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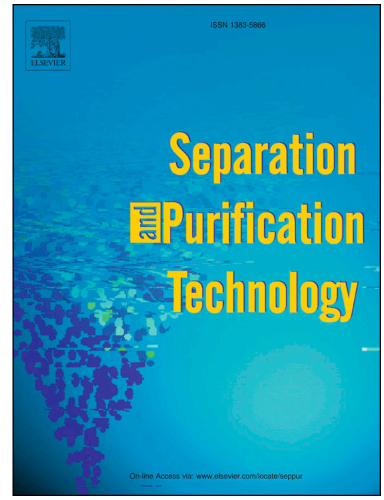

Review

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Research Review Manuscript

Sustainable recovery of microbial-derived natural pigments using deep eutectic solvents: advances, potential, and challenges

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Highlights

- DES-based methods enable milder, sustainable processing than traditional solvents
- Extraction methods include UAE and MAE, integrated SLE-LLE for selective extraction
- Task-specific DES improve selectivity and efficiency in pigment extraction process
- Ready-to-use pigment-rich extract is viable, and solvent recovery method is needed
- Challenges for broader adoption include DES recovery, scalability, and toxicity

Declaration of Interest statement

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 article.

Author contributions

Sze Ying Lee: Conceptualization; Writing - original draft. **João A.P. Coutinho**: Writing - review & editing. **Melanie Weingarten**: Writing - review & editing; Funding acquisition.

Abstract

Microbial-derived natural pigments present a promising alternative to synthetic colorants, offering diverse benefits and a more sustainable approach. This review explores the potential of deep eutectic solvents (DESs) as sustainable alternatives for recovering natural pigments derived from microbial sources, addressing the environmental and safety concerns associated with conventional extraction methods. Specifically, the microbial pigments discussed in this work include carotenoids (e.g., fucoxanthin, astaxanthin, canthaxanthin, bacterioruberin, lutein, and β-carotene), chlorophylls, and phycobiliproteins (phycocyanin and phycoerythrin). The review covers the fundamental properties and classifications of DESs, emphasizing their versatility and tunability for specific applications. The main stages of downstream processing, including biomass pretreatment, pigment extraction, and purification, are examined in detail, with DESs demonstrating better efficiency and selectivity than traditional solvents. The review also discusses the challenges and opportunities in developing task-specific DESs, optimizing extraction techniques, and achieving scalable, eco-friendly processes. The potential for using DES-based systems to produce ready-to-use pigment-rich extracts is highlighted, creating potentially new applications in food, cosmetics, and pharmaceuticals. The findings underscore the DESs' role in advancing the sustainable production of microbial pigments, and their implications for broader industrial adoption and the needs for further research.

Keywords: Natural pigment, microbial pigment, eutectic solvent, biomass pretreatment, pigment extraction, pigment purification

1. Introduction

The demand for natural colorants has increased in recent years, driven by growing consumer preference for safe, sustainable, and health-promoting ingredients in food, cosmetics, and pharmaceuticals. Synthetic colorants, have been associated with various health and environmental concerns, such as hyperactivity in children, allergenicity for sensitive individuals, and potential carcinogenicity [1]. The European Regulation (EC) No 1333/2008 requires that food containing synthetic colorants such as sunset yellow, quinoline yellow, carmoisine, allura red, tartrazine, and ponceau 4R must be labelled with a warning that they may have an adverse effect on activity and attention in children. Meanwhile natural pigments offer a biodegradable and safer alternative. Microorganisms, including bacteria, fungi, and algae, have been recognized as viable sources of these pigments, offering the added benefits of sustainability and reduced reliance on agricultural resources. A diverse array of natural pigments with different colors can be derived from microbial cell factories (**Figure 1A**). Examples are astaxanthin from *Haematococcus pluvialis*, canthaxanthin from *Chromochloris zofingiensis,* β-carotene from *Blakeslea trispora* and *Dunaliella salina*, fucoxanthin from *Tisochrysis lutea*, lutein from *Chlorella pyrenoidosa,* chlorophylls from *Chlorella vulgaris*, allophycocyanin and phycocyanin from *Arthrospira platensis*, and phycoerythrin from *Gelidium pusillum*. Apart from serving as food colorant, natural pigments possess health-promoting benefits like antioxidant, anti-inflammatory, and antimicrobial properties with large economic potential.

However, the production of microbial pigments is generally more complex and resource-intensive compared to synthetic colorants (**Figure 1B**). Since these pigments are produced intracellularly, downstream processing (DSP) involving multiple steps is required to efficiently recover them from the cell. Additionally, compared to synthetic colorants, natural pigments of microbial origin are more susceptible to environmental factors, including heat, light, oxygen, and pH, which can result in the loss of color intensity or alterations in hue [2]. Therefore, the extraction and purification of microbial-derived pigments present significant challenges. Traditional extraction methods often rely on volatile organic solvents and harsh chemicals that not only pose environmental risks but also compromise the quality and safety of the final products. These methods are energy-intensive and can result in the degradation of sensitive bioactive compounds, further limiting the appeal of natural pigments in various applications. There have been efforts to explore alternative eco-friendly solvents, preferably those with the preservation capability, for the recovery of microbial pigments. In this context, deep eutectic solvents (DESs) have emerged as a promising solution, offering an innovative approach to pigment extraction that aligns with the principles of green chemistry. The utilization of DESs in the food industry has primarily focused on the extraction of polar metabolites (e.g. phenolic compounds), since most DESs are intrinsically hydrophilic and water miscible [3]. The development of hydrophobic DESs have further expanded the spectrum of applications for extracting lipophilic compounds like carotenoids and chlorophylls. In addition, they have been used to lyse microbial cells to facilitate the release of intracellular pigments, and some have been employed for the purification/concentration of pigments [4, 5]. By the judicious selection of their constituents, DESs seems can be designed to be task-specific and selective to extract a specific compound of interest.

This review explores the use of DESs in the recovery of natural pigments from microbial biomass, examining their potential to enhance extraction efficiency, reduce environmental impact, and produce high-quality pigment extracts. Examples of microbial pigments discussed in this work are carotenoids (e.g., fucoxanthin, astaxanthin, canthaxanthin, bacterioruberin, lutein, and β-carotene), chlorophylls, and phycobiliproteins (phycocyanin and phycoerythrin). The review systematically evaluates the application of DESs across various stages of DSP, including biomass pretreatment, pigment extraction, and purification. The possibility of using pigment-rich crude extract obtained from extraction process as ready-to-use extract for various applications are discussed. Additionally, it addresses the challenges and opportunities associated with scaling up DES-based processes for industrial applications, ultimately highlighting the potential of DESs to revolutionize the production of natural pigments and contribute to a more sustainable future.

2. Deep eutectic solvents

Since their introduction, DESs have rapidly gained attention as "green" solvent alternatives, driven by their unique properties that make them attractive for a wide range of applications [6].

2.1 Definition and classification

A clear and standardized definition of DESs remains elusive. They are typically described as mixtures of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) linked by hydrogen bonds. Martins et al. [7] proposed that a DES should be defined as an eutectic mixture with a lower eutectic point temperature than its ideal liquid mixture, with "deep" referring to the significant depression of the melting point. Mixtures that do not meet this criterion should be termed as eutectic solvents. Despite the ambiguity in the definition, DESs and eutectic solvents are highly effective for extracting natural products due to their strong solvation capability. In this review, the term DES refers to all HBA and HBD combinations presented.

DESs are generally classified into five groups based on the chemical nature of their parent compounds. The most common, type III, consists of a quaternary ammonium salt (e.g., choline chloride [Ch]Cl) as a HBA and a HBD (e.g., amine, alcohol, and carboxylic acid). Type III DESs are water-soluble due to their favorable interactions with water molecules. The term natural deep eutectic solvent (NADES) has also been used to designate liquids made entirely of naturally derived metabolites like sugars, polyols, amino acids, and carboxylic acids [8]. On the other hand, type V DESs are prepared from terpenes (e.g., camphor, fenchyl alcohol, and coumarin), long-chain fatty acids (e.g., octanoic acid, decanoic acid, and dodecanoic acid), or by coupling a terpene (e.g., menthol and thymol) with a carboxylic acid (C1 to C12) or with a long-chain fatty acid [9]. These DESs tend to be water-immiscible due to the presence of one or more hydrophobic precursors. For sustainable applications, hydrophobic DESs should have low viscosity (< 100 mPa·s), a density differing from water by at least 50 $kg \cdot m^{-3}$, and avoid leaching or altering pH in water [10]. Notably, DESs with higher hydrophobicity show enhanced water stability, making them suitable for aqueous environments and solvent recycling [11].

2.2 Physicochemical properties

Because of the influence of their component combinations, hydrophilic and hydrophobic DESs exhibit distinct physicochemical properties. Most hydrophilic DESs have densities greater than water, while the majority of hydrophobic DESs possess densities smaller than water.

The viscosity of DES is another critical physicochemical property, as it directly influences mass transfer. Low viscosity is favored to promote faster mass transport for more efficient processes. Hydrophobic DESs typically display lower viscosities than hydrophilic DESs, with viscosities as low as 7 mPa·s at 25 ºC observed in dodecanoic acid/octanoic acid (1:3), although some may have exceptionally high viscosities depending on their specific compositions [10, 12]. Among hydrophobic DESs, non-ionic types have relatively lower viscosities compared to the ionic counterparts. For instance, terpene-based DESs typically have viscosities ranging from 10 – 60 mPa·s at 25 ºC, while DESs based on long chain quaternary ammonium salts (e.g., tetraheptylammonium chloride) exhibit high viscosities, at 173 mPa·s and above [13, 14]. Besides, the DESs prepared from lidocaine are more viscous than those made from thymol and menthol. On the other hand, high viscosity values have been reported for hydrophilic DESs, particularly those composed of sugars (e.g., glucose) [15]. It was also observed that the viscosity of carboxylic acid-based DES, with [Ch]Cl as the common HBA, increases with the number of carboxyl and hydroxyl groups in the structure of carboxylic acid, irrespective of the molar ratios, following the increasing order: [Ch]Cl/acetic acid < [Ch]Cl/lactic acid < [Ch]Cl/malic acid < [Ch]Cl/citric acid [16]. The high viscosity of the solvent reduces molecular mobility, and co-solvents (e.g., water and alcohols) can be added to lower the solvent's viscosity and facilitate its applications. However, the amount of co-solvent added needs to be optimized to balance viscosity reduction with the solvent's effectiveness. Alternatively, heating decreases the viscosity of DESs by increasing molecular movement, but the temperature must remain moderate to prevent pigment degradation.

3. Biomass pretreatment by deep eutectic solvents for pigment recovery

DESs have been investigated for their feasibility in improving the efficiency, sustainability, and safety of DSP for microbial-derived natural pigments. **Figure 2** depicts various techniques based on DESs used at different stages of DSP.

