Green Chemistry

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Effective lipid extraction from algae cultures using switchable solvents†

Cite this: *Green Chem.*, 2013, **15**, 353

Received 31st October 2012,
Accepted 5th December 2012

DOI: 10.1039/c2gc36730k

www.rsc.org/greenchem

Chiara Samorì,*[a] Diego López Barreiro,[b] Robin Vet,[c] Laura Pezzolesi,[a] Derk W. F. Brilman,[c] Paola Galletti[a,d] and Emilio Tagliavini[a,d]

A new procedure based on switchable polarity solvents (SPS) was proposed for lipid extraction of wet algal samples or cultures, thereby circumventing the need for an energy intensive drying step and facilitating easy recovery of the lipids from the extraction liquid. Lipids were extracted by using *N*,*N*-dimethylcyclohexylamine (DMCHA) and recovered by adding CO_2, thereby switching DMCHA into a hydrogen carbonate ammonium salt and resulting in the formation of a separate liquid lipid phase.

In the last decades, microalgal biomass has been suggested as a very promising energy source thanks to some important advantages over terrestrial biomass, including high photosynthetic efficiency, higher yields per cultured area compared with terrestrial crops, high lipid content in some strains and the possibility of marginal lands exploitation (*e.g.* desert regions).[1] However, in spite of these attractive benefits, economically viable microalgal biofuel production systems have not yet been demonstrated and the most appropriate and cost-effective set of technologies suitable for algae treatment are yet to be successfully integrated and optimized.[2] Due to this, the industrial exploitation of microalgae for biofuels applications is at the moment still hampered by too high costs for labor, nutrients and various process steps within the entire process.

Harvesting, dewatering and drying are a crucial part of microalgae cultivation and processing. The algae can be harvested by a variety of techniques such as filtration, centrifugation, flocculation (by using inorganic salts, polyelectrolytes or bio-flocculants), sedimentation or ultrasound separation, but the efficiencies of these processes are far from satisfactory.[3] The main problem is the energy required to handle these large volumes of cultivation media, which are very dilute suspensions (<0.5 kg m^{-3} dry biomass in some commercial production systems) of algae with cell diameters often below 20 μm.[4] Direct extraction of the desired lipid fraction from the algal culture media, or at least from the wet biomass after a pre-concentration step, would be an attractive solution. However, with current organic solvent systems the results are still unsatisfactory. This is partly due to their limited extraction efficiency, as the lipids to be extracted are included within the algal cells which are, in turn, surrounded by water. Furthermore, the subsequent solvent separation and lipids recovery is energy intensive.

Recently, some successful improvements have been made in the area of extraction efficiency; Deng *et al.*[5] treated cultures of *Nannochloropsis* sp. by using water as a tunable co-solvent for a supercritical methanol extraction process; Yang *et al.*[6] applied an osmotic shock pretreatment followed by solvent extraction to extract lipids from *Chlamydomonas reinhardtii* cultures; Satish *et al.*[7] developed a lipid extraction procedure on wet *Scenedesmus* sp. and *Chlorella* sp. samples (84% water content) *via* acid and base hydrolysis; Teixera[8] reported the successful weakening of various microalgae cell walls by exploiting the ability of imidazolium chloride ionic liquids to break the H-bonds of polysaccharides, causing the release of cellular components which may be directly extracted from the algal cultures with organic solvents. However in all of these applications, organic solvents have been used, thus a subsequent, energy-intensive solvent-lipid separation step *via* distillation or evaporation is needed.

In a previous paper, we developed a novel lipid extraction process from *Botryococcus braunii* diluted cultures by using switchable polarity solvents (SPS).[9] This new fascinating class of extracting media is capable of turning from a non-ionic, non-polar form into an ionic liquid by simply bubbling and removing CO_2.[10] Our system, based on an equimolar mixture of 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) and octanol, was more efficient in the extraction of hydrocarbons from

[a]*Centro Interdipartimentale di Ricerca Industriale (CIRI), University of Bologna, via S. Alberto 163, Ravenna, Italy. E-mail: chiara.samori3@unibo.it; Fax: +0039-0544-937411; Tel: +0039-0544-937353*
[b]*Department of Biosystems Engineering, Ghent University, Ghent, Belgium*
[c]*Thermo-Chemical Conversion of Biomass Group, Faculty of Science and Technology, University of Twente, Enschede, The Netherlands*
[d]*Department of Chemistry "Ciamician", University of Bologna, via F. Selmi 2, Bologna, Italy*

