

 **PATH** Spring Workshop
3rd Edition

MULTI**BI****REFINERY**



PATh Spring Workshop

June 8th 2018

Meliã Ria Hotel & Spa, Aveiro

BOOK OF ABSTRACTS

Organizing Committee:

João A. P. Coutinho

Mara G. Freire

Sónia P. M. Ventura

Pedro J. Carvalho

Armando J. D. Silvestre

Scientific Program

9:00 – Opening session (Sónia Ventura & Mara G. Freire)

9:10 – Invited Lecture

Can Advanced Materials from Renewable Resources Replace Plastics in a Sustainable Society?

Prof. Robin D. Rogers, McGill University, Montreal, QC, United States

9:50 – Discussion

Recovery of added-value compounds from biomass

10:00 – *Polyols as efficient bio-based solvents for the extraction of phenolic compounds from *Juglans regia* L. (Vanessa Vieira)*

10:10 – *Hydrotrope- and micelle-mediated phenomena of ionic liquids in aqueous solutions: their role in the extraction of added-value compounds from biomass (Emanuelle Faria)*

10:20 – *Integrated production-separation platforms of HMF applying switchable aqueous biphasic systems (Ana Maria Ferreira)*

10:30 – *Separation of ϵ -polylysine from culture broth using ionic-liquid-based aqueous biphasic systems (Mukesh Sharma)*

10:40 – *Fractionation of value-added compounds from honey using ionic-liquid-based systems (Sónia Pedro)*

10:50 – Discussion

11:10-11:40 – Coffee Break

11:40 – *New nanomaterials from protein fibrils: from fibrillation processes to innovative applications (Nuno Silva)*

11:50 – *Integrated process to extract and purify violacein from *Yarrowia* cells (Mariam Kholany)*

12:00 – *Extraction of intracellular carotenoids from yeast *Rhodotorula glutinis* CCT-2686 using protic ionic liquids as cell disrupting agents (Jorge F. B. Pereira)*

12:10 – *Integrated enzymatic catalysis: product recovery and enzyme reuse with thermoreversible aqueous biphasic systems (Helena Passos)*

12:20 – *(Egg)refinery: Egg fractionation into added-value proteins and functional materials (Mara G. Freire)*

12:30 – Discussion

12:50-14:30 – Lunch

14:30 – Invited Lecture

Ultra-low cost ionic liquids for lignocellulose deconstruction of contaminated waste wood

Dr. Jason P. Hallett, Imperial College, United Kingdom

15:10 – Discussion

Biomass deconstruction/fractionation

15:20 – *Insights on the mechanisms of wood delignification mediated by DES and DES aqueous solutions (André Lopes)*

15:30 – *Fractionation of wood using deep eutectic solvents aqueous solutions (Belinda Soares)*

15:40 – *Sustainable hemicelluloses extraction using Deep Eutectic Solvents (Eduarda Morais)*

15:50 – Discussion

16:10-16:40 – Coffee Break

Blue biorefinery

16:40 – *Technological development towards the industrial marine biomass valorization (Sónia Ventura)*

16:50 – *Phycobiliproteins purification by induced precipitation (Margarida Martins)*

17:00 – *Continuous purification of phycobiliproteins incorporating aqueous micellar two-phase systems in a continuous tubular separator (Filipa Vicente)*

17:10 – *Extraction of collagen from codfish skin (Meena Bisht)*

17:20 – Discussion

17:40 – Closing session

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Can Advanced Materials from Renewable Resources Replace Plastics in a Sustainable Society?

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Humanity has always relied on Nature for survival, but today we take oil as a feedstock and use it to produce chemicals to suit any purpose, from fuels and medicines to plastics. By reducing our reliance on Nature, we have forgotten our respect of Nature in the process. The world is now covered in oil in the form of non-biodegradable synthetic plastics which mar our landscapes and threaten our oceans. By 2050 there is predicted to be more plastic in the ocean than fish. At the same time, plastics represent a \$654 billion market worldwide and are ubiquitous in modern life. Simply eliminating synthetic plastic use with no viable alternatives would be almost impossible and catastrophic from both a social and economic point of view.