Pigment-producing microorganisms, especially microalgae and yeast, have rigid cell walls that are highly resistant to lysis. Therefore, biomass pretreatment approaches aimed at disrupting microbial cells have been applied prior to extraction steps. The cell lysis operation is essential for breaking open microbial cell walls, facilitating the penetration of solvent into cellular compartments to interact with pigment molecules for their liberation. There is a wide range of cell lysis methods available, including mechanical, physical, chemical, and biological techniques. Mechanical operations such as milling disintegrate cells by reducing them into smaller particles, and thus enhancing extractability. On the other hand, traditional chemical pretreatment methods, which involve the use of substantial amounts of corrosive acid and alkaline solutions, not only pollute the environment but also denature bioactive pigments. Aiming at reducing energy-intensive processes and toxic waste generation, DESs have been examined for the effectiveness in lysing cell walls and improving subsequent organic solvent-based extraction processes. The cell lysis process by DESs targets to rupture the cell membrane to release specific intracellular contents without causing complete disintegration that involves the breakdown of the entire cell and fragmentation.

The ability of DESs to lyse microbial cell wall is closely related to their potent capacity to dissolve cellulose and polysaccharide, which are the main structural constituents providing mechanical strength to microbial cell wall. For instance, the cell wall of lutein-producing microalgae *C. pyrenoidosa* is composed of 15% α-cellulose, 31% hemicellulose, 3% glucosamine, 27% protein, 9% lipid, and 5% ash [17]. The proteins, potentially present as glycoproteins, are linked with hemicelluloses and together with these polysaccharides form the major parts of the strong, continuous matrix. It has also been demonstrated that polysaccharides constitute 51 and 43% of the carbon atoms in the cell walls of *C. vulgaris* and *H. pluvialis*, respectively [14]. Glucose and rhamnose and are the most abundant monosaccharides found in the cell wall of both *C. vulgaris* and *H. pluvialis*, with mannose also present in high amounts specifically in *H. pluvialis* [18]. It was reported that the trends in the cell disruption ability of the DESs were consistent with their computed dissolution capability for cell wall components, including glucose, galactose, xylose, and glucuronic acid, which was validated by the analysis of total carbohydrate content in crude extracts [19]. The cell disruption ability of glycerol-based DESs followed the increasing order: water < glucose/glycerol (1:5) < proline/glycerol (2:5) < [Ch]Cl/glycerol (1:2) < betaine/glycerol/water (1:2:2) < trimethylammonium chloride/glycerol (1:2) [19]. Furthermore, a recent molecular dynamic (MD) simulation study elucidated that cellulose chains can be disrupted by DES species such as choline and chloride ions through their interactions, leading to the loss of their crystalline structure [20].

For cell lysis pretreatment, it is crucial to select DESs that efficiently disrupt the cell wall while preserving the bioactivity of the pigments, ensuring their integrity for subsequent extraction. Several [Ch]Cl-based DESs with HDB such as ethylene glycol, glycerol, and urea have displayed their effectiveness in lysing microalgae including *C. pyrenoidosa* [20, 21], *H. pluvialis* [22], and *D. salina* [23] for the recovery of various pigments, as presented in **Table 1**. It was reported that the DESs based on polyols (e.g., ethylene glycol and glycerol) were more effective than acidic DESs in the biomass pretreatment for enhancing lutein recovery [20, 21]. Low lutein yields were obtained from *C. pyrenoidosa* pretreated with acidic DESs (e.g., [Ch]Cl/malonic acid, [Ch]Cl/formic acid, and [Ch]Cl/acetic acid). Similarly, a poor yield of carotenoids was achieved when using [Ch]Cl/oxalic acid for the pretreatment of *D. salina* [23]. This likely occurs because acidic pretreatment conditions can accelerate the undesired trans-cis isomerization of pigment molecules when they are released during cell lysis, leading to their degradation [21]. The findings suggest that, despite their strong ability to dissolve cell wall components, organic acid-containing DESs are not ideal for biomass pretreatment specifically aimed at pigment recovery due to their extreme pHs (**Figure 3**) [24]. In contrast, DESs with the pHs close to neutral and slightly alkaline are less destructive to pH-sensitive pigments like lutein. However, there are exceptions where acidic DESs have been reported to effectively microbial biomass and extract astaxanthin, which are discussed in Section 4.1.2.

Besides pigment degradation, migration of pigment into DES solution during pretreatment process can result in product loss in the subsequent extraction. Asevedo et al. [23] observed the loss of carotenoids during pretreatment process of wet cells of *D. salina* using both neat and aqueous [Ch]Cl-based DESs. The authors then proposed a one-pot extraction method by adding the extraction solvent (a mixture of ethyl acetate and ethanol) directly into the pretreatment mixture containing DES and biomass instantly after the pretreatment process for extracting carotenoids. The approach improved carotenoid recovery by 20% compared to two-step method [23]. On the other hand, some hydrophobic DESs based on DL-menthol have exhibited their capacity to disrupt cells during biomass pretreatment process, leading to the increased lutein yields in subsequent extraction compared to untreated cells [20]. However, they are preferred to be used directly as the real extraction solvents for pigments, considering their good dissolution capability towards pigments. It is therefore suggested that the appropriate DESs used for cell pretreatment should be capable of weakening microbial cell wall without dissolving the pigment of interest to prevent its loss to pretreatment solvent environment, while also preserving pigment integrity throughout the process.

Table 1. Microbial biomass pretreatment using DESs for the recovery of pigments.

Microbial species	Biomass form	Optimal pretreatment conditions					Pigment extraction and yield			Comparison yield of control (mg g^{-1} DW)	Reference
		DES (molar ratio)	Method	SLR (g mL^{-1})	T (°C)	Time (h)	Pigment	Extraction solvent	Yield (mg g^{-1} DW) (solvent)		
C. pyrenoidosa	Dry	[Ch]Cl/ethylene glycol (1:2)	Stirring	3:40	RT	5 min	Lutein	Ethanol	2.40	0.32 (untreated)	[20]
C. pyrenoidosa	Dry	[Ch]Cl /glycerol (1:2)	Stirring	1:33.3	40	20 min	Lutein	Ethyl acetate	1.35	0.91 ([1] traditional alkaline pretreatment)	[21]
H. pluvialis	Wet cell residual after chlorophyll removal	[Ch]Cl /urea (1:2)	ATPS formed by 35% DES, 30% K_2HPO_4	-	50	1	Astaxanthin	2-propanol	99%	-	[22]

			and H_2O at pH 7.5								
D. salina	Wet	[Ch]Cl /urea (1:2)	Stirring	1:5	29	24	Carote noid	Ethyl acetate and ethanol (ratio of 1:1)	84.1%	62.6% (untreated biomass)	[23]

Abbreviations: ATPS, aqueous two-phase system; [Ch]Cl, choline chloride; DES, deep eutectic solvent; DW, dry weight; RT, room temperature; SLR, solid to liquid ratio; T, temperature; -, not described.

[1] The control study was conducted by applying alkaline hydrolysis biomass pretreatment followed by pigment extraction using tetrahydrofuran.

4. Extraction and purification of microbial pigments by deep eutectic solvents

Solid-liquid extraction (SLE) is the most widely used solvent-based technique for extracting microbial pigments. It involves the mixing of microbial biomass (whether pretreated or not) with an extraction solvent under continuous stirring. Selecting an appropriate extraction solvent is a crucial step, requiring consideration of several factors, including solubility and selectivity towards product of interest, solvent cost, and safety. Traditional trial-and-error methods for optimizing DESs are costly and inefficient. Computational approaches like Hansen solubility parameters and conductor like screening model for realistic solvation (COSMO-RS) are frequently used to screen a wide range of solvent and predict the most effective solvent for a specific pigment [25]. Additionally, the growing application of machine learning algorithms uncovers essential material structure-property relationships, facilitating the tailored design of optimal DES compositions for specific tasks [26]. These approaches not only assist in shortlisting potential solvents for experimental validation, saving time and resources, but also promote the discovery and development of new solvents, guiding the design of task-specific DES.

In addition to traditional heating and stirring protocols, intensified extraction approaches with the assistance of sonication and microwave heating have been applied to enhance extraction efficiency. During ultrasound-assisted extraction (UAE), the extraction solvent, with the aids of cavitation bubbles induced by high-frequency sound waves, penetrate efficiently into microbial cells and solubilize the target compound. On the other hand, microwave-assisted extraction (MAE) employs microwave energy to rapidly and uniformly heat both solvent and microbial biomass, enhancing mass transfer and facilitating efficient extraction. At present, UAE is more relatively used in extracting pigments compared to MAE due to its milder processing conditions. Viñas-Ospino et. al [27] reported that UAE achieved more than a 2-fold higher yield than that of obtained using traditional procedure and MAE, when comparing the use of different techniques for extracting carotenoids from orange peels using L-Menthol/D, L-Camphor (1:1). The poor yield of MAE, even lower than that of the traditional method, could possibly be due to the degradation of heat-sensitive carotenoids by microwave heating [27]. The findings reveal the need to optimize process parameters of MAE, especially temperature and processing time. Similarly, UAE needs to be optimized for their parameters, including duty cycle, electrical acoustic intensity, solid to liquid ratio (SLR), and the solution's pH. It was demonstrated that the yields of phycobiliproteins from the lyophilized biomass of cyanobacteria *Oscillatoria* sp. BTA170 could be enhanced by up to 95% through the optimization of these factors compared to that obtained under unoptimized conditions [28].

Apart from typical methods of SLE using a single solvent or a mixture of solvents in a homogeneous phase, SLE has been explored to integrate with aqueous two-phase system (ATPS), where the immiscible aqueous phases are brought into direct contact with microbial biomass, allowing for concurrent extraction and purification. The ATPS can be designed to selectively extract the target pigment into an appropriate phase, which in turn separating it from solid microbial matrix and other co-extracted impurities that migrate into the opposite aqueous phase [5, 29]. Furthermore, the combined use of SLE method with liquid-liquid extraction (LLE) concept, using two immiscible liquids, has been assessed for extracting pigment directly from microbial biomass. For example, a water-stable hydrophobic DES can form an immiscible LLE system with an aqueous solution containing a buffer salt or a hydrophilic DES. Meanwhile, in common purification protocols, ATPS and LLE are usually loaded with the pigment-rich crude extract, which has been cleared of cell debris, to minimize interferences from cells or their debris and enhance the purity of the final product [30]. Another technique, dispersive liquid-liquid microextraction (DLLME), which involves extracting product from an aqueous solution into a dispersion of extraction phase droplets, has also been assessed for the extraction of microbial pigments [29].

Adhering to the guiding principle of "like dissolves like", it can be inferred that, in general, hydrophobic DESs are likely more efficient for extracting non-polar carotenoids, while highly polar, free carotenoids and phycobiliproteins are expected to be more favorably extracted by hydrophilic DESs, as summarized in **Table 2** and **Figure 4** [31, 32]. Based on the literature in **Table 2**, thymol-based DESs were found to be effective for extracting carotenoids (including fucoxanthin, astaxanthin, and lutein), particularly from dry microbial biomass in most studies. The π-electron-rich benzene ring in thymol enhances its ability to interact with carotenoids containing conjugated double bonds and functional groups, facilitating their solubilization and extraction from microbial biomass. These interactions were elucidated in the MD study, which showed that thymol establishes van der Waals interactions with the ε-ionone ring of the lutein molecule through its aromatic ring [13]. The interactions also stabilize carotenoids within the thymol-based DES matrix, especially at high temperatures [33]. These findings show that the DESs containing π-electron donor groups seems promising [34]. Despite that, the state of microbial biomass, whether it is dry or wet, and whether it has been subjected to cell lysis pretreatment prior to extraction process, significantly influence the selection of solvent and extraction technique. The DESs used must be capable of improving miscibility and cell wall permeabilization particularly when working with wet cells. Additionally, when processing untreated cells, DESs need to serve a dual role to achieve efficient extraction: they need to possess the capacity to weaken microbial cell walls, while having the ability to dissolve the pigment of interest. The use of DESs for the extraction and purification of each pigment is discussed in the sub-sections.