†Electronic supplementary information (ESI) available: Experimental details, microorganism cultivation and characterization, NMR spectra. See DOI: 10.1039/c2gc36730k

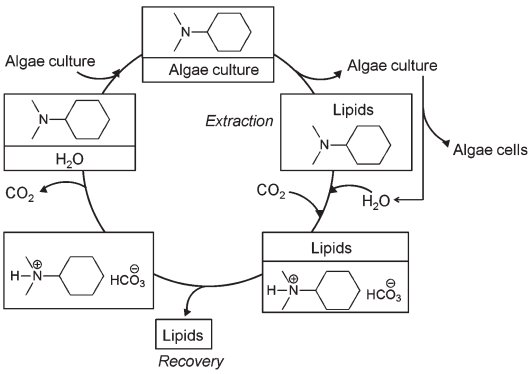

Fig. 1 Scheme of algae cultures extraction based on DMCHA.

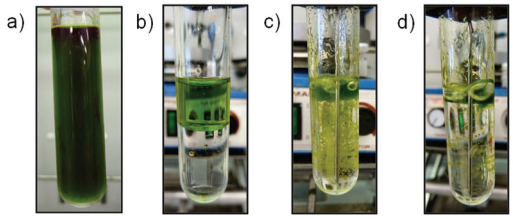

Fig. 2 Extraction of algal wet biomass with DMCHA (50 mg mL^{-1} extraction system): (a) DMCHA containing algal lipid (green layer) after 24 h of extraction (algal biomass was removed by centrifugation); (b) on the top DMCHA, on the bottom H_2O; (c) CO_2 bubbling; (d) after formation of $DMCHAH^+$ HCO_3^-, lipids (green layer and drops) float on the surface of the system.

B. braunii cultures than traditional organic solvents such as hexane. Independently, Jessop *et al.*[11] have introduced a new SPS based on *N*,*N*-dimethylcyclohexylamine (DMCHA) to extract freeze-dried samples of *B. braunii*.

In this work, we want to report the use of a novel SPS system, based on DMCHA, for extracting and recovering lipids directly from wet samples (about 80% water content) and cultures (biomass concentration around 2 g L^{-1}) of three microalgal strains: two marine species, *Nannochloropsis gaditana* and *Tetraselmis suecica*, and a freshwater species, *Desmodesmus communis* (Fig. 1). Our system achieved for the first time the extraction of lipids from dilute cultivation media without any treatment for cell disruption and avoiding the use of volatile organic solvents and their associated evaporation energy costs for the lipid recovery.

Our choice for DMCHA was based on its low volatility, intermediate polarity, and relatively low water solubility (18 g L^{-1}) which are good properties from a technical and environmental point of view. In particular, amines like DMCHA have a switchable hydrophilicity: this means that they can be switched from a hydrophobic form, with a low water-solubility, into a water-soluble hydrophilic form.[12]

The efficiency of DMCHA in extracting algal lipids from *wet biomass* was tested by varying the biomass/DMCHA ratio from 2 mg mL^{-1} to 50 mg mL^{-1}. The systems were stirred at room temperature for 24 h, and then, after having removed the algal residual biomass by centrifugation, the organic layer was split into two parts: one was used to determine the *lipid extraction efficiency* of DMCHA by evaporating the amine, determining total lipids (TLs) content by weight and analyzing both free and bounded fatty acids amount in the extract through conversion into fatty acid methyl esters (FAMEs); the other one was used to determine the *lipid recovery efficiency* after switching the SPS into the polar form, namely into the hydrogen carbonate ammonium salt ($DMCHAH^+$ HCO_3^-) through addition of CO_2 and H_2O as shown in Fig. 2, and analyzing also in this case the FAMEs yield (detailed experimental procedure in ESI†).

