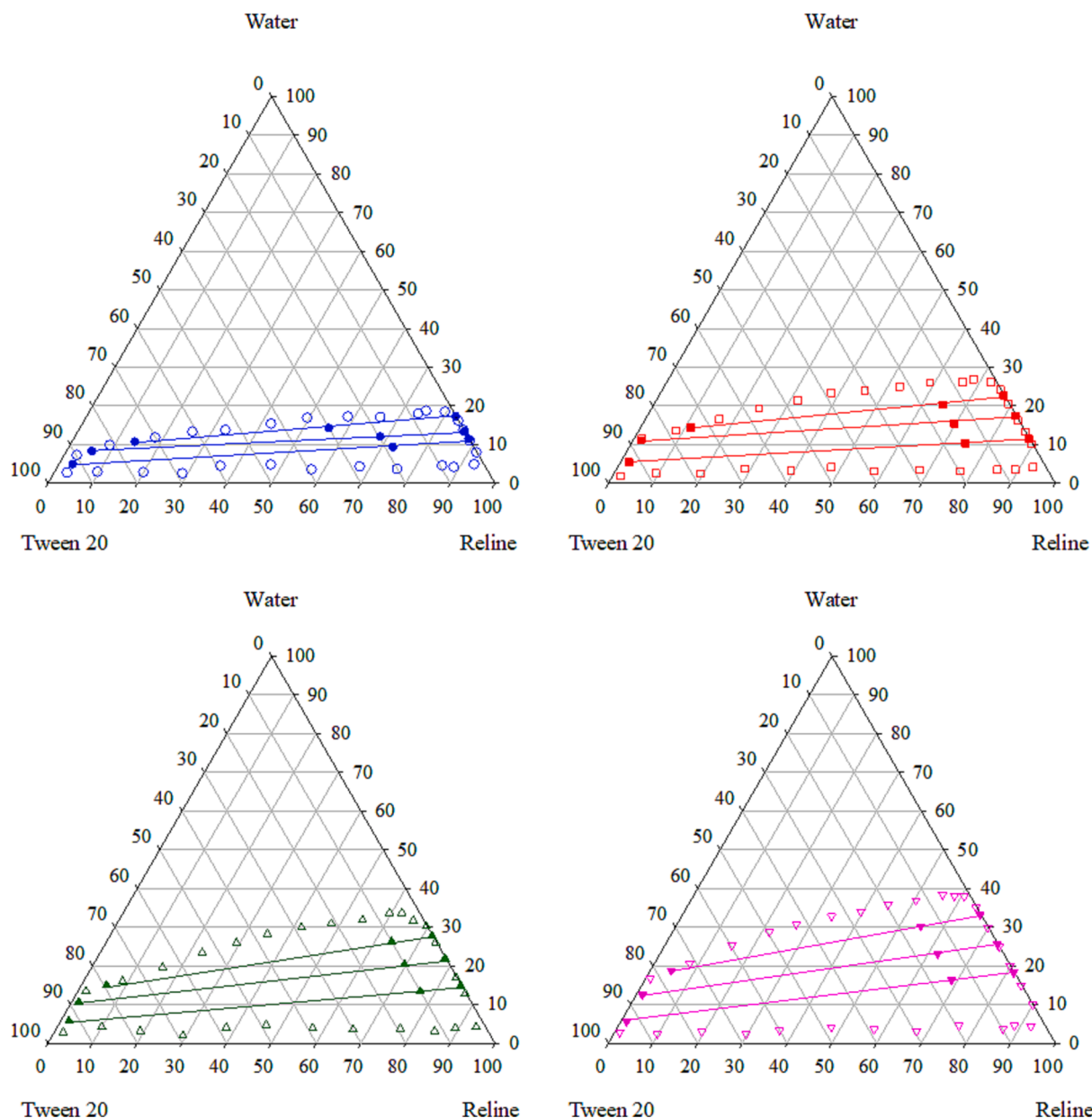


Fig. 2. Tie-lines for: (Tween 80 (1) + reline (2) + H₂O (3)) at 298.2 K (○), 313.2 K (□), 323.2 K (△), 333.2 K (▽) and 0.1 MPa. Experimental solubility data are represented by void symbols and experimental tie-line data are represented by full symbols.

