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A sustainable and efficient method for sequential extraction of lutein and lipid from deep eutectic solvent pretreated *Chlorella pyrenoidosa*†

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Microalgae biomass is regarded as a potential feedstock for valuable compounds such as pigments, lipids and proteins. However, development of single molecule extraction processes is the most common practice. A green multiproduct extraction approach is needed for economically sustainable process development of the microalgal industry. Therefore, this study aims to investigate the sequential extraction of lutein and lipid from dry and wet *Chlorella pyrenoidosa* biomass pretreated with a choline chloride-based deep eutectic solvent (DES) under a sustainable biorefinery scheme. In this context, we have assessed the kinetic modeling of the solid–liquid extraction process for the aforementioned compounds, focusing on the effects of temperature and time. The maximum lutein (3.80 mg g^{-1}) and lipid (95.0 mg g^{-1}) contents from dry biomass were obtained at 45 °C in 40 min and at 70 °C in 90 min, respectively. From wet biomass, the maximum lutein (2.57 mg g^{-1}) and lipid contents (87.47 mg g^{-1}) were obtained at 35 °C in 40 min and at 70 °C in 90 min, respectively. The kinetics of the solvent-based extraction process for lutein and lipids were assessed *via* first-order and second-order kinetic models with an associated investigation of kinetic parameters, such as rate constants, saturation concentration and activation energies. We found that temperature is an important parameter that influences the extraction of all compounds and also has a significant impact on the kinetic parameters. Toxicity evaluation of the DES and economic assessment of DES *vs.* ionic liquids (ILs) were performed. The synthesis cost of the DES is lower than that of ILs, and *Escherichia coli* JM109 survivability assessment confirms the DES as a non-toxic solvent. The present study provides valuable insights into the sequential extraction for a high-value multiproduct biorefinery.

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1. Introduction

Microalgae are photosynthetic microorganisms relying on light and nutrients to produce a vast group of compounds such as carbohydrates, proteins, lutein, and lipids.[1,2] Lutein has human health benefits, such as ameliorating cardiovascular diseases, age-related macular degeneration (AMD), and cancer.[3,4] Lipids with great interest are triacylglycerols (TAGs), which are used in a wide range of applications, from biodiesel to cosmetics, food ingredients, and personal care products. The largest source of TAGs is palm oil, which has led to massive deforestation.[5,6] Lipids from microalgae also offer an alternative to palm oil, and omega-3 long-chain polyunsaturated fatty acids, *i.e.*, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), are regarded as alternatives to fish oil and essential fatty acids in both human nutrition and aquaculture.[6]

Significant progress has been achieved in extracting valuable compounds from microalgae. Various studies have suggested different extraction approaches including ball milling, French press, high-pressure homogenization (HPH), ultrasound, grinding with liquid nitrogen, enzymatic hydrolysis, and acid–alkali hydrolysis.[4,7,8] However, most of these approaches focus on extracting a single product (lutein, lipids, and protein), which becomes economically unfavorable. Some studies have focused on the coproduction of high-value (carotenoids) and low-value products (protein). For example, lutein and β-carotene have been extracted using pressurised liquid extraction (PLE), which involves high pressure (103 bar) and high temperature (40–110 °C), resulting in a low yield (0.08 mg g^{-1}).[9] In another study, lutein

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and lipids were extracted in a single-step process where 85.0% of lutein and 58.8% of lipids were recovered from the pretreated biomass.[10] However, the involvement of sophisticated methods and subsequently high energy consumption emerge as major challenges. To address these issues, a simple, fast, eco-friendly, and cost-effective method is urgently required.

To overcome the aforementioned challenges, deep eutectic solvents (DESs) have received more attention in biomass processing due to their high performance, cost-effectiveness, low toxicity, sustainability, simple preparation, biodegradability, and recyclability.[11–15] In our earlier studies, biomass of several microalgal species, such as *Chlorella pyrenoidosa*, *C. vulgaris*, and *Chlorococcum* sp. (GN38), were pretreated using DESs.[16–18] DESs-based pretreatment approach overcame the disadvantages of conventional methods, facilitating the successful extraction of lutein or lipids in an environmentally and economically friendly manner. Thus, this study attempts the sequential extraction for multiproduct biorefinery from DES-pretreated algal biomass.