The *lipid extraction efficiency* of the system applied to wet biomass, expressed as *TLs extracted* on algal dry weight basis, was higher than that obtained through a typical hot extraction

Table 1 TLs and FAMEs content expressed on algal dry weight basis (means ± standard deviation, n = 3), obtained through $CHCl_3$–MeOH hot extraction of dried samples and DMCHA extraction of wet samples (50 mg mL^{-1}, 24 h extraction)

	TLs (wt%)		FAMEs (wt%)	
	$CHCl_3$–MeOH	DMCHA	$CHCl_3$–MeOH	DMCHA
D. communis	17.8 ± 0.1	29.2 ± 0.9	6.0 ± 0.1	6.1 ± 0.7
N. gaditana	45.1 ± 0.9	57.9 ± 1.3	10.6 ± 0.1	11.0 ± 0.9
T. suecica	25.4 ± 2.6	31.9 ± 1.5	4.5 ± 0.5	5.4 ± 0.6

procedure with $CHCl_3$–MeOH (Table 1); the observed differences in comparison with $CHCl_3$–MeOH could probably be due to additional non-volatile material extracted by DMCHA. The composition of the oils extracted by the two methods, in terms of neutral lipids (NL), glycolipids (GL) and phospholipids (PL) (see ESI†), revealed some differences, especially concerning *D. communis*; for this alga the amount of each lipid class (on algal dry weight basis) was much higher than that obtained with $CHCl_3$–MeOH, suggesting that DMCHA could have access to lipids which were not extractable by $CHCl_3$–MeOH (Table 2).

We also examined the *lipid extraction efficiency* of the system on wet samples, *expressed as FAMEs yield* (wt% on algal dry weight basis) as a function of the extraction time (Fig. 3). The FAMEs yield was very good for all the tested algae, independent of the biomass/DMCHA ratio; after 24 h FAMEs content was slightly higher than that obtained by submitting algal pellets to $CHCl_3$–MeOH extraction: this could probably be due to the fact that DMCHA had access to structural lipids which are resistant to extraction with $CHCl_3$–MeOH (Table 1).

The *lipid recovery efficiency* upon converting the DMCHA with H_2O and CO_2 into $DMCHAH^+$ HCO_3^- was around 70–80% independent of the DMCHA/biomass ratio, the time and the algal species (due to the small scale of the experiments, hexane was used to recover the lipid layer, see ESI†). These findings could be due to both the small scale of the system and to the presence of a certain amount of polar lipids (*e.g.* for *N. gaditana* polar lipids represent more than 50% of the total) which could be partially retained in the water phase. The

Table 2 Lipids fractionation of oils obtained through extraction of dried algae samples ($CHCl_3$–MeOH) and wet algal biomass (DMCHA), expressed on algal dry weight basis (NL: neutral lipids; GL: glycolipids; PL: phospholipids)

	$CHCl_3$–MeOH			DMCHA		
Lipid (wt%)	*D. communis*	*N. gaditana*	*T. suecica*	*D. communis*	*N. gaditana*	*T. suecica*
NL	1.6	7.2	2.8	4.1	5.2	3.5
GL	10.3	12.6	12.7	14.3	29.5	14.7
PL	5.9	25.3	9.9	10.8	23.2	13.7

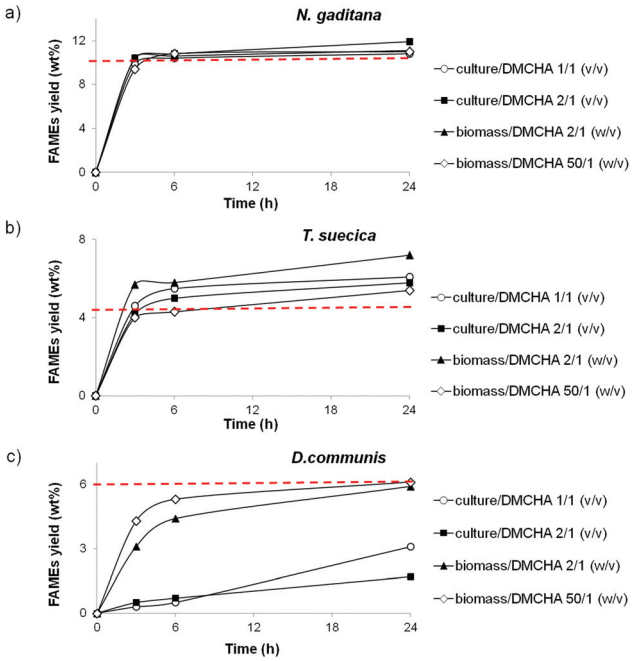

Fig. 3 FAMEs yields in dependence of time, obtained through DMCHA extraction of the three algae cultures or wet biomass (for comparison, the red dotted line is FAMEs yield obtained with $CHCl_3$–MeOH hot extraction).