L of the selected model pollutants. These were sterilized by filtration through a 20- μ m filter prior to the addition to the sterilized medium (121 °C and 20 min), in order to avoid any possible alteration of the chemical structure of the pollutant. Flask inoculation (3%) was carried out with cell pellets previously obtained, and they were then grown in an orbital shaker (Innova 44, New Brunswick Scientific,) at 100 rpm and 65 °C.

2.4. Binodal curves determination

The binodal data were obtained through the cloud point method at four different temperatures (298.2, 313.2, 323.2 and 333.2) K and atmospheric pressure (0.1 MPa) [27]. Mixtures of reline and non-ionic surfactant (Tween 20 or Tween 80) with various known compositions (covering the full range) were introduced into glass cells. These cells

were placed in a thermostatted bath (F200 ASL digital thermometer, \pm 0.01 K) and were stirred. Water was drop-wisely added to each tube until a turbid solution was achieved. The composition of the solution was ascertained by weighing on an analytical balance (Sartorius Cubis MSA balance, 125P-100-DA, \pm 10⁻⁵ g). Subsequently, additional water was added until a transparent solution could be visually observed, and the composition was once again recorded after weighing.

2.5. Tie-lines determination

The tie-lines (TLs) were carried after introducing reline, water and Tween surfactant in a glass ampoule at a composition within the biphasic area. The mixture was initially agitated and maintained in static conditions for 24 h at the corresponding temperature in a thermostatted cabin to get the thermodynamic equilibrium. Dr. Kernchen

Table 5

Parameters of Eq. (8) and Eq. (9) for: Surfactant (1) + reline (2) + H₂O (3) at several temperatures and 0.1 MPa.

Eq. (8)	T/K	K	n	R ²
Tween 20	298.2	24.04	2.7414	0.988
	313.2	2.7544	1.7931	0.961
	323.2	0.9627	1.4498	0.977
	333.2	1.0567	1.7852	0.999
Tween 80	298.2	0.6195	0.9421	0.961
	313.2	0.7085	1.1551	0.953
	323.2	1.6313	1.5380	0.986
	333.2	0.7168	1.5467	0.981
Eq. (9)	T/K	K ₁	r	R ²
Tween 20	298.2	0.5081	0.4776	0.927
	313.2	0.8783	0.6762	0.997
	323.2	1.2492	0.7077	0.999
	333.2	1.0613	0.5591	0.989
Tween 80	298.2	1.9815	0.9398	0.997
	313.2	2.0977	0.9400	0.999
	323.2	1.1134	0.7923	0.999
	333.2	1.2619	0.6034	0.997

ABBEMAT WR refractometer (uncertainty of $\pm 4 \cdot 10^{-5}$) and Anton Paar DSA-48 digital vibrating tube densimeter (uncertainty of $\pm 2 \cdot 10^{-4}$ g·cm⁻³) were employed to characterize the bottom and upper layers after a calibration stage. The densimeter underwent daily calibration using ambient air and high-quality Millipore water to ensure the reliability of the data. Through this process, combined expanded uncertainty for density was achieved at 5×10^{-4} g/cm³ with a confidence level of 95% and a k-factor of 2. The reliability of refractometer hinges on prior calibration using Millipore water, which yielded an uncertainty of 4×10^{-5} .

2.6. Pollutants quantification

Anthraquinone, thiazine, and triarylmethane dyes (AB48, MB and CV, respectively) were dissolved at a concentration of 4 g·cm⁻³ in a ternary mixture including Tween surfactant, reline eutectic mixture, and water. The solution was stirred for one hour and left for two days at room temperature to attain equilibrium. Dye content of the layers was

determined through UV-vis spectrophotometry (Unicam Helios β , Thermo Electron Corp.) at the corresponding wavelength (663 nm for AB48, 662 nm for MB and 590 nm for CV) once the calibration was carried out (Absorbance_(663 nm) = 9.1613.[AB48]; Absorbance_(662 nm) = 212.34.[MB]; Absorbance_(590 nm) = 218.84.[VC]). The same procedure was applied to quantify the dye content in microbiological degradation process. Each degradation value was the mean of two parallel experiments (deviations always lower than 3%) and abiotic controls (without microorganism) were included.