Table 2. Microbial-derived pigments by extraction (and selective extraction) methods using DESs.

Pigment	Microbial species	Type of DESs tested (molar ratio)	Biomass form and pretreatment	Optimal extraction conditions						Comparison on yield of control (mg g^{-1} DW) (solvent)	Reference
				DES (molar ratio)	Method	SLR (g mL^{-1})	T (°C)	Time (h)	Yield (mg g^{-1} DW)		
Fucoxanthin	*T. lutea*	Thymol/dodecanoic acid (1.25:1); thymol/decanoic acid (1:1); thymol/octanoic acid (1:1.38); tetrabutylammonium chloride/decanoic acid (1:2); tetrabutylammonium chloride/octanoic acid (1:2); tetrabutylammonium chloride/acetic acid (1:2)	Dry, milling to 200 µm	Thymol/dodecanoic acid (15:1)	Heating and stirring	1:8.8	36.2	2.58	22.0	6.8 (ethanol)	[35]
Astaxanthin	*H. pluvialis*	DL-menthol/oleic acid (2:1); thymol/oleic acid (3:1, 1:1); geraniol/oleic acid (13:1, 2:1)	Dry	Thymol/oleic acid (3:1)	Stirring	1:40	RT	24	83%	[1]100% (mixed organic solvent)	[36]
Astaxanthin	*Xanthophyllomyces*	Thymol/octanoic acid (1:1); thymol/menthol (1:1); thymol/salol (1:1); thymol/camphor (1:1);	Dry, [3]PEF-	Thymol/salol (0.3:1)	Shaking	1:100	20	24	79%	[2]100% (DMSO)	[37]

	dendrorhous	menthol/octanoic acid (1:1); menthol/camphor (2:1)	treatment								
Astaxanthin, β-carotene	*Phaffia rhodozyma*	[Ch]Cl/lactic acid (1:1, 1:2); [Ch]Cl/butyric acid (1:2, 1:5)	Wet	[Ch]Cl/ butyric acid (1:5) aqueous solution (50% (w/w) in H_2O)	Heating and stirring	1:5	65	1	81.8% astaxanthin, 82.3% β-carotene	38.6% astaxanthin, 43.1% β-carotene (DMSO)	[38]
Astaxanthin	*Paracoccus carotinifaciens*	[Ch]Cl/acetic acid (1:3); [Ch]Cl/butyric acid (1:3); [Ch]Cl/glycerol (1:3); [Ch]Cl/ethylene glycol (1:3); [Ch]Cl/propylene glycol (1:3)	Dry	[Ch]Cl/ acetic acid (1:3)	UAE	1:20	65	8 min	1500 μg mL^{-1} extract	-	[39]
Astaxanthin	*H. pluvialis*	[Ch]Cl/D-ribose; [Ch]Cl/ D-fructose; [Ch]Cl/sucrose; [Ch]Cl/D-glucose	Dry	[Ch]Cl/ D-fructose	ATPS formed by IL (tributyloctylphosphine chlorid	1:20	35	3	2.35 partitioned to IL-rich phase	2.23 (acetone)	[5]

					e), DES and H_2O						
Canthaxanthin	*C. zofingiensis*	[Ch]Cl/1, 3-butanediol (1:3); [Ch]Cl/1, 2-propanediol (1:2); [Ch]Cl/1, 4-butanediol (1:6); [Ch]Cl/1, 3-propanediol (1:2); [Ch]Cl/ethylene glycol (1:2); [Ch]Cl/glycerol (1:2); ethylene glycol/D, L-malic acid (1:1); glycerol/D, L-malic acid (1:1); decanoic acid/octanoic acid (1:3); lauric acid/octanoic acid (1:3); lauric acid/nonanoic acid (1:3); D, L-menthol/decanoic acid (3:1); D, L-menthol/lauric acid (3:1); D, L-menthol/lactic acid (3:1); lauric acid/decanoic acid (1:3)	Dry	Octanoic acid/decanoic acid (2.3:1)	UAE	1:15	50	49 min	70.4 µg mL^{-1} extract	62.2 µg mL^{-1} extract (ethanol)	[40]
Bacteriorubеrin	*Haloferax mediterranei*	Menthol/acetic acid (1:1); menthol/butyric acid (1:1); menthol/decanoic acid (1:1); menthol/lactic acid (1:1); menthol/levulinic	Wet	Menthol/levulinic acid (1:1)	Stirring	1:10	25	1.8	5.2 mg g^{-1} wet	0.76 mg g^{-1} wet biomass (ethanol)	[41]

		acid (1:1); menthol/thymol (1:1)							biomass		
Lutein	*Scenedesmus* sp.	Fenchyl alcohol/thymol (1:1); menthol/camphor (1:1); thymol/menthol (1:1); α-terpineol/fenchyl alcohol (1:1); menthol/fenchyl alcohol (1:1); thymol/camphor (1:1); camphor/α-terpineol (1:1); thymol/coumarin (1:1); menthol/α-terpineol (1:1); thymol/α-terpineol (1:1)	Dry	Fenchyl alcohol/thymol (1:1)	Heating and shaking	1:50	60	1.17	6.3	5.4 (mixed solvent of n-heptane, ethanol and H_2O)	[13]
C-phycocyanin	*A. platensis*	Glycerol/glucose (2:1); glycerol/betaine (2:1); glycerol/proline (4:1); glycerol/glucose/betaine (4:1:1); glycerol/glucose/proline (4:1:1)	Wet	Glycerol/glucose (2:1) aqueous solution (≈70 % (w/w) in H_2O)	UAE	1:10 g g^{-1}	35	1	8.12	-	[42]

C-phycocyanin	*A. platensis*	Glycerol/glucose (1:1); glycerol/xylose (1:1); glycerol/fructose (1:1); glycerol/sucrose (1:1); glycerol/maltose/H_2O (1:1:0.5)	Dry	Glycerol/xylose (1:1) aqueous solution (70 % (v/v) in H_2O)	MAE-DLLME formed by DES, sodium sulphate and H_2O	1:4.35 (addition of salt)	74.85	6 min	86%	-	[29]
B-phycoerythrin	*Porphyridium cruentum*	Glycerol/glucose (2:1); glycerol/betaine (2:1); glycerol/proline (2:1); glycerol/glucose/betaine (4:1:1)	Wet	Glycerol/glucose (2:1), glycerol/betaine (2:1) and glycerol/glucose/betaine (4:1:1) aqueous solutions (70% in H_2O)	UAE	1:10	-	1	~12.5	23.9 (H_2O)	[43]

B-Phycoerythrin	*Porphyridium purpureum*	Trimethylammonium chloride/glycerol (1:2); [Ch]Cl/glycerol (1:2); betaine/glycerol/water (1:2:2); L-proline/glycerol (2:5); glucose/glycerol (1:5)	Wet	L-proline/glycerol (2:5) aqueous solution (75% in H_2O)	UAE	1:8.5 g g^{-1}	25	10 min	1.258	0.093 mg g^{-1} (H_2O)	[19]

Abbreviations: ATPS, aqueous two-phase system; [Ch]Cl, choline chloride; DES, deep eutectic solvent; DLLME, dispersive liquid-liquid microextraction; DMSO, dimethyl sulfoxide; DW, dry weight; MAE, microwave-assisted extraction; PEF, pulsed-electric field; RT, room temperature; SLR, solid to liquid ratio; T, temperature; UAE, ultrasound-assisted extraction; -, not described.

[1] The total content of astaxanthin in microbial cells was determined using a mixed solvent of cyclohexane, ethanol, and acetone, and this value was taken as 100% for control and comparison purposes in the study.

[2] The total content of astaxanthin in microbial cell was determined by bead-beating-assisted extraction using DMSO, and this value was taken as 100% for control and comparison purposes in the study.

[3] The fresh biomass was pretreated by PEF followed by 24 h-aqueous incubation before freeze drying.

4.1 Carotenoids

Carotenoids are grouped into two major classes, namely oxygen-containing xanthophylls (e.g., fucoxanthin, astaxanthin, canthaxanthin, and lutein) and carotenes that are purely hydrocarbons (e.g., β-carotene, torulene, and lycopene). They give yellowish-orange-reddish colors to food products. Typically, the processing of polar carotenoids involves the use of polar solvents like acetone and ethanol, whereas non-polar carotenoids and esterified xanthophylls are more effectively extracted by non-polar solvents such as petroleum ether, *n*-hexane, and tetrahydrofuran [44].

4.1.1 Fucoxanthin

Fucoxanthin, a yellow-orange pigment, is exclusively sourced from the aquatic environment, specifically from brown macroalgae and microalgae species. Because of its long-conjugated double-bond chain, fucoxanthin displays relatively hydrophobic in nature. However, the presence of oxygen-containing functional groups such as epoxide and hydroxyl groups confer it polar character and hydrogen-bonding ability [45]. A recent COSMO-RS findings showed that the DESs composed of thymol and medium-chain carboxylic acids have superior extraction capacities for fucoxanthin compared to the commonly used organic solvents (e.g., ethanol and methanol), while [Ch]Cl-based DESs were predicted to be the least effective in solubilizing fucoxanthin [35]. Further experimental results validated that the extraction ability of thymol/carboxylic acid-based DESs followed the trends of increased carbon chain's length of carboxylic acids, in the order: thymol/octanoic acid < thymol/decanoic acid < thymol/dodecanoic acid. The best DES successfully yielded 22.0 mg g^{-1} dry weight (DW) of fucoxanthin from the dried and milled *T. lutea* under the optimized conditions, which was significantly higher than the 6.8 mg g^{-1} DW obtained using ethanol [35]. In contrast, as predicted, hydrophilic DESs such as [Ch]Cl/lactic acid and aqueous glucose/lactic acid, as shown in another work, achieved relatively low yields at approximately 0.2 mg g^{-1} DW from seaweed *Fucus vesiculosus* even with the assistance of sonication, compared to traditional maceration protocol using ethanol yielding 1.1 mg g^{-1} DW [46]. This may also be due to the degradation of fucoxanthin under acidic conditions, since fucoxanthin is unstable at pH < 4 [47]. The development of DES for extracting fucoxanthin is still at a very early stage, and hence traditional solvents like acetone and ethanol are still widely used.

4.1.2 Astaxanthin

The red pigment astaxanthin can be found in microalgae *H. pluvialis* in esterified form as monoester with long-chain fatty acids for preserving its stability, while those synthesized in yeast *X. dendrorhous* (formerly *P. rhodozyma*) and bacterium *P. carotinifaciens* are in the free form. Free astaxanthin is bioavailable and readily absorbed by organisms during consumption, and hence dried astaxanthin-rich biomass of *P. rhodozyma* and *P. carotinifaciens* are widely used as additives in aquaculture and animal feed [48, 49]. On the other hand, astaxanthin derived from *H. pluvialis* occupies market segment of dietary supplements and food [50]. Nevertheless, there is ongoing research development of astaxanthin-rich extract from *P. carotinifaciens* for human nutrition applications [51].