The present study evaluates the implementation of sequential extraction of lutein and lipid with ethanol, as well as a combination of methanol (M) and ethyl acetate (EA) (2 : 1). As reported in our previous study, this solvent combination showed promising results for the recovery of the neutral lipids as compared to the use of a single solvent (M, EA). These neutral lipids are considered as the ideal feedstock for biodiesel.[19] Moreover, this study investigates the effects of temperature and time, and the best fitting of a kinetic model for the extraction of lutein and lipids from *Chlorella pyrenoidosa*. The first-order kinetic model (FOKM) and second-order kinetic model (SOKM) were applied for the extraction process. The FOKM and SOKM under non-equilibrium conditions were used to simulate the solid–liquid extraction process of lutein and lipid compounds from microalgae biomass. The kinetic parameters (*i.e.*, rate constant of extraction, saturation concentration, and activation energies) were determined to show the influence of parameters such as temperature and time on lutein and lipids extraction from dry and wet biomass and to describe the extraction mechanism of the process.

2. Experimental section

2.1. Materials

Microalgae biomass (*Chlorella pyrenoidosa*) was provided by Yunnan Boshan Zeyuan Microalgae Health Technology Co. Ltd, China. Choline chloride (ChCl, 98%) and ethylene glycol (EG, 98%) were obtained from Macklin, China. Methanol (≤100%) and ethyl acetate (99.5%) were obtained from Thermo Fisher, China. Ethanol (99.7%) was obtained from Tianjin Fengchuan Chemical Reagent Technology Co., Ltd. The lutein standard (purity 90%) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd.

2.2. Synthesis of the DES and the toxicity test

A ChCl-based DES was synthesized using ChCl as a hydrogen bond acceptor (HBA) and EG as a hydrogen bond donor (HBD) in a molar ratio of 1 : 2 followed by heating (80 °C) and stirring with a magnetic stirrer as reported elsewhere.[18] DES formation was confirmed using Fourier transform infrared (FTIR) spectroscopy (BRUKER-TENSOR II-USA) and the measurement of the spectral region was recorded between 400 and 4000 cm^{-1}. The synthesized DES was sealed and stored in a desiccator to avoid the absorption of external water.

DES toxicity was detected according to the method reported in ref. 20, and *Escherichia coli* JM109 (*E. coli*) was chosen as a target. Furthermore, to observe the *E. coli* cells, 400 μL bacterial solutions of the cells grown in the standard LB medium and the medium supplemented with 150 mM of DES [ChCl : EG (1 : 2)] were observed under a 40× microscope after being dyed by crystal violet staining solution.

2.3. Pretreatment of microalgae biomass and sequential extraction procedure for lutein and lipid

5 g of biomass and 66.67 g of DES (*i.e.*, a biomass to DES ratio of 5 : 66.67) were added to a beaker. The solution was magnetically stirred for 5 min (pre-treatment) according to our previously optimised study.[21] After that, the pretreated biomass was separated from the DES *via* centrifugation, washed multiple times with water and then freeze dried for 48 h.

Ethanol was used for the solid–liquid extraction of lutein, and M : EA (2 : 1) was used for lipid extraction. 0.5 g of pretreated biomass in ethanol solvent (ratio of 1 : 23.34, g : mL) was used in a 20 mL glass tube at 300 rpm for lutein extraction. For the kinetic studies, the experiment was carried out at three different temperatures (25, 35, and 45 °C) for lutein. After lutein extraction, the solvent was removed, and the remaining biomass was dried at room temperature for 48 h. The dried biomass was weighed, and the lipid extraction experiment was performed at 50, 60, and 70 °C. In all mentioned experiments, the time for the extraction of the lutein was assessed (10, 20, 30, 40, 50, and 60 min) along with that for lipid extraction (10, 20, 30, 60, 90, 120, and 150 min).

2.4. Biochemical analysis of lutein and lipid content

Lutein concentration in each sample was analyzed using a high-performance liquid chromatography (HPLC) system (1200 Infinity, Agilent Technologies) coupled with a variable wavelength detector, according to the previous study.[18] Lutein content was calculated according to eqn (1).

$$\text{Lutein content (mg g}^{-1}) = \text{Lutein concentration (mg L}^{-1}) \times \text{Solvent volume (L)/Pretreated biomass (g)} \quad (1)$$

Lipid content was determined by the gravimetric method as mg g^{-1} of the dry weight biomass and calculated as follows:

$$\text{Lipid content (mg g}^{-1}) = (W_2 - W_1)/W_0 \quad (2)$$

W_2 is the weight of the flask after the solvent evaporation, W_1 is the initial weight of the flask, and W_0 is the weight of the biomass after lutein extraction.