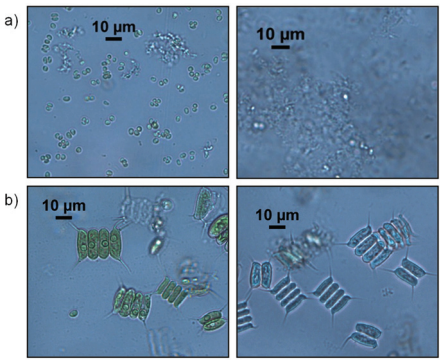

Fig. 4 Light microscope images (320×) of (a) *N. gaditana* and (b) *D. communis* cultures before (left) and after (right) 24 h extraction with DMCHA.

amount of amine lost in the lipid recovered fraction (see ESI†) was 0.9% of the DMCHA used for the extraction.

The efficiency of DMCHA in extracting *algal cultures* (biomass concentration around 2 g L^{-1}) was tested by varying the culture/DMCHA volume ratio from 2/1 to 1/1 (this means biomass/DMCHA ratios of 4 mg mL^{-1} and 2 mg mL^{-1}, respectively); the heterogeneous system was stirred at room temperature for 24 h and, after settling, the organic layer on the top was separated and split into two parts, as for the wet biomass experiments. We can see that *N. gaditana* (Fig. 3a) and *T. suecica* (Fig. 3b) cultures were easily and quickly extracted with both the culture/DMCHA ratios affording high FAMEs yields after 3 h contact time that slightly increased after 24 h. Even in this extraction system the FAMEs amount obtained was slightly higher than that obtained by submitting algal pellets to $CHCl_3$–MeOH extraction. In contrast, our protocol was less suitable for the extraction of *D. communis* cultures (Fig. 3c): after 24 h, FAMEs yield was just 3.1 and 1.7 wt% (culture/DMCHA ratio 1/1 and 2/1, respectively), less than a half of FAMEs extracted with $CHCl_3$–MeOH (6.0 wt%). However, as shown before, good results were obtained for this type of alga from the extraction of the wet biomass slurry, where the required contact between cellular lipids and the SPS, due to a lower amount of water, can be achieved. Water in fact could act as a barrier between hydrophobic DMCHA and the dispersed algal biomass.

The reason for the different behavior of the three algae can be found in the features of their cell walls. In the case of *N. gaditana* and *T. suecica*, we could hypothesize that the amine was able to disrupt their thin polysaccharide cell walls, having access to a fraction of lipids which are not accessible to the $CHCl_3$–MeOH mixture. The low efficiency in extracting *D. communis* cultures was not surprising: *D. communis*, in fact, has a very thick cell wall mainly composed of algaenans, linear C_{22}–C_{34} hydrocarbon chains cross-linked through ether and ester bonds, which confer to the cell wall an extraordinarily high resistance to many treatments (such as solvent extraction, heating, sonication and milling).[13] This hypothesis was confirmed by light microscope observation of *N. gaditana* and *D. communis* cultures after 24 h of extraction with DMCHA: in the first case, cells were completely broken indicating that DMCHA was able to destroy cellular structures and thus have access to and extract all the lipid content (Fig. 4a). In the second case, cells appeared empty (without chlorophyll) but intact (surrounded by the cell wall), suggesting that the thick cell wall was only slightly affected by DMCHA; in contrast we think that cell membranes were easily destroyed by the amine, allowing the release of water soluble compounds such as chlorophyll (Fig. 4b).

A major concern regarding the use of amines as extracting solvents is the possible toxic effects to humans and environmentally valuable organisms. Indeed DMCHA possesses significant water solubility (18 g L^{-1}) and adverse biological effects (*e.g.* 50% of the maximum adverse effect, EC_{50}, is 88.5 mg L^{-1} towards *Desmodesmus* sp.).[14] As a consequence, residual water at the end of the process should be depurated and could not be reused as a medium for algal growth. We have also tried to decrease the amount of DMCHA losses in the water phase by increasing the salinity of the medium; in this case we found that the solubility of DMCHA in water decreased from 18 g L^{-1} to 1.2 g L^{-1} (see ESI†) by increasing the salinity up to 70 psu.