Similar to fucoxanthin, astaxanthin displays a complex polar-nonpolar-polar nature because of the presence of two terminal β-ionone rings linked with conjugated double bonds. To date, astaxanthin from various microbial hosts has been extensively explored for the extraction using DESs compared to other carotenoids. Hydrophobic DESs (e.g., menthol/myristic acid [52], thymol/oleic acid [36], and thymol/salol [37]) displayed good extraction ability towards astaxanthin. There are exception of two studies reporting the effective use of hydrophilic [Ch]Cl/butyric acid aqueous solution [38] and [Ch]Cl/acetic acid [39] for extracting astaxanthin from *X. dendrorhous* and *P. carotinifaciens*, respectively. The findings may be attributed to the high cell wall solubilizing capacity of these DESs towards wet cells and the different form of carotenoids present in microbial host.

Rodrigues et al. [52] reported that the best DES, menthol/myristic acid, at 60 °C and 2 h extraction process, achieved a yield of astaxanthin from crab shell waste comparable to that of a 6-h Soxhlet extraction with acetone. Additionally, the authors observed a 3- to 657-fold increase in yields compared to the Soxhlet approach using acetone when the identical DES-based extraction method was applied to *H. pluvialis* biomass and mussels. The DESs composed of terpene (e.g., menthol, thymol, and geraniol) and oleic acid have also been explored for their applicability to extract astaxanthin, which was predominantly in the monoester form, from both freeze-dried *H. pluvialis* biomass and culture broth [36]. The three DESs tested recovered 60% of astaxanthin from freeze-dried biomass after stirring at room temperature for 6 h, and the yields increased to 83% when the extraction was prolonged to 24 h. However, LLE approach applied directly to culture broth using the same DESs yielded only 30% after 6 h and at most 68% after 48 h. These findings evidenced that the presence of water in culture broth hinders the efficiency of solvent-cell contact, and thus restricting extraction yields [36]. Besides, the findings prompt further inquiry into the effectiveness of hydrophobic DESs to disrupt and penetrate untreated wet cells for the extraction of intracellular products. In accordance with the findings, Artigas-Hernández et al. [37] reported that the pretreatment of fresh cells of *X. dendrorhous* with electroporation using pulsed-electric field (PEF), followed by a 24-h aqueous incubation before lyophilization, enhanced the extraction efficiency of astaxanthin from freeze-dried cells using thymol/salol from 55 (for untreated cells) to 79%. In the study, thymol/salol appeared to be the best solvent to extract astaxanthin from both untreated and treated biomass over the other five terpene-based DESs evaluated [37]. On the other hand, Mussagy et al. [38] elucidated that the solvent with combined polar (e.g., [Ch]Cl) and non-polar (e.g., butyric acid) constituents was more effective for extracting astaxanthin from wet cells of *P. rhodozyma*, with the assistance of heating at 65 ºC for 1 h. The extraction ability of the solvents tested in the presence of 50% H_2O followed the order: [Ch]Cl < butyric acid ≈ [Ch]Cl/butyric acid (1:2) < [Ch]Cl/butyric acid (1:5). It was suggested that the solvent with an amphiphilic nature is favored when using wet biomass, since its hydrophilic parts can promote miscibility with wet biomass facilitating cell wall lysis, while its hydrophobic domains enable the solubilization of the targeted pigment molecules. Likewise, another work reported that [Ch]Cl/carboxylic acid-based DESs, such as [Ch]Cl/butyric acid and [Ch]Cl/acetic acid, displayed superior capacity in extracting astaxanthin from dried bacterium cells of *P. carotinifaciens* using UAE at 65 ºC for 8 min [39]. In contrast, alcohol-based counterparts (e.g., propylene glycol, ethylene glycol, and glycerol) were found to lack the capability to extract astaxanthin from these cells, though bacterium cells have less rigid cell walls compared to microalgae and yeast.

In most of the studies, moderate temperatures around 60 – 65 °C were used to enhance extraction efficiency by increasing the solubility of astaxanthin and reducing the viscosity of DESs. It was reported that the increasing of the extraction temperature to 60 °C resulted in a 1.2-fold increase in yield compared to that of achieved at room temperature [36]. However, extraction temperature is always limited to 65 °C and below to prevent isomerization and degradation of astaxanthin. UAE has also been used to enhance extraction efficiency by leveraging mechanical and thermal effects of cavitation formed. It was shown that, by applying the identical extraction solvent, SLR, and temperature, UAE operating at 65 °C and an amplitude of 15% for 8 min increased the astaxanthin yield by 900% compared to that obtained using conventional heating and stirring approach operating at 300 rpm using a stirrer hot plate for 10 min [39].

Apart from typical SLE technique, selective extraction using one-step integrated SLE and ATPS platform has been explored. The ATPS composed of ionic liquid (IL) and

[Ch]Cl/sugar-based DES has been proposed to selectively extract astaxanthin ester and free astaxanthin from *H. pluvialis* [5]. It is worth noting that in the study, although the IL tributyloctylphosphine chloride appeared to have played a crucial role as the main extractant for astaxanthin ester (the main form of astaxanthin found in *H. pluvialis* biomass), the presence of [Ch]Cl/fructose improved the IL's extraction capability significantly. [Ch]Cl/fructose alone was incapable of extracting any astaxanthin from microalgal biomass but combining it with the IL to form an ATPS and applied the system for extraction has achieved high yield of astaxanthin ester at 2.35 mg g^{-1} in IL-rich phase than that of obtained using IL only (0.20 mg g^{-1}). The yield was even comparable to that of obtained using organic solvents such as ethanol (1.96 mg g^{-1}), methanol (2.19 mg g^{-1}), and acetone (2.23 mg g^{-1}) [5]. The system also facilitated a certain degree of product purification, but it's full separation potential might not have been fully examined, since the majority of astaxanthin from *H. pluvialis* exists in the ester form with limited free astaxanthin available for separation. The integrated SLE-ATPS technique is worth exploring further to expand its applicability, especially for simultaneous extraction and purification of microbial pigment. It is a pre-requisite to first investigate the aptitude of DESs to form ATPS with different phase constituents before the extraction investigation.

4.1.3 Canthaxanthin

A ketocarotenoid similar to astaxanthin, canthaxanthin is a strong reddish-orange pigment considered safe for use as a food colorant by the United States Food and Drug Administration (FDA) [53] and the European Food Safety Authority (EFSA) with E161g classification [54]. While canthaxanthin is present in very low concentrations in natural sources, the vast majority of commercially available canthaxanthin is chemically synthesized from petrochemicals. The increased preference for natural canthaxanthin has promoted advancement in genetic engineering of microbial hosts for canthaxanthin production [55, 56].

Lately, Yang et al. [40] demonstrated that all seven hydrophobic eutectic solvents tested displayed comparable or even superior capacities to ethanol and *n*-hexane in extracting canthaxanthin from lyophilized *C. zofingiensis* using UAE at 50 °C. Under the optimized conditions, the yield obtained from the unmilled dry biomass using the most promising solvent, namely octanoic acid/decanoic acid (70.4 μg mL^{-1} extract), surpassed that of achieved using ethanol applied to the ground biomass (62.2 μg mL^{-1} extract). The results reveal that octanoic acid/decanoic acid was capable of performing dual functions, that were cell lysis and canthaxanthin extraction [40]. On the other hand, aqueous hydrophilic eutectic solvents containing 30% of water had poor extraction capability for canthaxanthin [40]. However, another report showed that ethanol and n-hexane achieved lower extraction efficiency for canthaxanthin from bead-beaten disrupted yeast cells compared to chloroform [57], suggesting the need for future research using more appropriate organic solvents for comparative analysis.

4.1.4 Bacterioruberin

Bacterioruberin, a C50 carotenoid, is among the rare carotenoids synthesized exclusively by a limited number of microorganisms such as haloarchaea. It is comprised of an isoprenoid chain with thirteen conjugated double-bond carbon units and four functional OH-groups arising terminally. Kholany et al. [41] demonstrated that menthol/levulinic acid (1:1) obtained a 4-fold yield improvement over ethanol for extracting bacterioruberin from wet biomass of *Haloferax mediterranei*. In contrast, other menthol-based counterparts, such as menthol/acetic acid, menthol/butyric acid, menthol/decanoic acid, menthol/lactic acid, and menthol/thymol, showed no ability to extract bacterioruberin from wet cells in the absence of water. The authors also observed an excessive clamping of wet biomass during the extraction process when applying the solvents that were formed by two hydrophobic parent compounds (e.g., menthol/decanoic acid and menthol/thymol). It was therefore suggested by the authors that the presence of a small hydrophilic carboxylic acid in the menthol-based solvents is essential to act as an adjuvant for weakening cell wall of wet biomass facilitating the extraction of intracellular pigment. The finding was in a good agreement with the work of Mussagy et al. [38] on wet *P. rhodozyma* cells for astaxanthin extraction, highlighting the crucial role of an amphiphilic extraction solvent especially when processing wet biomass.

4.1.5 Lutein

Lutein is a yellow xanthophyll carotenoid containing a C40 isoprenoid backbone with oxygen-containing rings at both ends. It is applied as feed and food additives (E161b) [58]. At present, lutein used for commercially marketed dietary supplements and nutraceuticals is exclusively derived from marigold flowers (*Tagetes erecta*) that are a rich natural source of lutein esters. However, lutein from microalgae shows appealing development and commercial potential [59].

Similar to other xanthophylls having oxygen-containing functional groups, lutein is polar to some extent. Fan et al. [13] reported the use of hydrophobic DESs that possessing lutein-stabilizing capability for extracting lutein from dry *Scenedesmus* sp. biomass. Amongst the DESs prepared from the combination of menthol, thymol, α-terpineol, camphor, fenchyl alcohol, and coumarin, the equimolar fenchyl alcohol/thymol displayed the greatest capability to extract lutein at 60 °C for 70 min, and its yield was significantly higher than that of obtained using ethyl acetate. The experimental results were in a good agreement with the solubility data of lutein in the solvents predicted by COSMO-RS. Interestingly, apart from microbial biomass, Gabriella et al. [60] demonstrated the viability of integrating the use of hydrophobic eutectic solvents and enzymatic lysis, forming a LLE system comprised of a hydrophobic phase and an aqueous phase for the recovery of carotenoid and phenolic compounds from sunflower waste, respectively. With the assistance of enzymatic lysis, D,L-menthol/D,L-lactic acid (1:2) yielded a 2.3-fold recovery of carotenoids composed of mainly lutein when compared to that of acquired by *n*-hexane under the same process conditions [60]. The compelling findings highlight the potential application of this technique to process microbial biomass, especially those with rigid cell walls that require enzymatic hydrolysis of cell wall to liberate intracellular compounds.

4.1.6 β-carotene

Carotenes are the unoxidized compound group of carotenoids. One of the most well-known carotenes is β-carotene, which is responsible for the orange color of many fruits and vegetables. It serves as a food colorant (E160a) and also as a provitamin A in dietary supplements. Apart from plant source, natural β-carotene is industrially produced by fermentation of *B. trispora* and approved for food use. On the other hand, microalgae *D. salina* is a significant bio-producer, since it can accumulate β-carotene up to 14% of its DW [61]. β-carotene is lipophilic, and therefore non-polar solvents are used for its extraction, with vegetable oils occasionally being explored.