For fatty acids, transesterification of extracted lipids was done according to ref. 22 and the composition was determined using our previous report.[1]

2.5. Kinetic modelling

In this study, FO and SOKM were used in modelling the extraction of lutein and lipid from the microalgae biomass using ethanol and M : EA (2 : 1) as solvents. In brief, first-order extraction (KM), which was suggested by Harouna-Oumararou *et al.*,[23] was evaluated in such a way that the rate of leaching (r_e) is proportional to the driving force ($C_s - C_t$) and the first order rate equation corresponds to the linear driving force as shown in eqn (3).

$$r_e = dC_t/dt = k(C_s - C_t) \quad (3)$$

Here C_t (mg g^{-1}) is lutein and lipid concentration at a time (t), C_s (mg g^{-1}) is the concentration of the compounds at saturation, and k (m^{-1}) is the FO extraction rate constant.

Eqn (4) was obtained by integrating eqn (3) by using the boundary conditions as $C_t = 0$ at $t = 0$ and $C_t = C_t$ at $t = t$, in such a way that plotting ln values *vs.* t gives the slope, which is used to determine the FO extraction rate:

$$\ln[C_s/(C_s - C_t)] = kt \quad (4)$$

The Arrhenius model is used in solid–liquid extraction to investigate the relationship between the extraction rate and temperature. Therefore, the temperature depends on the extraction kinetics.[24]

The Arrhenius equation, as shown in eqn (5) and (6), was used to determine the kinetic parameters. KPs (A_e and E_a) calculated by plotting (ln k *vs.* $1/T$) [eqn (5)]. A_e and E_a are obtained from the slope and intercept.

$$k = A_e e^{-(E_a/RT)} \quad (5)$$

$$\ln k = \ln A_e - E_a/RT \quad (6)$$

In the above eqn (5) and (6) k, A_e, E_a, R and T represent the rate constant (m^{-1}), Arrhenius constant (m^{-1}), activation energy (kJ mol^{-1}), universal gas constant specified as 8.314 J mol^{-1} K^{-1}, and temperature (K). SO extraction kinetics is modelled using eqn (7).[24]

$$r_e = dC_t/dt = k(C_s - C_t)^2 \quad (7)$$

Integrating eqn (7) considering the boundary conditions $C_t = 0$ at $t = 0$ and $C_t = C_t$ at $t = t$ gives

$$1/(C_s - C_t) - 1/C_s = k_t \quad (8)$$

Or

$$C_t = C_s^2 k_t/(1 + C_s kt) \quad (9)$$

Rearranging eqn (8) in a linearized form gives eqn (10) and (11) as below.

$$t/C_t = t/C_s + 1/C_s^2 \quad (10)$$

$$t/C_t = t/C_s + 1/m \quad (11)$$

Here, m denotes the initial extraction rate coefficient, which is equal to kC_s^2, while SO extraction rate is calculated from the intercept *via* plotting t/C_t *vs.* t following eqn (11).

Using eqn (5) and (6), KPs (A_a, E_a) were found, where g (mg min)$^{-1}$ is the unit of k and A_e of SO.

3. Results and discussion

3.1. DES validation and toxicity

FTIR has been proven to be a very important test in confirming the formation of DES. DES was synthesized by combining ChCl and EG. FTIR spectra of ChCl, EG, and their derived DES were investigated to understand the modification as depicted in Fig. 1. In the FTIR spectra, a broad band was noticed at 3300 cm^{-1} in the synthesized DES, which is the characteristic of the hydrogen bonding formation and the same observation is found in the literature.[25] The spectrum of the DES was dominated by EG; however, an additional characteristic band at 953 cm^{-1} originating from ChCl was noticed. This new band was attributed to the C–N$^+$ stretching.[26] In addition, the DES presented vibrational bands at 2938 and 2873 cm^{-1} corresponding to C–H stretching. Furthermore, the presence of vibrational peaks between 800 and 1100 cm^{-1} situated at 1080 cm^{-1}, 1035 cm^{-1}, and 882 cm^{-1} is associated with functional groups, namely, C–O stretching, C–C–O asymmetric stretching, and C–C–O symmetric stretching, respectively.[26] These results confirm the successful synthesis of the ChCl:EG DES.