In conclusion, we have demonstrated that SPS based on a lipophilic tertiary amine, such as DMCHA, can be successfully applied to the extraction of wet algal biomass and, most relevantly, of cultures. A complete life cycle assessment and an economic analysis will correctly weigh all contributions but we believe that the proposed process could help in reducing the costs related to algae treatments because of low energy consumption, above all in the case of culture extraction systems: no energy is used for de-watering the biomass, the extractions are performed at room temperature, the algal lipids are recovered by the addition of CO_2 and the solvent system is recycled (switched-back) by CO_2 removal. Certainly, one of the major drawbacks is the toxicity: even if DMCHA has a low volatility (b.p. 158 °C), its (eco)-toxicity is quite high and we are currently working on possible structural modifications, such as the introduction of polyethoxylated chains, which could reduce the impact on biological systems.[15] Although other aspects of our process need further improvements (*e.g.* the increase of lipid recovery efficiency and the reduction of amine loss), we are confident that enhanced research will overcome these drawbacks to make the SPS extraction of wet algal biomass and cultures a profitable tool in the exploitation of algae for a biorefinery-based energy economy.

Acknowledgements

We thank Dr Fabio Moretti of the University of Bologna, Italy, for NMR spectra; we are grateful to Robert Nachenius of Ghent University, Belgium, for English revision of the manuscript. We thank the University of Bologna (RFO program) and CIRI Energia e Ambiente for funding and fellowship grants.

Notes and references

1 J. R. Benemann, J. C. Weissman, B. L. Koopman and W. J. Oswald, *Nature*, 1977, **268**, 19.
2 E. Stephens, I. L. Ross, Z. King, J. H. Mussgnug, O. Kruse, C. Posten, M. A. Borowitzka and B. Hankamer, *Nat. Biotechnol.*, 2010, **28**(2), 126.
3 E. Molina Grima, E. H. Belarbi, F. G. Acien Fernandez, A. Robles Medina and Y. Chisti, *Biotechnol. Adv.*, 2003, **20**, 491.
4 L. Xu, D. W. F. Brilman, J. A. M. Withag, G. Brem and S. Kersten, *Bioresour. Technol.*, 2011, **102**, 5113.
5 P. D. Patil, V. G. Gude, A. Mannarswamy, S. G. Deng, P. Cooke, S. Munson-McGee, I. Rhodes, P. Lammers and N. Nirmalakhandan, *Bioresour. Technol.*, 2011, **102**(1), 118.
6 G. Yoo, W. K. Park, C. Woong Kim, Y. Choi and J. W. Yang, *Bioresour. Technol.*, 2012, **123**, 717.
7 A. Sathish and R. C. Sims, *Bioresour. Technol.*, 2012, **118**, 643.
8 R. E. Teixeira, *Green Chem.*, 2012, **14**, 419.
9 C. Samorì, C. Torri, G. Samorì, D. Fabbri, P. Galletti, F. Guerrini, R. Pistocchi and E. Tagliavini, *Bioresour. Technol.*, 2010, **101**, 3274.
10 P. G. Jessop, D. J. Heldebrant, X. Li, C. A. Eckert and C. L. Liotta, *Nature*, 2005, **436**, 1102.
11 A. R. Boyd, P. Champagne, P. J. McGinn, K. M. MacDougall, J. E. Melanson and P. G. Jessop, *Bioresour. Technol.*, 2012, **118**, 628.
12 P. G. Jessop, L. Kozycz, Z. Ghoshouni Rahami, D. Schoenmakers, A. R. Boyd, D. Wechsler and A. M. Holland, *Green Chem.*, 2011, **13**, 619.
13 L. G. Alba, C. Torri, C. Samorì, J. van der Spek, D. Fabbri, S. R. A. Kersten and D. W. F. Brilman, *Energy Fuels*, 2012, **26**, 642.
14 IUCLID dataset.
15 C. Samorì, G. Sciutto, L. Pezzolesi, P. Galletti, F. Guerrini, R. Mazzeo, R. Pistocchi, S. Prati and E. Tagliavini, *Chem. Res. Toxicol.*, 2011, **24**, 392.