The use of DESs specifically for extracting only β-carotene from microbial biomass has relatively underexplored. Mussagy et al. [38] reported that, along with astaxanthin, β-carotene was also extracted by [Ch]Cl/butyric acid at 65 ºC from wet *P. rhodozyma* biomass. The results demonstrated that the molar ratio of HBA and HBD in the concentrated solution of [Ch]Cl/butyric acid significantly influenced the selectivity of carotenoid extracted, with a HBA/HBD ratio of 1:5 showing a balanced recovery of β-carotene and astaxanthin, whereas an increase molarity of HBD (molar ratios of 1:6 – 1.8) increasingly favored the selective extraction of β-carotene over astaxanthin. This implies that an increase in the relative hydrophobicity of eutectic solvents promotes the extraction of the more non-polar compound.

Several works have explored the use of DESs for extracting β-carotene from vegetable and agricultural waste such as pumpkin [62] and its peels [63], and orange peels [64]. The findings of COSMOtherm conducted by Viñas-Ospino et al. [64] showed that β-carotene has a good solubility in hydrophobic DESs particularly L-menthol/D,L-camphor (1:1) and L-menthol/eucalyptol (1:1) at 60 °C after a screening of sixty-eight hydrophobic and hydrophilic DESs. The efficiency of the identified DESs was proven by experimental work using UAE applied to orange peels. Another work reported that, among the ten DESs based on C8 – C12 fatty acids tested, UAE with caprylic acid/capric acid (3:1) achieved the highest efficiency in extracting β-carotene from freeze-dried and pulverized pumpkin [62]. Given these supporting findings, it is worthwhile to investigate the use of DES for extracting microbial β-carotene. Despite the significant variability in the processing methods for microbial biomass compared to plant-based matrices, which is influenced by their unique cellular structure and associated biochemical composition, the efficacy of hydrophobic DESs in dissolving and stabilizing β-carotene has undoubtedly been proven in these works.

4.2 Chlorophylls

Chlorophylls are the most abundant bioactive pigments found in green plants, algae, and some bacteria. They are valuable in food as authorized green colorants (E141), with their vibrant green hue being widely appreciated by consumers.

Chlorophylls can be extracted either directly from their sources or from the residual biomass after extracting lipids [65] and pigmented proteins [66]. In instances where microbial hosts synthesize multiple groups of hydrophobic pigments such as carotenoids alongside chlorophylls, they are usually co-extracted in solvent-based extraction due to their shared cellular localization [67]. These co-extracted products need to be separated to achieve better purity for their intended application. Singh et al. [68] explored the use of LLE based on hydrophobic DES for separating chlorophylls from fucoxanthin in the crude extract that was prepared using SLE with a hydrophilic DES from diatom microalgae *Thalassiosira andamanica*. Benzyltrimethylammonium chloride/caprylic acid (1:2) was used due to its capability to form a LLE system with the aqueous hydrophilic DES, effectively transferring chlorophylls to the upper hydrophobic phase and thus allowing efficient recovery and reuse of the hydrophilic DES [68]. There are also several reports involving the assessment of DESs for extracting multiple classes of pigment, including chlorophylls, which are discussed in Section 4.4.

4.3 Phycobiliproteins

Phycobiliproteins are light-harvesting pigment-protein complexes responsible for the bright color of cyanobacteria, red algae, and cryptomonads. Based on the structure and chromophore composition, they are categorized into allophycocyanin, phycocyanin, and phycoerythrin which exhibit characteristics spectrum described as blue-green (650 – 655 nm), blue (610 – 620 nm), and pink (540 – 570 nm), respectively. Besides their use as food colorants, phycobiliproteins are highly recognized for their unique fluorescence characteristics and diverse nutraceutical benefits [69, 70]. The application of phycobiliproteins depends on their purity levels. A purity of phycocyanin at 0.7 is suitable for food use, 1.5 for cosmetics, and > 4.0 for therapeutic treatments [71].

These pigments are water-soluble, and hence their extraction from microbial host usually involves the use of distilled water or buffer solution accompanied with a cell disruption operation (e.g., high speed homogenization, freeze-thawing, and sonication) [30, 72]. After extraction, phycobiliprotein-rich extract typically undergoes purification processes such as ammonium sulfate precipitation, membrane separation, and chromatography to meet the desired level of purity [73]. Alternative methods such as ATPS using sustainable phase former like DESs have also been proposed.

4.3.1 Phycocyanin

Phycocyanin is regarded as a valuable natural food colorant because of its rare intense blue color and protein-based structure. The well-recognized form, C-phycocyanin, which binds only phycocyanobilin as the sole chromophore, is abundant in cyanobacteria such as *A. platensis* (formerly *Spirulina platensis*) and *Aphanizomenon flos-aquae*. It was suggested to use wet biomass for extracting phycocyanin, since the drying pretreatment (e.g., spray, oven, and cross flow drying) caused approximately 45% loss of pigment [74]. C-phycocyanin is sensitive to pH and temperature. It stays stable for longer periods at lower temperatures within the pH 5 – 7.5 but became unstable at temperatures exceeding 40 °C [74]. Therefore, the extraction process needs to be developed considering the limitations in temperature and pH.

The findings from studies using DESs for the extraction of C-phycocyanin are varied, likely depending on the specific DESs tested. Several aqueous solutions of DESs such as glycerol/glucose (2:1), glycerol/glucose/betaine (4:1:1), and glycerol/glucose/proline (4:1:1) have displayed abilities in extracting and stabilizing C-phycocyanin from wet *A. platensis* [42]. The extraction temperatures between 30 – 35 °C, with an extraction time of 60 min, and the addition of 20 – 30% water, were shown to be optimal for the UAE process using DESs [42]. In contrast, Fabre et al. [75] demonstrated that the UAE using both neat and diluted [Ch]Cl-based DESs with mixed buffer solution achieved much lower yields and selectivity compared to that of using only phosphate buffer solution. Besides, poor yields were obtained by [Ch]Cl-based DESs containing levulinic and itaconic acid, likely due to acid denaturation of C-phycocyanin [75]. Zhuang et al. [4] also observed the color change of phycocyanin from blue to yellow-green when the pH was adjusted gradually from 7 to 9, indicating the inactivation of protein-pigment structure under alkaline conditions. These findings highlight the critical need of maintaining a biocompatible pH environment for the extraction of C-phycocyanin, which must be considered when selecting a suitable solvent. The DESs formed by alkyl ammoniums (e.g., [Ch]Cl and betaine), polyols (e.g., glycerol, sorbitol, and xylitol) and carbohydrate (e.g., glucose, fructose, and sucrose) have mild pHs ranging from 4.5 to close to neutral, which seems appropriate [76]. Additionally, sugars like glucose and sucrose have shown to increase thermal stability of C-phycocyanin [77]. However, these DESs usually possess high viscosities that need to be overcome by dilution, which may in turn affect their extraction capacity. In most studies, the effective concentration of DESs in water is typically maintained at 70% and above to retain the DESs' supramolecular structure while improving solvent properties.

DESs have also been explored for their potential in selectively extracting and purifying C-phycocyanin using LLE techniques, such as DLLME composed of a hydrophilic DES with Na_2SO_4 [29] and ATPS formed by a water-miscible DES with K_2HPO_4 [4, 78]. It was reported that C-phycocyanin preferably partitioned into the aqueous DES-rich top phase during MAE with an ATPS composed of glycerol-based DES and Na_2SO_4, achieving a maximal yield of 86% from *A. platensis* powder [29]. The extraction temperature used in this work was high, close to 75 °C, but with a short processing time of 6 min, assisted by rapid microwave heating, to prevent product degradation [29]. On the other hand, ATPS based on [Ch]Cl/urea (1:2) and K_2HPO_4 achieved the highest purity and recovery yield of C-phycocyanin from crude extract over the other ATPSs formed by [Ch]Cl-based counterparts containing glucose, fructose, glycerol, and ethylene glycol [78]. The selected ATPS, under optimal conditions, yielded C-phycocyanin with a purity of 3.38 and a recovery rate of 66%. Likewise, another report validated the potential of ATPS based on [Ch]Cl/urea for purifying phycocyanin [4]. In addition to the high yield in its ATPS application, [Ch]Cl/urea showed good phase-forming capabilities, distinguishing it as a better option compared to [Ch]Cl/glycerol, which also had achieved a comparable extraction efficiency but exhibited a weaker phase-forming ability. Most [Ch]Cl-based DESs, with common HBA/HBD molar ratio of 1:1, showed better abilities to form ATPS with K_2HPO_4 compared to their glycerol counterparts, following the rankings: glycerol/glucose < [Ch]Cl/glycerol < glycerol/sucrose < [Ch]Cl/fructose < [Ch]Cl/glucose < [Ch]Cl/urea. In this work, the system composed of [Ch]Cl/urea (1:1) and K_2HPO_4 with adjusted pH 7, under optimal conditions, achieved a higher recovery yield of 92% but with a lower purity factor of 1.25 when compared to that of obtained from the previous report using [Ch]Cl/urea with a different molar ratio, that was 2:1 [4, 78].

Interestingly, very recently, LLE systems composed of hydrophobic DESs and phosphate buffer have been assessed for C-phycocyanin partitioning [32]. Not all hydrophobic DESs can form stable immiscible two-phase system with aqueous buffer solution. Furthermore, their biocompatibility with C-phycocyanin must be considered. The system comprised of decanoic acid/thymol (2:3) and 24 wt% phosphate buffer solution, at a volume ratio of 1:1, displayed ability to promote the migration of C-phycocyanin into hydrophobic DES phase, with a high partition coefficient of 29.4 and a recovery yield of 99% from the feed containing C-phycocyanin. The findings demonstrated the potential of hydrophobic DES-based LLE system to recover a polar pigment like C-phycocyanin from aqueous phase [32]. However, future work is needed to validate the system's purification capability using crude extract prepared from microalgae biomass, since the presence of contaminant proteins in crude extract may interfere with the partitioning behavior of protein of interest.

4.3.2 Phycoerythrin

Phycoerythrin is a red-colored pigment-protein complex found in red algae and cyanobacteria. Its different forms include R-phycoerythrin, C-phycoerythrin and B-phycoerythrin, which are distinguished by their different spectral properties and photosynthetic organism producers. R-phycoerythrin can be found in red marine macroalgae (e.g., *Gelidium pusillum*), while B-phycoerythrin is derived from single-celled red microalgae (e.g., *Porphyridium* sp.). Compared to the formers, C-phycoerythrin from cyanobacteria is still under early development [79].