To assess the toxicity of the ChCl : EG (1 : 2) DES, *E. coli* cells were grown in LB media supplemented with 150 mM to 450 mM DES from 5 h to 35 h, and compared with the standard LB media (pre-adapted cells). Experimental results (available only in the online version of ESI Fig. S1†) show that *E. coli* cells could grow in DES supplemented LB medium, showing that ChCl : EG (1 : 2) is not toxic to some extent and can be labelled as an environment friendly solvent.

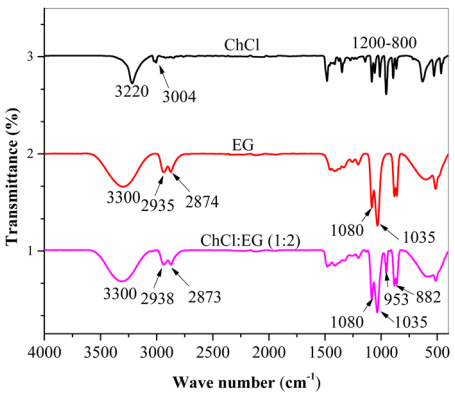

Fig. 1 ChCl : EG (1 : 2) DES confirmation *via* FT-IR spectra.

3.2. Extraction of lutein using ethanol as a solvent from dry and wet biomass

Fig. 2(a) presents the experimental results for the extraction kinetics of lutein at various temperatures (25, 35, and 45 °C) using ethanol as a solvent. A rapid increase in the lutein content at the beginning was observed (~10 min). As the extraction process continued, a slow and steady trend remained constant until the peak point was attained, where most of the lutein was extracted. This observation can be explained on the basis of Fick's law.[27] At the initial extraction stage, a high concentration gradient between the solid and liquid phases results in the high diffusion of lutein into the solvent. As the extraction process progresses, the gradient concentration decreases, resulting in an increase in the extraction yield until the maximum lutein recovery is achieved.

Fig. 2(a) also shows that extracted lutein beyond the peak point in all conditions was reduced in dry and wet biomass. This observation is attributed to the decomposition of lutein due to thermal degradation or the prolonged reaction time.[28–30] Lutein stability is recognized as a challenge for the food processing industry, as lutein is sensitive to light, temperature, and oxygen.[31] Therefore, values beyond the peak point were excluded when simulating the lutein extraction kinetics. In fact, they do not show how quickly lutein is extracted. Instead of that, they represent the thermal degradation rate of lutein. Fig. 2(a) shows that the highest lutein extraction from dry biomass at the highest temperature of 45 °C is 3.80 mg g^{-1} in 40 min, and lower lutein contents of 2.82 and 2.78 mg g^{-1} were achieved at lower temperatures of 35 and 25 °C. In the case of wet extraction, at 35 °C, 2.57 mg per g lutein content and at 25 and 45 °C, 2.27 and 2.11 mg per g lutein contents were recorded in 40 min. These values indicate that temperature is an important extraction parameter for the recovery of lutein. The positive effect of increasing the temperature on the extraction of lutein can be a consequence of the increase of the solubility of the lutein in the solvent and the improvement of diffusion rate and mass transfer from solid to solvent.[32]

3.2.1 First-order kinetic model for lutein extraction from microalgae using ethanol. The experimental data of the extraction kinetics shown in Fig. 2(a) for the FOKM were plotted as $\ln C_s/(C_s - C_t)$ *vs.* t. These resulting plots were then used to find the rate constant (k) and coefficient of determination R^2 (Fig. 3(a)). The plots show that the extraction of lutein can be modeled *via* a linear FOM. The k value declined with rising temperature (9.2×10^{-2} min^{-1}, 6.0×10^{-2} min^{-1}, and 2.5×10^{-2} min^{-1}) at the reaction temperatures of 25, 35, and 45 °C, respectively. Similarly, in the wet biomass case, the k value also decreased (1.25×10^{-1} min^{-1}, 7.3×10^{-2} min^{-1}, and 7.1×10^{-2} min^{-1} at 25, 35, and 45 °C, respectively). However, the declining extraction rate of lutein using ethanol as a solvent in both cases in response to increasing temperature confirms the high efficiency of ethanol for lutein extraction at low temperature. Following our results, a study carried out by Hobbi *et al.*[33] demonstrated that the k value decreased as the temperature increased from 20 to 60 °C using acetone–water as the solvent to extract polyphenolic compounds from apple pomace.