Chemists make new chemicals and that has led to the development of a chemical industry that knows how to sell these chemicals. The more chemicals we make, the more the industry seeks new and better markets to sell them into. New, more sustainable technologies have been forced into the business of 'replacements' and must follow the same commercialization pathways as the old technologies they are trying to replace. The unsustainable practices, however, have often had decades or more head start and thus the economics to replace them are often insurmountable without government or policy intervention. The technologies to directly replace synthetic plastics with renewable biopolymers from plants and animals are there, but the consistencies of supply and economies of scale to make them competitive are not.

With a plethora of abundant natural and renewable resources (in terms of both quantity and diversity), from water purification - to cosmetics - to medical applications, the quality of human life can greatly benefit (and improve at the same time) from new, innovative technologies using building blocks obtained directly from plants and animals. With this mission in mind, we are exploring the entire range of the "biorefinery concept," from dissolution, and conversion of plant and animal resources into value added chemicals and extraction of essential oils or vital chemicals, to isolation of pure biopolymers and production of new biomaterials from them. Because we can directly dissolve Nature's raw materials, we can shape them, functionalize them, blend them, and use other non-chemistry means to produce advanced functional materials. These new, innovative materials represent new market opportunities which we will develop and promote



Figure reproduced from Rogers, R. D. "Eliminating the need for chemistry," *C&E News* 2015, 93, 42–43 Credit: Steven Kelly/C&EN/Shutterstock

Polyols as efficient bio-based solvents for the extraction of phenolic compounds from *Juglans regia* L.

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In this work, a set of polyols were studied as promising green solvents to recover phenolic compounds from natural matrices. Some of them are already allowed to be included in food, pharmaceutical and cosmetic applications due to their biocompatibility, and can be obtained from renewable sources. Considering the natural matrix, walnut leaves (*Juglans regia*) were selected as a significant source of bioactive compounds, namely phenolic compounds (flavonols, phenolic acids). In this context, their extracts (or pure components) could be used as natural antioxidants in different applications, such as the replacement of synthetic antioxidants such as BHT (2,6-di-tert-butyl-4-methylphenol) or in dermatological bases preventing oxidative damage^{1,2}.

Glycerol and a series of diols (1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, 1,2-pentanediol, 1,5-pentanediol, 1,2-hexanediol, 1,6-hexanediol) were screened as solvents and the correspondent phenolic profile was characterized by HPLC-DAD and compared to the reference ethanol + water solvent. Finally, the bioactivity of the extracts was evaluated by measuring the total oxidant capacity by oxygen radical absorbance capacity (ORAC) and the anti-inflammatory activity (NO inhibition). The obtained results provide relevant information regarding the design of extraction processes using alternative solvents that could potentially be used as formulation media.

References

¹Jeong et al., *J. Clean. Prod.*, 2017, 151, 87-95.

²Ribeiro et al., *Cosmetics.*, 2015, 2, 48-65.

Acknowledgments: The authors thank the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013), V. Vieira (SFRH/BD/108487/2015) grant and L. Barros contract. To project POCI-01-0145-FEDER-006984 – Associate Laboratory LSRE-LCM funded by FEDER through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) – and by national funds through FCT – Fundação para a Ciência e a Tecnologia. To project CICECO-Aveiro Institute of Materials POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement.

Hydrotrope- and micelle-mediated phenomena of ionic liquids in aqueous solutions: their role in the extraction of added-value compounds from biomass