Hydrophilic DESs based on glycerol have been explored to extract phycoerythrin using UAE from wet biomass of *P. cruentum* and *P. purpureum*, respectively. However, significantly different findings were reported in these studies, even though some of the DESs tested had the same components but different molar ratios, such as glycerol/glucose and glycerol/proline [62, 63]. Gheluwe et al. [43] reported that the four glycerol-based DESs tested, regardless of the addition of water ranging from 20 to 40%, obtained a maximum yield of 12 mg g^{-1} dry biomass, which was only half of that acquired by using water as the extraction solvent under the same UAE process conditions for 1 h. On the other hand, Li et al. [19] showed that the aqueous solutions of five glycerol-based DESs assessed, at the concentrations of 70%, achieved higher yields than those obtained using water, up to 13.5 folds higher, when using UAE for 10 min. The crude extract obtained from the extraction using proline/glycerol (2:5) was further purified using an ATPS with polypropylene glycol 400, two-step ammonium sulfate precipitation and ultrafiltration processes to obtain a final purity ratio of 3.6 [19]. Apart from that, DES-based ATPS using [Ch]Cl/urea and K_2HPO_4 has also successfully applied for purifying R-phycoerythrin from marine algae, *Porphyra yezoensis* [80]. By using a two-step ammonium sulphate precipitation step followed by the optimized DES-based ATPS, the purity of R-phycoerythrin was improved to 3.825 from an initial of 0.713 in crude extract, with a yield of 70% [80]. The purity level achieved in these studies were comparable to that of commercially available analytical grade, revealing the potential of DES-based extraction and purification techniques.

4.4 Total carotenoids, chlorophylls, and phycobiliproteins

Several studies have explored the extraction capabilities of DESs towards multiple bioactive pigments in microalgae. Ozel et al. [81] found that the UAE using [Ch]Cl/acetic acid (1:2) and [Ch]Cl/urea (1:2), respectively, at 50 ºC for 1 h, yielded crude extracts containing not only carotenoid but also protein, phenolics, and carbohydrate from freeze-dried microalgal biomass. Another work discovered that the glucose/glycerol/water (1:2:4) yielded a crude extract with an interesting composition suitable for skincare product formulation, containing multiple polar and non-polar compounds including phycocyanin, carotenoids, chlorophylls, and polyunsaturated fatty acids (PUFAs), from *A. platensis* after UAE for 30 min [82]. These findings showed the feasibility of obtaining mixed extract enriched with multiple pigments and antioxidants, which are ideal for use in nutraceuticals and cosmetic formulations.

Alternatively, Hilali et al. [83] proposed the use of an integrated UAE-LLE platform using a hydrophilic DES (glycerol/glucose (2:1) with 20% water added) and a hydrophobic DES (menthol/1,2-octanediol (1:1)), forming a LLE system for concurrently extracting and separating both polar and nonpolar compounds from wet cells of *A. platensis* in a single-step. After extraction, the carotenoid-enriched fraction (along with PUFAs) and the C-phycocyanin-rich extract can be easily separated for their respective applications. The integrated method increased the productivity by four-fold for chlorophylls and free fatty acids, and two-fold for carotenoids compared to the sequential extraction method [83]. In view of the promising results, future work should focus on exploring its scalability and process optimization.

5. Development potential and challenges

Despite promising results reported in the literature, there have been no commercial breakthroughs. In particular, DES-based extraction methods for microbial pigments are a relatively new and emerging technology. The investigations covered in this review are limited to laboratory-scale, and pilot-scale operations have yet to be established. They have not progressed beyond technology readiness level (TRL) 3, which is experimental proof of concept. Several challenges need to be addressed to achieve widespread adoption, as illustrated in **Figure 5**.

5.1 Ready-to-use pigment-rich extract

DESs are often prepared from natural materials, many of which are approved for use by the FDA in the product formulation (**Table 3**). Fortunately, when these substances are used in combination, their mixtures exhibit superior capability in extracting natural pigment from microbial biomass. Utilizing common ingredients from food, cosmetics, and pharmaceutical fields as extractants facilitates the creation of pigment-rich extracts that can be seamlessly integrated into product formulations, and thus simplifying DSP and reducing the need for costly purification steps. In addition, the use of natural pigments in these products adds value compared to synthetic colorants, offering complementary health benefits beyond their role as colorants. The concept of ready-to-use extract could significantly enhance energy efficiency and reduce environmental impact compared to conventional methods, which often involve the use of energy-intensive processes such as solvent evaporation and the emission of toxic vapors. Wils et al. [82] showed that the microalgal pigment-rich extracts obtained using DESs had an anti-inflammatory effect on keratinocytes stimulated by *Staphylococcus aureus*, supporting their use as cosmetic ingredients. Besides, hydrophobic DESs composed of menthol and saturated fatty acids with varying chain lengths (e.g., stearic acid, myristic acid, and lauric acid) displayed therapeutic benefits in wound healing and antibacterial properties against *Staphylococcus epidermidis* and *S. aureus* strains [84]. Jin et al. [85], in their *in vivo* pharmacokinetic study, found that the aqueous solution of betaine/lactic acid (1:1) significantly improved the oral bioavailability of bioactive compounds extracted from Chinese medicine, including hydroxysafflor yellow (a pigment from safflower), compared to water extracts. The study was conducted by administering ready-to-use crude extracts to Sprague-Dawley rats. The findings support the application of DES as drug delivery system following extraction. More research in this area is needed, and further investigation could stimulate the development of new products that leverage both DESs and bioactive pigments for additional benefits. The amount of ready-to-use extract included in a product formulation varies based on its intended application. For example, in food formulations, the concentration may be adjusted to improve the visual color appeal and the desired sensory attributes of the products, while in skincare formulations, it can be tailored to boost specific benefits like hydration or anti-aging effects. It is suggested that, since an active ingredient constitutes ≤ 5 wt% of a cosmetic product, the ready to use extract can be incorporated into various formulations without dramatically altering their rheological properties or sensory profile [86].

Additionally, the availability of natural pigments in the form of ready-to-use extracts could benefit from the preservation effect provided by DES-based extractants. The integrity of B-phycoerythrin was effectively preserved in crude extracts obtained using aqueous DESs based on glycerol, glucose, and betaine, with only a 10% loss after 30 d of storage under accelerated aging conditions (40 °C, 75% humidity) that mimic a 6-month storage period at room temperature [43]. Fan et al. [13] also reported that a 1:1 mixture of fenchyl alcohol and thymol exerted a relatively good protective effect on lutein from being damaged by high temperature and light compared to methanol. Approximately 94% of lutein was retained in DES, compared to 81% in methanol, when heated at 40 °C for 120 min. Besides, when exposed to light irradiation at room temperature, lutein showed only 5% degradation in DES on the 3rd day, compared to 72% degradation in methanol, and on the 20th day, it was completely degraded in methanol while 78% of the lutein content remained in DES [13]. Moreover, lutein can be stored in DES with only 15 and 20% degradation after 60 d at –20 and 4 °C, respectively, compared to 40 and 82% degradation in methanol [13]. Another report showed that thymol/dodecanoic acid (1.25:1) was suitable for a short-term storage of fucoxanthin extract without requiring refrigeration, with no significant degradation observed during 11 d of storage in the dark at room temperature [35]. Moreover, benzyltriethyl ammonium chloride/caprylic acid (1:2) increased the photostability of chlorophylls up to 6-fold compared to methanol [68]. The data obtained so far reveal that DES-based extractants not only preserve pigment during storage but also provide a better protection against heat and light-induced degradation compared to traditional solvents and water. The findings underscore the potential benefits of DES in preserving pigment integrity throughout product formulation process and maintaining the quality and color of final pigment-based products.

The use of ready-to-use extracts also overcomes the limitations of incorporating lipophilic pigments like carotenoids into aqueous-based product formulations, which are hindered by their low water solubility and poor chemical stability. Their use boosts the practicality and effectiveness of pigments in pharmaceutical, food and skin care/cosmetic applications by improving their solubility, stability, and bioactivity. For instance, DL-menthol-based hydrophobic DES can form a microemulsion with Tween 80 and water, serving as an effective delivery system for natural carotenoids [87]. The microemulsion improved stability, solubility and antioxidant ability of carotenoids compared to organic solvents. Specifically, the maximum solubility of lutein in DES-based microemulsion at 35 °C reached 12.5 mg mL^{-1}, significantly higher than ethanol, methanol, and acetone (1.2, 0.7, and 3.7 mg mL^{-1}, respectively). Furthermore, it was found that 473.6 mg mL^{-1} of astaxanthin ester can be dissolved in DES-based microemulsions, whereas its maximum solubilities in ethanol, methanol, and acetone were relatively low, at 44.6, 20.0, and 329.4 mg mL^{-1}, respectively [87].

The findings to date support the potential of using DESs to prepare ready-to-use extracts. Nevertheless, although each component of the eutectic mixture is individually recognized as safe, the toxicity of the mixture as a whole must be evaluated to ensure that no synergistic toxicological effects occur before further application. It was reported that the toxicological behavior of DES could differ from that of its individual components [88, 89]. Several studies have reported that the [Ch]Cl-based DES containing organic acids (e.g., oxalic acid, malonic acid) displayed moderate levels of cytotoxicity compared to the other counterparts containing sugars and polyols [90]. On the other hand, the cytotoxicity of menthol-based DESs towards HaCaT cells was reported to follow the increasing order: menthol/stearic acid (8:1) < menthol/myristic acid (8:1) < menthol/lauric acid (4:1) [84]. Menthol/lauric acid exhibited cytotoxicity comparable to that of pure menthol. However, menthol/stearic acid and menthol/myristic acid showed reduced cytotoxicity, revealing that the inclusion of myristic acid and stearic acid in the DES formulations lowers their cytotoxicity [84]. The study showed that none of the saturated fatty acids exhibited cytotoxicity within the concentration range tested. Apart from DES constituents, there are many other factors influencing the toxicity of a DES, including its concentration, viscosity, and pH. Further studies are needed to confirm the safety of DESs in their intended forms of use. Standard protocols should be designed to assess the toxicity of DESs at various trophic levels, along with the use of appropriate techniques and bio-models, for confirming the product toxicity [91].

From both the technological point of view and the aspect of product quality, the creation of ready-to-use extracts, without additional purification of the extracts, appears to be an ideal opportunity. Nonetheless, the variations in solvent composition and pigment concentration in ready-to-use extracts, along with their interactions with other formulation components, and their impact on product attributes (e.g., color intensity, appearance, texture, and sensory qualities), require additional effort on the product formulation. Additionally, the establishment of recommended daily intake and upper safety limits for DESs is vital for their inclusion in commercial products for human consumption. Appropriate legislative recommendations from relevant government agencies would undoubtedly facilitate broader adoption of the ready-to-use extracts in food, pharmaceutical and cosmetic/personal care industries.

Table 3. Examples of DES precursors approved by the FDA along with their functions and uses in various sectors.