2.7. Modelling and simulation

The experimental data were fitted to the proposed equations through SOLVER function in Microsoft EXCEL. Flowsheet simulation was carried out by means of SuperPro Designer® v8.5 (Intelligen Inc.).

3. Results and discussion

3.1. Binodal curves determination

The potential of DES to be used as salting-out agents in aqueous solutions of non-ionic surfactants is a topic to be researched, and no data are available in the literature regarding ATPS based on Tween family and DES. In light of this, reline was added into aqueous solutions containing Tween 20 and Tween 80 to assess its segregation capacity. The resulting binodal data were recorded as indicated in the experimental section.

The ATPS for the ternary systems consisting of Tween 20 or Tween 80, reline, and H₂O at four temperatures (298.2, 313.2, 323.2 and 333.2 K) are shown in Fig. S2 and Fig. S3 (and the data can be consulted in Tables S2 and S3). Since the salting out agent employed is the same across these experiments, the data obtained can be interpreted in the context of the chemical properties of the surfactants and the specific operating conditions.

On the one hand, the selected surfactants differ in the length of their lateral alkyl chains; Tween 20 contains a laurate chain (12 carbon atoms) while Tween 80 includes an oleate chain (18 carbon atoms) (Fig. S1). These differences explain the variations in the hydrophobicity

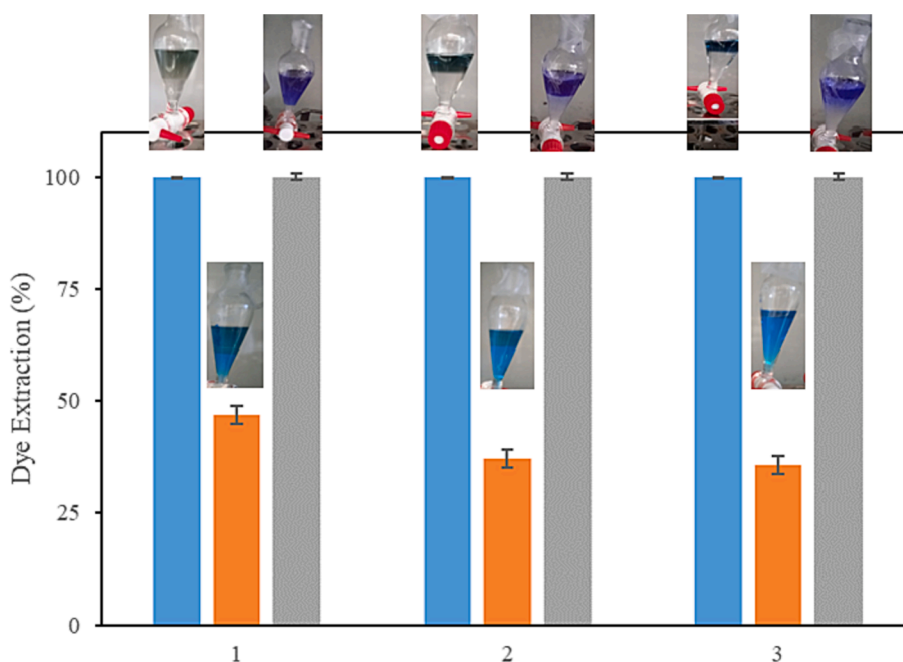


Fig. 3. Extraction percentages at 298.2 K for AB48 (blue bars), MB (orange bars) and CV (grey bars). Mass percentages of feed: 1) $F_1, F_2 = 72, 20$; 2) $F_1, F_2 = 45, 45$; and 3) $F_1, F_2 = (10,77)$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