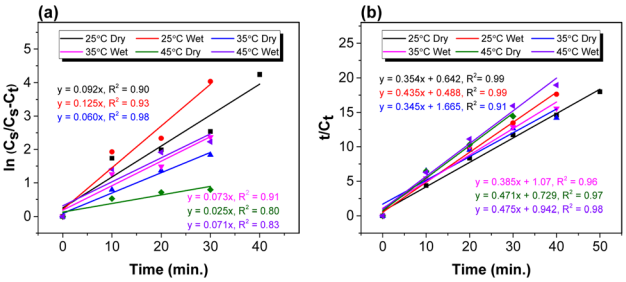

Fig. 3 First-order kinetic models (a) and second-order kinetic models (b) for extraction of lutein from dry and wet microalgae biomass.

3.2.2 Second-order kinetic model for lutein extraction from microalgae using ethanol. The SOKM was also used to explain the kinetics of lutein extraction from biomass. Using eqn (10) and (11), t/C_t *vs.* t plots were generated, k and R^2 were finally determined (Fig. 3(b)). The k values were found to be 1.95×10^{-1} g (mg min)$^{-1}$, 7.2×10^{-2} g (mg min)$^{-1}$, and 3.05×10^{-1} g (mg min)$^{-1}$ at 25, 35, and 45 °C, respectively, indicating its positive correlation with temperature as depicted in Fig. 3(b). In the case of the wet biomass, the k value decreases from 3.88×10^{-1} g (mg min)$^{-1}$ to 1.39×10^{-1} g (mg min)$^{-1}$ with increasing the temperature from 25 to 35 °C, but at 45 °C, the k value increases to 2.40×10^{-1} g (mg min)$^{-1}$. These findings indicate no positive correlation between temperature and the k value.

3.3. Kinetics of lipid extraction using M : EA (2 : 1) from microalgae

The experimental results of extraction kinetics of lipids from biomass at different temperatures (50, 60, and 70 °C) are depicted in Fig. 2(b). Fig. 2(b) shows a rapid increase in the initial 10 min of the reaction, and then a gradual rise in the lipid content was noticed. The highest lipid content was 95 mg g^{-1}, achieved at 70 °C after 90 min. At the temperatures of 50 and 60 °C, the lipid content was 63.92 and 77.63 mg g^{-1} at the time of 120 min, respectively. These results show the effect of temperature on the extraction process. Meanwhile, in the wet biomass, 55.52 mg g^{-1}, 78.66 mg g^{-1}, and 87.47 mg g^{-1} lipids were achieved at 120, 60, and 90 min at 50, 60 and 70 °C, respectively.

3.3.1 First-order kinetic model for the extraction of lipid from microalgae using M : EA (2 : 1). The FOKM was achieved based on the experimental data of the extraction kinetics

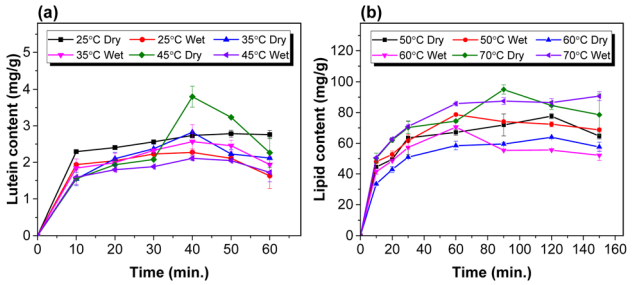

Fig. 2 Lutein (a) and lipid (b) extraction from dry and wet microalgae biomass with ethanol and methanol : ethyl acetate at solid–liquid ratio of 1 : 23.34 (g mL^{-1}) under continuous magnetic stirring at 300 rpm.