DES precursor	Class	FDA approval	Function	Examples of products with precursor formulations
Thymol (thyme)	Essential oil	☑	Alleviating symptoms of respiratory diseases, antiseptic, antibacterial, and anti-inflammatory agents	- Cough syrup - Mouthwash, toothpaste, skin and hair care products - Sweet confectionery, herb and spice blends, ready-to-eat soup
Menthol	Essential oil	☑	Providing cooling effect, antibacterial, antifungal, and anti-inflammatory agents	- Cough syrup, decongestant - Mouthwash, toothpaste, skin and hair care products - Sweet confectionery
Octanoic acid (caprylic acid), decanoic acid (capric acid), dodecanoic acid (lauric acid), myristic acid, oleic acid	Fatty acid	☑	Antimicrobial and anti-foaming agents, emulsifier, texture and flavor enhancers	- Drug delivery/penetration enhancer - Skin and hair care products - Confectionery creams, ice-cream, margarine, bakery products
Levulinic acid	Organic acid	☑	Fragrance, flavoring agent, skin-conditioning and antimicrobial agents	- Drug delivery/penetration enhancer, precursor for pharmaceutical active ingredients - Skin and hair care products, fragrance and perfume - Food and beverage with a whiskey-odor

Glycerol (glycerin), sorbitol, xylitol	Sugar alcohol	☑	Antibacterial, emollient, moisturizer/humectant, sweetener, thickener	- Allergen immunotherapy, cough syrup, excipient in drug formulation, capsule coating - Skin and hair care products, cosmetics - Beverage, energy bar, cake, soft candy, chewing gum, condiment, cream, marshmallow, dairy products
[Ch]Cl	Quaternary ammonium salt	☑	Nutritional supplement	- Drug formulation for prevention of choline deficiency - Choline-fortified food
L-proline	Amino acid	☑	Osmoprotectant, nutritional supplement, flavor enhancer	- Precursor for pharmaceutical peptides and proteins - Skin and hair care products, cosmetics - Dietary supplement, gelatine foods and desserts, dairy products, processed meats and vegetables

5.2 Pigment polishing and solvent recovery

If the crude extracts are not intended for direct use, the next challenge is to separate the pigment from the DES-based extractant to produce high-quality pigments and enable solvent recycling. The isolation of pigment from a DES-based extractant is difficult due to the low vapour pressure and high viscosity of DES, and strong interactions between pigments and DES molecules. Several methods are currently being explored, including the addition of antisolvent (e.g., water, ethanol, and acetone), the use of macroporous resin, and back extraction method through LLE [92]. Particularly for hydrophilic DESs, water is frequently used as an anti-solvent to disrupt the bonding between HBA and HBD molecules, which can result in the loss of the DES's properties, consequently inducing the precipitation of extracted pigment [93]. On the other hand, adding water to the crude extract containing hydrophobic DESs could form a two-phase system, which can facilitate the recovery of co-extracted proteins at the interface [41]. Nonetheless, the potential migration of DES components into water complicates their recovery and recycling, increasing the cost and difficulty of reuse. Another work demonstrated the separation of carotenoids from a hydrophobic fatty acid-based DES extract through pigment precipitation by altering solvent's polarity [62]. Besides, C-phycocyanin, which has been partially purified using DES-based ATPS, can be subsequently precipitated using ammonium sulfate method to increase its purity level [19]. While these methods effectively isolate pigments from crude extracts, more efforts are needed to enhance process efficiency and the recovery of DES for recycling.

Another important factor in optimizing the use of DESs as extractants is their reuse. DESs have been shown to be effectively reused across multiple cycles (≥ 3 cycles) without compromising their efficiency for biomass pretreatment [20, 21] and pigment extraction [35, 41] processes. For example, the concentration of fucoxanthin reached up to 13.1 mg mL^{-1} DES after the 6th cycle of extraction, which is much higher than the loading of 0.1 – 0.3 mg mL^{-1} concentration recommended for therapeutic applications of fucoxanthin [35]. This reusability reduces the need for fresh solvent and also enhances the ultimate concentration of pigment in the crude extract, and thus minimizing waste and reducing overall production costs.

5.3 Process sustainability and scale-up

The DES-based approach for pigment recovery, which combines green solvents with efficient DSP techniques, appears to align with sustainability principles. Yang et al. [40] adopted the Eco-Scale methodology to evaluate the greenness of extracting microbial canthaxanthin using octanoic acid/decanoic acid compared to *n*-hexane and ethanol. The Eco-Scale methodology, introduced by Gałuszka et al. [94], assesses the greenness of an analytical method by assigning penalty points to parameters that deviate from ideal green analysis, where a score of 100 represents an ideal green process. The findings reveal that the DES-based extraction process is a green method, achieving an Eco-Scale score of 94.8, which surpassed the scores of *n*-hexane (85.8) and ethanol (83.3) [40]. Another metric, ComplexGAPI, has also been used to assess the environmental friendliness of the extraction processes involving DESs, since the sustainability of solvent preparation is included in the analysis. The ComplexGAPI pictogram, which features five pentagrams and an additional hexagonal field, allows for a quick assessment of the eco-friendliness of the entire protocol [95]. Hilali et al. [83] applied the ComplexGAPI tool to evaluate the sustainability of both the sequential SLE and integrated SLE-LLE approaches for recovering multiple pigments from *A. platensis* using hydrophilic and hydrophobic DESs. Overall, both strategies showed positive environmental impacts, with the SLE-LLE strategy having a more favorable effect due to the reduced energy consumption in its process. On the other hand, a life cycle assessment by Zaib et al. [96] reported that [Ch]Cl/urea has lower environmental impacts compared to dichloromethane and ethyl acetate, but it posed higher adverse environmental impacts than methanol and ethanol.

The ecotoxicity of DESs depends on the nature of the mixture. It was reported that the [Ch]Cl-based DESs with organic acids as the HBD exhibited moderate ecotoxicity towards *Aliivibrio fischeri*, with the toxicity dominated by acid concentration [97]. In contrast, the DESs composed of [Ch]Cl as the HBA and glycerol-derived ethers/glycerol as the HBD were classified as non-toxic towards *A. fischeri* [98]. The polarizability and hydrophobicity of solvents significantly affect their ecotoxicity. However, limited studies exist on hydrophobic DESs, and further research is needed on their ecotoxicology and biodegradability.

Apart from process sustainability, several critical factors need to be considered to optimize the use of DES in industrial-scale pigment extraction and ensure that their superior capacity achieved in lab-scale is effectively realized in larger-scale process. Many lab-scale extraction studies reported have applied mild conditions, with some have been conducted at room temperature while others have employed external heating to achieve optimal results. The heating and stirring process in large-scale and continuous operations is well-established, with proven methodologies and readily available equipment. In the context where the UAE is being proposed to enhance extraction efficiency, its scale-up needs to be evaluated more thoroughly. Although several ultrasonic reactors are commercially available, scale-up of UAE reactors is difficult due to complex physical nature of high-frequency wave propagation in multiphase media and acoustically induced cavitation [99]. It is also essential to assess the dissipated ultrasonic power and associated energy cost from an industrial perspective. Tamminen et al. [100], in their study on scaling up continuous UAE of pigments from spinach using an aqueous ethanol solution, reported that the total ultrasound power required in the pilot-scale module was 2.5-fold higher to achieve the same extraction yield obtained in the lab-scale setup. Moreover, the physicochemical properties of DESs need to be considered, since they could pose limitations during the process scale-up. Specifically, DESs such as DL-menthol/levulinic acid (1:1), DL-menthol/thymol (1:1), and 70% aqueous glycerol/glucose (3:1) have viscosities of 30.1, 36.1, and 32.4 mPa·s at 25 °C, respectively. These viscosities are higher than those of water, ethanol, and hexane, which are 0.9, 1.1, and 0.3 mPa·s at 25 °C, but still are comparable to other solvents like sunflower oil and 70% aqueous glycerol, which have viscosities of 48.2 and 26.9 mPa·s, respectively [76, 101, 102]. The high viscosity of DESs complicates their handling at industrial scale, making processes such as pumping, mixing, and stirring more difficult and energy-intensive. Moreover, solid-liquid separation after extraction process can lead to the loss of crude extract, since viscous liquids stick to residual biomass and equipment surfaces. Also, the non-volatility of DESs presents a drawback when drying residual biomass wetted by these solvents for subsequent biomass valorization, since DESs cannot be removed through evaporation and may require an additional washing step.

Current research emphasizes the design and technological performance of solvents and their process optimization, with insufficient focus on economic aspects. Nevertheless, process economics are vital for assessing the feasibility of scaling operations to a commercial level. There is lack of cost-benefit analyses to thoroughly evaluate the economic viability of these techniques. The key obstacle to the industrial production of microbial pigments is the high cost of pigment's DSP processes, which can constitute 50 – 80% of the total production expenses due to energy and equipment requirements. Traditional recovery methods use dry microbial biomass for effective pigment extraction with organic solvents. Current approaches favor the use of wet biomass to avoid the costly and energy-intensive drying process. However, there is still limited research on developing wet extraction protocols using DESs, highlighting the need for further investigation in this area. The economic evaluation of DESs indicated that variations in material prices were significantly governed by the components used in their combination. It was reported that dodecanoic acid/octanoic acid (1:3) costs 58 € kg^{-1}, while the estimated price for L-menthol/eucalyptol (1:1) was 184 € kg^{-1} [27]. Moreover, the preparation of DESs was identified as the primary contributor to the impacts of energy consumption [103]. Hence, efficient recovery of DESs is crucial to minimize raw material costs and reduce operational expenditure [104]. In summary, to promote the adoption of these solvents in industrial processes, a holistic approach must be developed, addressing all concerns including sustainability, scale-up, economics, solvent reusability, and recovery if a ready-to-use extract is not feasible.

6. Conclusions

Natural pigments of microbial origin offer a safer alternative to synthetic colorants while also providing versatility and supporting current sustainability trends. However, conventional DSP of microbial pigments involves hazardous solvents and energy-intensive processes, and low yields. The use of DESs for the extraction of pigments from microbial biomass represents a promising advance towards more sustainable processing methods, with benefits such as selective extraction, reduced environmental impact, and milder processing conditions. Their tunability allows for the design of task-specific solvents that can effectively dissolve and stabilize various classes of pigments, potentially leading to more efficient and higher-quality extractions.

However, while DESs hold significant potential, several challenges must be critically addressed before their widespread adoption. One of the primary concerns is the scalability of DES-based extraction methods. The transition from laboratory-scale experiments to industrial applications requires rigorous optimization of solvent systems, energy efficiency, and cost-effectiveness. While the recovery and reuse of DESs are difficult due to their high viscosity and low volatility, the possibility of producing ready-to-use pigment extracts that do not require solvent removal offers a promising alternative. These DES-based extracts could streamline production, reduce waste, and lower costs by eliminating the need for additional purification steps. Nevertheless, ensuring the stability and safety of these solvent-containing extracts is crucial for their commercial viability and consumer safety. Developing robust protocols to assess the long-term effects of DESs in these extracts is essential, in addition to examining the potential toxicity of DESs. While many DESs are composed of generally recognized as safe (GRAS) substances, the synergistic effects of their components in complex mixtures are not fully understood and thus require thorough toxicological assessments. This is especially important if DESs are to be used in food, cosmetic, and pharmaceutical applications where human exposure is direct and substantial. If these challenges are resolved, the approach of using ready-to-use pigment extracts could revolutionize pigment extraction by offering a more sustainable and economically feasible solution. Otherwise, developing efficient and cost-effective methods for solvent recovery and recycling is essential to enhance the overall sustainability and economic viability of DES-based processes. Lastly, although DESs have a lower environmental impact than traditional solvents, their biodegradability and the environmental fate of their components still need comprehensive evaluation. Overcoming these obstacles could enable DESs to transform pigment production, driving more eco-friendly practices in industries such as food, cosmetics, and pharmaceuticals.

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Figure captions

Figure 1. Natural pigments of microbial origin with different colors **(A)**; and their main production steps **(B)** in a comparison with that of synthetic colorant shown in red dash box. The dashed arrow line shows an alternative energy-saving recovery path that omits drying operation.