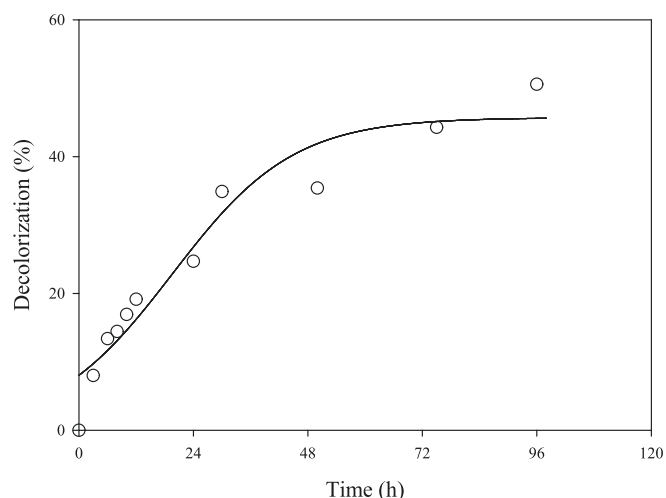


Fig. 4. Biological Decolorization of real effluent containing AB48. Symbols represent the experimental data and solid line the fitting to theoretical logistic model.

of both components, with Tween 80 exhibiting the least hydrophilic character. This observation is corroborated by the hydrophilic-lipophilic balance (HLB), a quantitative measurement of chemicals hydrophobicity and varies between 0 (high) and 20 (low). In this context, the HLB value for Tween 80 (15.0) is lower than that reported for Tween 20 (16.7) [28], indicating the greater hydrophobicity of the former.

This discrepancy in hydrophobicity translates a greater capacity for Tween 80 to be salted out by reline, which in turn explains the larger biphasic regions associated to this Tween, as observed in Fig. S3. Furthermore, this can be attributed to Tween 80 diminished capacity to interact with water molecules, making it more susceptible to be hydrated by reline. This behaviour is coincident with previous findings regarding neoteric solvents like DES and ionic liquids [18,29,30].

Regarding operating conditions, the influence of temperature

variation can be analysed for two distinct ATPS: one comprising Tween 20 and the other involving Tween 80. The data presented in Figs. S2 and S3 evidence a consistent trend across both systems: higher temperatures result in larger immiscibility regions for both surfactants.

The analysis of literature suggests the existence of different trends in ATPS behaviour with respect to the temperature changes: i) ATPS that are minimally affected by temperature changes, such as those constituted by polymers (PEG 400 or PEG 6000) and sulphate salts [31] and ii) ATPS that are more influenced and follow both proportional (polyethoxylated-based polymers and salts) [32] and inverse (ionic liquids and salts) relationship between temperature and the size of the biphasic region [33]. Hence, it becomes evident that the chemical nature of the phase-forming compounds plays a crucial role in determining their response to temperature variations.

In this work, Tween molecules contain a hydrophobic tail responsible for micellization phenomena. This trend becomes more pronounced at higher temperatures due to the dehydration ethylene oxide blocks. As highlighted Kerwin (2007) [34], this, in turn lead results in an increased hydrophobicity in the molecule. Conversely, the ethoxylated moiety also play a major role in phase disengagement. Conformers bearing the highest dipole moment are prevalent at low temperature, while those with very low dipole moments are predominant at elevated temperatures [35]. Under these conditions, the ethoxylated chains exhibit a more apolar character, contributing to an overall increase in the hydrophobicity of the non-ionic surfactant under study.