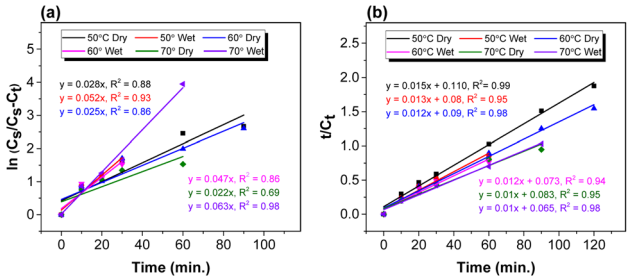

Fig. 4 First-order kinetic models (a) and second-order kinetic models (b) for the extraction of lipids from dry and wet microalgae biomass.

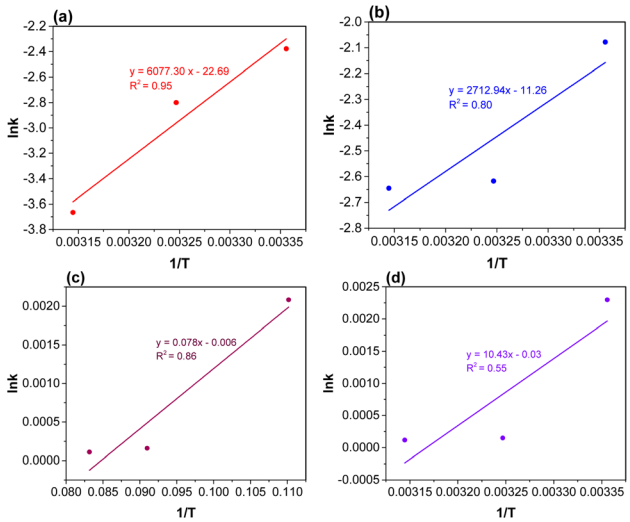

Fig. 5 Arrhenius plots obtained from the first-order kinetic model (a and b) for lutein and from the second-order kinetic model (c and d) for the extraction for lipid compounds.

[Fig. 2(b)]. The k and R^2 were determined by plotting ln $C_s/(C_s - C_t)$ *vs.* t (Fig. 4a). The lipid extraction can be represented using the linear form of the first-order model. The k value decreased from 2.8 × 10^{-2} min^{-1} to 2.5 × 10^{-2} min^{-1} and 2.2 × 10^{-2} min^{-1} when the temperature was increased from 50 to 70 °C with R^2 values of 0.88, 0.86, and 0.69, respectively. In contrast, in the case of wet biomass, k decreased from 5.2 × 10^{-2} to 4.7 × 10^{-2} min^{-1} and then increased to 6.3 × 10^{-2} min^{-1}, showing no positive correlation between the temperature and the k value.

3.3.2 Second-order kinetic model for extraction of lipid from microalgae using M : EA (2 : 1). The SOKM was employed to examine the release kinetics of lipids from lutein free biomass using k and R^2 (Fig. 4(b)). The best fits were observed when compared to the first-order and k values were determined to be 2.08 × 10^{-3} g $(mg\ min)^{-1}$, 1.6 × 10^{-4} g $(mg\ min)^{-1}$, and 1.1 × 10^{-4} g $(mg\ min)^{-1}$ at the reaction temperatures of 50, 60 and 70 °C, respectively, with higher R^2 values of 0.97, showing that the second-order model can be used to extract the lipid. However, the k values showed a decreasing trend in response to the temperature increase, which could be confirmed by the high efficiency of the solvent (M : EA, 2 : 1). For the wet biomass, the same trend was observed, 2.3 × 10^{-3} g $(mg\ min)^{-1}$, 1.5 × 10^{-4} g $(mg\ min)^{-1}$, and 1.2 × 10^{-4} g $(mg\ min)^{-1}$ at 50, 60 and 70 °C, respectively, with the rise of the temperature could be confirmed with the high efficiency of the used solvent.

3.4. Kinetic parameters determination

Related kinetic parameters were found using the Arrhenius equation discussed in Section 2.5.