Figure 2. The use of DESs across various DSP stages of microbial pigment, including biomass pretreatment, pigment extraction (using different SLE techniques, including traditional heating and the intensified approaches such as UAE and MAE), and pigment purification (using LLE particularly ATPS with hydrophilic DESs). The integrated SLE-LLE strategies, involving the use of the immiscible two-phase system facilitated by a hydrophobic DES (i); and the ATPS with a hydrophilic DES (ii), are presented. These strategies can be used for the selective extraction of multiple classes of pigments or a specific pigment of interest.

Figure 3. Different types of aqueous hydrophilic DESs with their different pH ranges [21].

Figure 4. Common components used for preparing hydrophobic DESs **(A)**; and hydrophilic DESs **(B)**; and the approximate order of the increasing hydrophobicity of pigments **(C)**. The chemical structures and values of the octanol-water partition coefficient, log *P* (provided in brackets), are obtained from ChemSpider [27]. The values with an asterisk (*) are acquired from the reference [28].

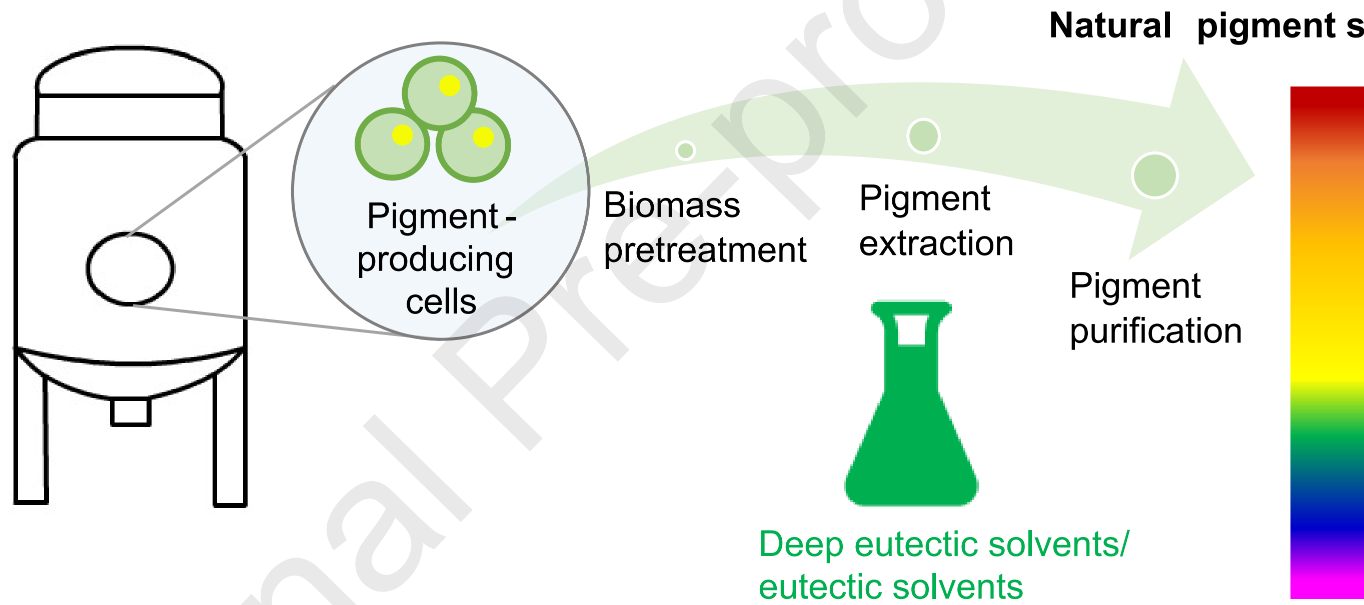
Figure 5. The benefits of DES-assisted extraction of microbial pigments claimed in the literaure and the challenges of implementing these methods on an industrial scale.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Graphical Abstract

Graphical abstract

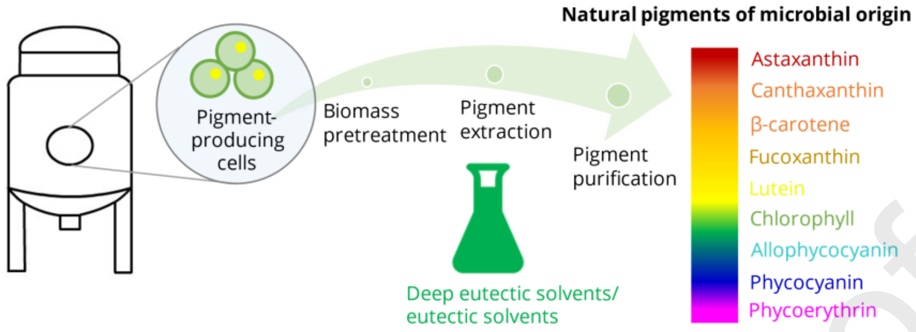


Figure_1

Click here to access/download;Figure;Figure_1_r2.docx

Figure 1

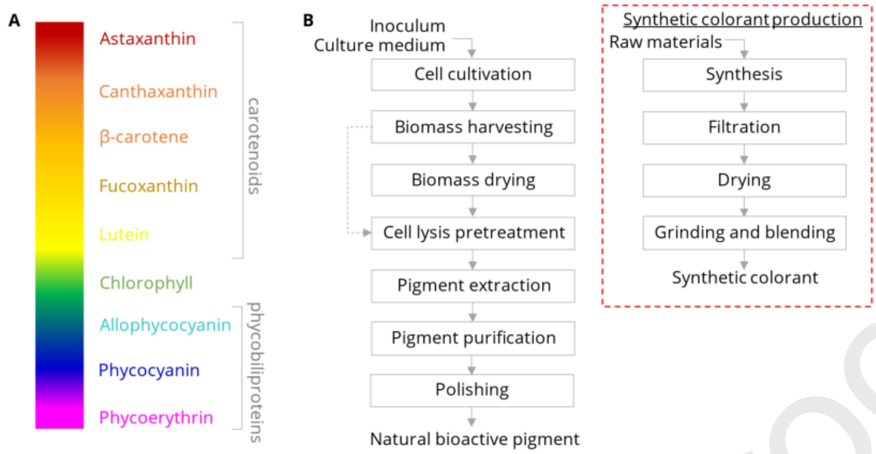


Figure_2

Click here to access/download;Figure;Figure_2.docx

Figure 2

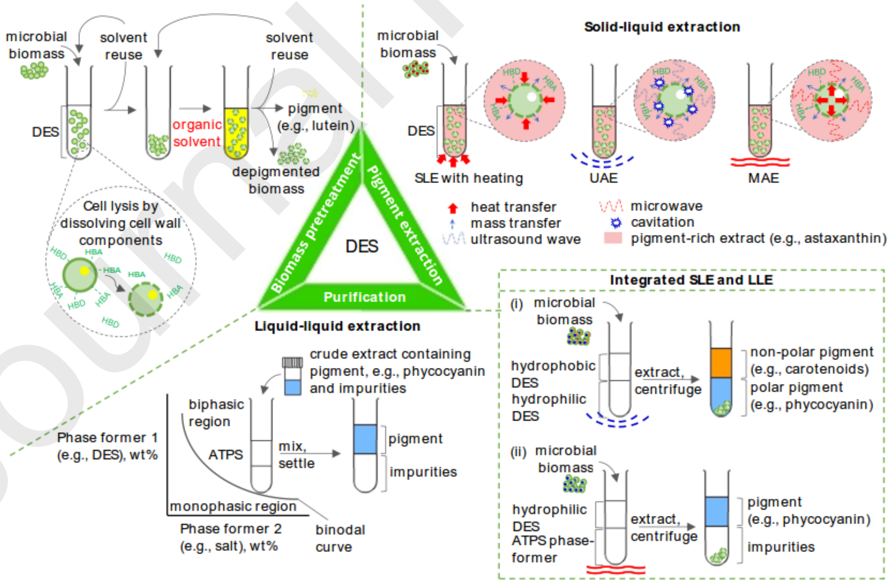


Figure 3

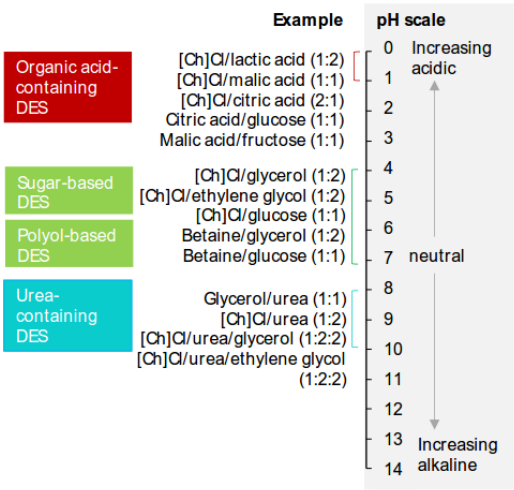


Figure 4

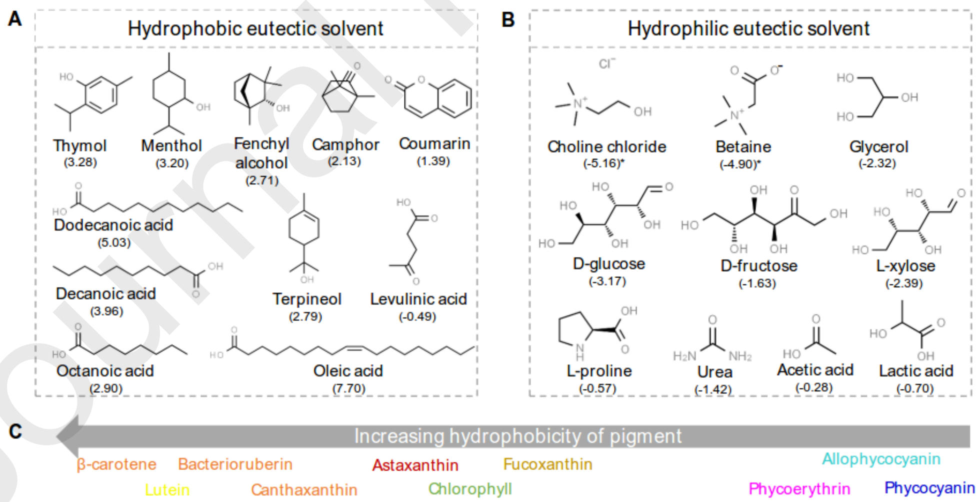
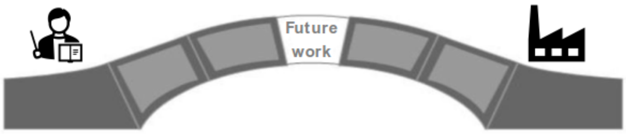


Figure_5

Figure 5



Academic claims:
- Low vapor pressure
- Non-flammable
- Eco-friendly
- Made of natural substances
- Tunable properties
- High extraction capacity
- Ready-to-use extracts

Industrial applicability concerns:
- Process scalability
- Economic feasibility
- Complicated product isolation
- Difficult to recycle solvent
- Lack of long-term stability and safety data of ready-to-use extracts, and their market potential
- Limited environmental impact assessment