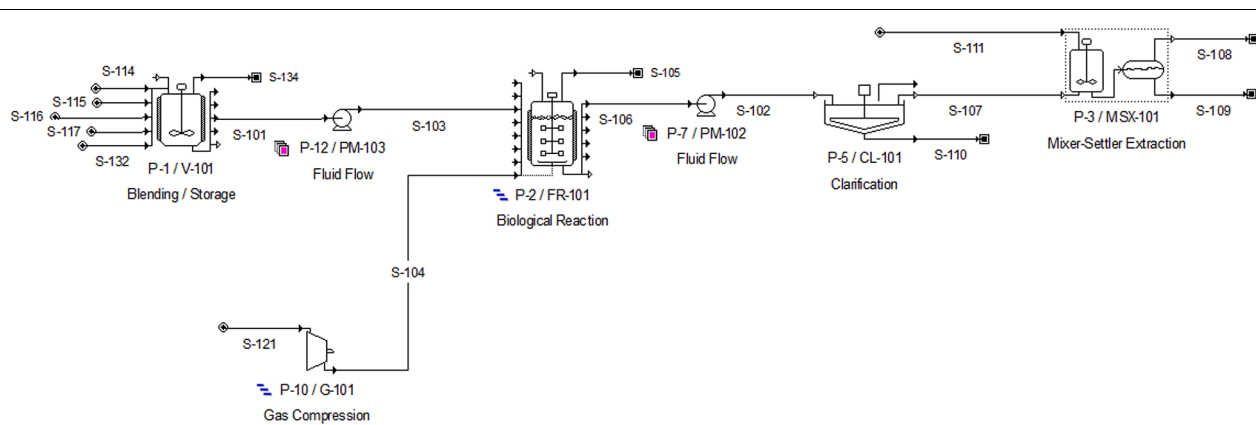
Once the data have been analysed, several polynomial and exponential expressions have been employed to correlate the binodal data [36]:

$$F_1 = a \cdot \exp(bF_2^{0.5} - cF_2^3) \tag{1}$$

$$F_1 = (a + bF_2^{0.5} + cF_2 + dF_2^2) \tag{2}$$

$$F_1 = \exp(a + bF_2^{0.5} + cF_2 + dF_2^2) \tag{3}$$

$$F_1 = a \cdot \exp(bF_2^d - cF_2^c) \tag{4}$$



Components	S-101 (Kg/batch)	S-106 (Kg/batch)	S-109 (Kg/batch)
AB48	3.01	1.62	0.01
Tween 80	50.00	10.04	114.20
Water	43000.00	43147.37	20218.31
Reline	0	0	86401.91
Biomass	0	29.30	1.09
Medium compounds	676.63	541.31	505.57
Total	43729.64	43729.64	107241.10

Fig. 5. Process flowsheet diagram for a sequential treatment of AB48-polluted effluent and main components composition in representative streams.

where F_1 and F_2 are the mass fraction percentages of Tween surfactant and reline, respectively. Furthermore, the fitting parameters a , b , c , d , and e were ascertained through minimization of the standard deviation (σ) applied with the help of SOLVER tool in Microsoft Excel:

$$\sigma = \left(\sum_{i=1}^{n_{DAT}} (z_{exp} - z_{adj})^2 / n_{DAT} \right)^{1/2} \quad (5)$$

being z_{exp} , and z_{adj} the experimental and theoretical data, respectively, and n_{DAT} the number of experimental values.

The parameters and standard deviations for each model have been listed in Tables 1-4. Upon analysing the deviation values, it becomes evident the low suitability of the polynomial model when compared to the exponential models. In this sense, both the Merchuk model (Eq. (1)) and the modified Merchuk model (Eq. (4)) provide more accurate descriptions of the experimental solubility data.

3.2. Tie-lines characterization and pollutants extraction

Before approaching on the extraction of the selected dyes, the tie-lines (TLs) of the systems composed of Tween 20 or Tween 80 + reline + H₂O at four different temperatures (298.2, 313.2, 323.2 and 333.2) K were undertaken. These TLs are illustrated in Fig. 1 and Fig. 2, while the empirical data has been included in Table S4. Furthermore, this table includes significant parameters that describe the extraction process, such as tie-line length (TLL) and slope of TLs (S), which were determined as indicated below:

$$TLL = \left[(F_1^I - F_1^{II})^2 + (F_2^I - F_2^{II})^2 \right]^{0.5} \quad (6)$$

$$S = F_1^I - F_1^{II} / F_2^I - F_2^{II} \quad (7)$$

where F_1 and F_2 bear the same meaning as that described previously. I and II refer to Tween-rich phase (top) and reline-rich phase (bottom), respectively.