3.4.1 Lutein extraction from dry and wet microalgae. To obtain the kinetic parameters for the FOKM, ln k was plotted against $1/T$. The E_a value was determined to be 50.53 kJ mol^{-1} on the basis of the fitted line using the coefficient of determination value of ~0.95. In comparison to the wet biomass, an activation energy (E_a) of 22.61 kJ mol^{-1} was determined with a R^2 value of 0.80. In contrast, the SOKM showed no significant correlation and was therefore not considered. The value of E_a depends on various factors (targeted compound, material, solvent, and sample pretreatment). The value of E_a could be employed in identifying the mechanism that controls the extraction process. Thus if $E_a > 40$, the extraction mechanism is controlled by solubilization; if $E_a < 20$ the extraction is due to diffusion, if $20 < E_a < 40$, the extraction process is governed by diffusion and solubilization.[34] Therefore the E_a value indicates that the extraction process of dry biomass is governed by solubilization, and that of wet biomass is governed by diffusion and solubilization. However, the opposite trend was observed in both cases, as shown in Fig. 5(a and b), indicating that lutein extraction does not obey the Arrhenius equation.

3.4.2 Lipid extraction from dry and wet microalgae. Fig. 5(c and d) are generated using the Arrhenius equation. E_a and A_e were found to be −136.41 kJ mol^{-1} and 0 $(g\ min^{-1})$, respectively, based on the fitted line characterized by an R^2 value of 0.85, and for the wet biomass, −138.20 kJ mol^{-1} and 0 $(g\ min^{-1})$, respectively, with an R^2 value of 0.82. The opposite trend of the fitted line was observed, indicating that the extraction of lipids using M:EA does not obey the Arrhenius equation. This confirms that an increase in temperature does not translate to higher lipid extraction when the M:EA solvent is used. The same observation was noticed by Hobbi *et al.*[33] with E_a = −17.0 kJ mol^{-1}, and A_e = 0.00 g min^{-1}, during the extraction of phenolic compounds using the acetone–water solvent, showing that there was no correlation between temperature and k.

3.5. Fatty acid analysis of lutein extracted biomass

The results of the fatty acids are presented in Fig. 6, which shows that the major composition of fatty acids consists of C16 : 0, C16 : 2, C16 : 3, C18 : 2, C18 : 3 and C19 : 0 in dry and wet biomass. The same fatty acids were found in other studies reported by other researchers.[1,35,36] In addition, the dominant fatty acid components were C16 : 0 (27%), C18 : 2 (30%) and C18 : 3 (23%). This indicates that the major component in fatty acids lies between C16 and C18, which provides several advantages such as low viscosity, quality ignition and higher oxidative stability for longer storage.[35,37,38] These types of fatty acids are the most suitable for biodiesel.[1] Fig. 6 depicts the major composition of the fatty acids, including 28.0% of saturated fatty acids (SFAs) and 72.0% of polyunsaturated fatty acids (PUFAs), while for the wet biomass 32.0% SFAs and 70.0%

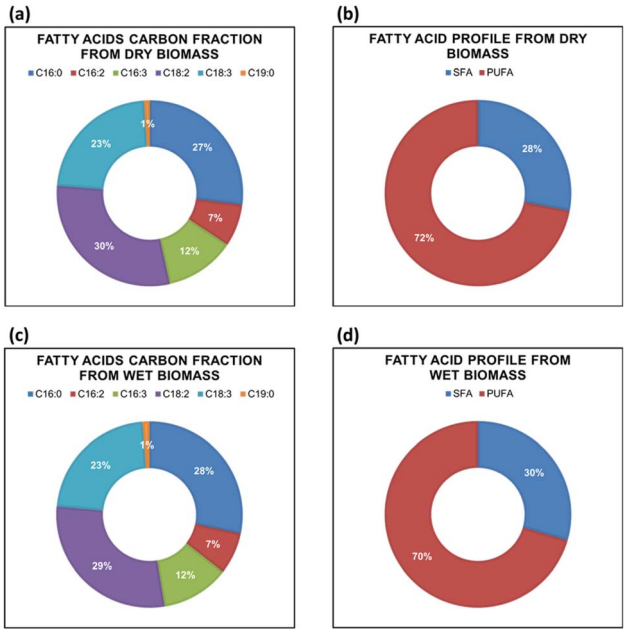

Fig. 6 Fatty acid (C16–C19) fraction (a) and (c) and profile (b) and (d) of microalgae biodiesel from dry and wet biomass.