The data presented in Table S4 enable us to conclude that higher concentrations of reline in the bottom phases are consistently associated with surfactant-rich layers of higher purity, for all temperatures and no matter the non-ionic surfactant under study. This trend is also reflected in the larger TLL values for those tie-lines with lower water concentrations. The reason for this phenomenon may be that when there are more reline molecules in the system, a greater amount of water is required to solvate them, resulting in higher purity in Tween-rich layer. These findings are valuable for determining the minimum quantity of reline required to achieve a desired level of purity in the upper layer.

Othmer-Tobias and Bancroft equations have also been applied to get a deeper characterization of the experimental tie-line data [24,25]:

$$(100 - F_1^I / F_1^I) = K \cdot (100 - F_2^II / F_2^II) \quad (8)$$

$$(F_3^II / F_2^II) = K_1 \cdot (F_3^I / F_1^I)^r \quad (9)$$

being F_1 , F_2 and F_3 the Tween, reline and water mass fraction percentages, respectively, and being K , n , K_1 and r the fitting parameters. Once again, the parameters were optimized by means the minimization of standard deviation, as previously mentioned. These parameters are compiled in Table 5, together with the determination coefficients obtained for each fitting.

The values of the determination coefficients, all exceeding 0.9, support the statement that both models are adequate tools for describing the TLs of the investigated ATPS. Remarkably, nine of these fittings achieved R^2 values over 0.99. Additionally, it is evident that Bancroft equation tends to provide closer descriptions of the liquid-liquid equilibrium data, as almost 88% of the data are correlated at levels near the unit.

Finally, given the robustness of the systems at all studied tempera-

tures, 298.2 K was chosen for assessing the feasibility of this DES-based ATPS for dye extraction. Furthermore, since surfactants are usually present in wastewater effluents originating from tanneries [18], and considering the larger immiscibility region obtained with the system containing Tween 80, this surface-active compound was selected as model for evaluating the applicability of this reline-based ATPS for the extraction of AB48, MB, and CV. Dye extraction percentage (E) was defined as follows:

$$E (\%) = (A^{II} \cdot V^I / A^I \cdot V^{II}) \cdot 100 \quad (10)$$

where A and V are the dye concentration (g/L) and the phase volume (L), and the superscripts I and II refer to initial conditions and surfactant-rich phase, respectively.

The data depicted in Fig. 3 reveal the excellent suitability of the proposed system at room temperature for the removal of both acidic and basic dyes, with extraction percentages over 99% /for AB48 and CV, no matter the tie-line feed composition. However, the results for MB differ quantitatively, with maximum extraction percentages of 50% observed when feed compositions contain low DES contents.

As previously stated, one of the key factors governing molecule partitioning is hydrogen bonding capacity. The presence of heteroatoms in the selected dyes will confer different abilities to interact with the components forming the ATPS. In this context, the findings from Xu and coworkers [37], who investigated MB partition in hydrophobic and hydrophilic DES, are also coincident with our data. They observed that a significant portion of MB remained in the more hydrophilic phase. Therefore, the hydrophilicity of the dye seems to be a crucial in understanding how the partition works in this DES-based ATPS.

The analysis of log Kow (n-octanol/water partition coefficient) of each dye can provide valuable insights into the differential partition of the selected compounds. In this regards, both AB48 and CV exhibit log Kow values (5.20 and 5.90, respectively) higher than that of MB (0.75). This implies that the most hydrophobic dyes entirely migrate to the surfactant-rich layer, while MB is distributed between the two phases, no matter the amount of DES and surfactant in the system.