PUFAs were noticed which was very similar to *Chlorella* sp. (34% SFA and 66% PUFAs) and *Scenedesmus* sp. culture (36.5% SFAs and 63.5% PUFAs).[39] Besides, Ngangkham *et al.* reported 31.8% SFAs and 60.2% PUFAs from *Chlorella sorokiniana*.[40]

3.6. Economic assessment of DES *vs.* ILs

The price of the solvent is a key problem in its industrialisation; whenever a new solvent is proposed, one of the most asked questions is its cost. Up to now, it can be confirmed that the success of DES lays on its simplicity of the preparation method with the parent compounds like organic salts and ionic liquids. Quaternary Ammonium Salts (QASs) and phosphonium salts are used to prepare the DES, which are cheap compared to imidazolium-based ionic liquids. One of the most attractive features of DES systems is their straightforward preparation process is just mixing, and heating without purification, which is the real benefit not only from the synthesis point of view but also from the economic point of view. The introduction of all the DES as the raw material is cheap, environmentally friendly, and economical.[14] Therefore, the cost of DES and ILs was assessed using the equation reported by Dugoni *et al.*[41]

$$\text{DES or ILs price} = M_1P_1 + M_2P_2/M_1 + M_2 \quad (12)$$

Here M_1 is the molecular weight of component 1, M_2 is molecular weight of component 2, while P_1 and P_2 are the prices of components 1 and 2, respectively.

To compare the price of DES [ChCl : EG (1 : 2)] and ILs, ILs were selected from the previous reports,[28,42] which were used for the pretreatment of microalgae biomass for carotenoid extraction. Five ILs [1-ethyl-3-methylimidazolium chloride (EMIM)Cl, 1-ethyl-3-methyl-imidazolium-ethylsulphate (EMIM) $EtOSO_3$, 1-butyl-3-methylimidazoliumbromide, 1-butyl-3-methyl-imidazolium-chloride (BMIM) Cl, and diallylammonium diallylcarbamate (DACARB)] were used and labelled as IL-01 to IL-05. Fig. 7 shows the comparison of the DES and ILs, and it can be observed that the price of DES (18.72 USD per kg) is 13 times less than that of IL-02 [1-ethyl-3-methyl-imidazolium-ethylsulphate (EMIM $EtOSO_3$)]. In the same way the cost of DES compared to other ILs is also less (*i.e.*, 28, 10, 8, and 2 times). This shows a significant cost reduction, which can play a vital role in biomass valorization to extract lutein and other value-added compounds.

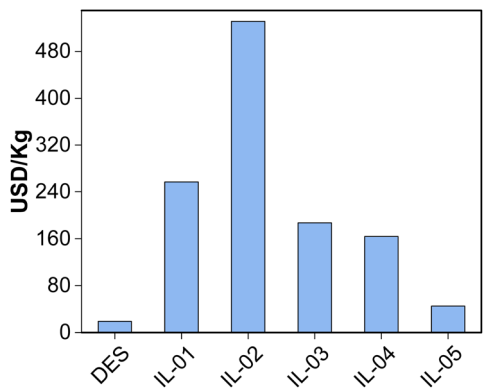

Fig. 7 Price comparison of DES [ChCl : EG (1 : 2)] and other ILs.

4. Conclusion

In summary, microalgae are a promising source of lutein and other value-added chemicals for food applications. To improve the cost effectiveness of the algal biomass, a biorefinery approach was applied. Sequential extraction of lutein and lipid from DES-pretreated dry and wet biomass was attempted, representing a stepwise extraction of the compounds. The kinetic study of solid liquid extraction process of compounds were performed. Kinetic parameters showed that a lower temperature is needed for the lutein and lipid extraction using ethanol and M : EA (2 : 1) solvent, indicating the lower energy requirement, which can improve the economics in a large-scale process. Furthermore, the solvent demonstrated lower toxicity, as evidenced by the survival of *E. coli*, indicating environmental friendliness.

Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Author contributions

Conceptualization: BZ, GM, JX, methodology: BZ, GM; investigation: BZ, GM, MAA, LD; supervision: JX, GM, XZ; writing—original draft: BZ, GM; writing—review & editing: BZ, GM, AZ, MAA, ZL, TB, JX; funding: JX, GM.

Conflicts of interest

The authors declare no conflicts of interest.

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