3.3. Integration of DES-based ATPS in a biological degradation process

The obtained data suggests that the proposed process could be suitable for treating pollutants with high hydrophobicity, such as many of the commonly encountered contaminants faced nowadays, including as polycyclic aromatic hydrocarbons, herbicides, pesticides, phthalates or polychlorinated biphenyls. While previous research works has explored the capacity of microorganisms to degrade these types of pollutants [38,39], it is often challenging to achieve complete mineralization through such treatment. Therefore, incorporating an extra stage involving an ATPS could present a solution to establish biotechnological remediation methods that are competitive with other physical or chemical alternatives.

To demonstrate this fact, AB48 was chosen as a model pollutant to be firstly biodegraded from an aqueous effluent by *Anoxybacillus flavithermus*. As shown in Fig. 4, nearly 50% degradation can be obtained once the stationary phase is reached. The experimental data can be suitably modelled through the following logistic equation [26]:

$$D = D_{max} / 1 + e^{\left[\ln \left(\frac{D_{max} - 1}{D_0 - 1} \right) \mu_D t \right]} \quad (11)$$

where D is the pollutant removal percentage at a specific time t , D_0 and D_{max} are the initial and maximum pollutant removal percentage, respectively, and μ_D is the maximum specific pollutant removal rate. The parameters values were obtained through the minimization of standard deviation, calculated using Eq. (5), resulting in the values: $D_0 = 8.0$ %, $D_{max} = 45.7\%$, and $\mu_D = 0.079 \text{ h}^{-1}$ ($R^2 = 0.933$). These data reaffirm the need for adding an extra stage to eliminate the undegraded pollutant. Consequently, a tentative process flowsheet diagram is proposed in

Fig. 5, in which a reline-based ATPS is integrated following the biotechnological treatment.

The experimental data served as the basis for simulating the process using SuperPro Designer 8.5 software. This tool was selected for its specialized design tailored for biotechnological process simulation, including features for modelling bioprocesses like bioreactors and the ability to track materials meticulously. Its user-friendly interface also streamlines tasks like economic analysis, environmental assessment, and scheduling, crucial for assessing biotechnological process viability. Mass balances were performed, and the most significant streams are illustrated in Fig. 4, which highlights the suitability of the proposed strategy. Only 0.01 Kg out of the initial 3.01 Kg of pollutant remains in the final stream, indicating nearly complete removal of the selected model pollutant (resulting in a global remediation efficiency of 99.7%). This behaviour outperforms the removal percentages recently reported by Chu et al (2020) [40], achieved through ultrasound, advanced oxidation processes and sorption, with maximum levels of 70% when employing individual or combined methods. The integration of all three strategies was the sole option capable of achieving approximately 100% removal.

4. Conclusions

This work has demonstrated the capacity of the DES reline to act as phase segregation agent in aqueous solutions of polymeric non-ionic surfactants. Liquid-liquid equilibrium data were determined at (298.2, 313.2, 323.2 and 333.2) K and 0.1 MPa for the ATPS composed of Tween 20 or Tween 80 + reline + water. Various exponential and polynomial equations were employed to correlate these data. Additionally, experimental *T*Ls were determined and appropriately modelled with Othmer-Tobias and Bancroft equations to provide an in-depth description of the studied systems.

The excellent extraction capacity of the proposed DES-based system was demonstrated, particularly for highly hydrophobic and persistent pollutants like AB48 and CV, where remediation levels higher than 99% were achieved at room temperature. These results were the basis for implementing this strategy to enhance the removal rates obtained after biological treatment of an AB48-polluted effluent. The remediation results were significantly improved, increasing from about 45.7% to 99.7%, through the integration of both the biological and physical treatment stages. A process flowsheet diagram was developed to facilitate simulation using commercial simulation software.

CRedit authorship contribution statement

N.M.: Methodology, Data curation, Investigation, Software. **F.J.D.:** Funding acquisition, Project administration, Resources, Writing original draft, Writing - review & editing. **M.A.L.:** Funding acquisition, Project administration, Resources. **A.R.:** Conceptualization, Formal analysis, Investigation, Supervision, Validation, Writing original draft. **M.S.:** Funding acquisition, Project administration, Resources, Conceptualization, Formal analysis, Investigation, Supervision, Validation.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molliq.2023.123471>.

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