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Ionic liquids (ILs) aqueous solutions have a high potential for the extraction of value-added compounds from biomass.¹ Some studies demonstrated that ILs in aqueous media can act either as hydrotropes or as surface-active agents, playing a key role in the solubility of bioactive compounds.^{2,3} Surface-active ILs correspond to compounds presenting long alkyl chains and able to form aggregates in aqueous solutions, while hydrotropes, although also presenting a non-polar and polar part, do not form self-aggregates but instead form aggregates with other compounds, such as bioactive compounds. Taking advantage of these features, this work shows the advantages of using hydrotrope ILs aqueous solutions for the extraction of phenolic acids versus the use of surface-active ILs aqueous solutions for the extraction of triterpenic acids from biomass samples. The solubility of phenolic acids (syringic acid) and triterpenic acids (ursolic, oleanolic and betulinic acids) in aqueous solutions of different ILs was determined, and the best identified solvents were applied in the extraction of these value-added compounds from *Rocha* pear and apple peels. The performance of conventional volatile organic solvents in the extraction of the target compounds was also evaluated for comparison purposes. If properly chosen, ILs aqueous solutions are remarkable solvents to improve the solubility and extraction of bioactive compounds from agro-food byproducts, contributing therefore to their valorization in a Biorefinery context.

References

¹Passo, H., Freire M.G.; Coutinho J.A.P.; *Green Chem.*; 2014;16(12):4786-4815

²Łuczak J.; Hupka J.; Thöming J.; Jungnickel, C; *Colloids Surfaces A Physicochem. Eng. Asp.*; 2008;329(3):125-133

³Cláudio A.F.M.; Neves M.N.; Shimizi K.; Lopes J.N.C.; Freire M.G.; Coutinho J.A.P.; *R. Soc. Chem.*; 2015;17(7):3948-3963

Acknowledgments: This work was developed in the scope of the project CICECO-Aveiro Institute of Materials (Ref. FCT UID/CTM/50011/2013), financed by national funds through the FCT/MEC and FEDER under the PT2020 Partnership Agreement. E. L. P. de Faria acknowledges financial support from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the PhD grant 200908/2014-6.

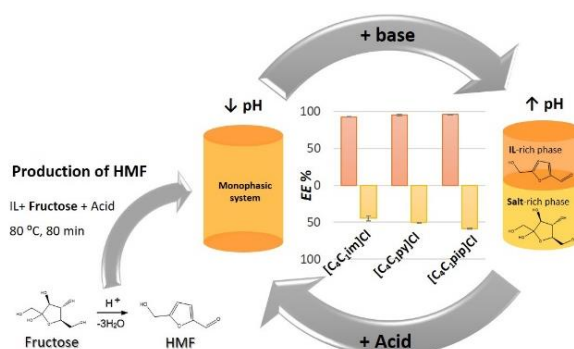
Switchable (pH-Driven) aqueous biphasic systems constituted by ionic liquids as integrated production-separation platforms

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Recently, a large interest has been devoted to dynamic and reversible biphasic systems constituted by ionic liquids (ILs) in separation processes. It was previously demonstrated that mixtures involving ILs and other solvents can be switched between the homogeneous regime and a two-phase system either by a temperature-driven phenomenon or by adding CO₂/N₂. In this work, we show that aqueous biphasic systems (ABS) composed of ILs, i.e., ternary systems where the major solvent is water, may have their reversible behavior triggered by small changes in pH. These systems were investigated as integrated reaction-separation platforms (Fig. 1), in which reaction and separation steps can be carried out sequentially by taking advantage of their switchable behavior. The proposed systems are able to produce hydroxymethylfurfural (HMF) from fructose dehydration at acidic pH and in homogeneous medium, and then allow the separation of HMF from unreacted fructose by the creation of two-phase systems through an increase in the system pH.¹



References

¹Ferreira et. al., *Green Chem.*, 2017, 19, 2768-2773.

Acknowledgments: This work was developed in the scope of the project CICECO-Aveiro Institute of Materials (Ref. FCT UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when applicable co-financed by FEDER under the PT2020 Partnership Agreement. A. M. Ferreira acknowledges FCT for the PhD grant SFRH/BD/92200/2013. This research was undertaken, in part, thanks to funding from the Canada Excellence Research Chairs Program. The research leading to reported results has received funding from the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ERC grant agreement no. 337753.

Separation of ϵ -poly-L-lysine from culture broth using ionic-liquid-based aqueous biphasic systems

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ϵ -Poly-L-lysine (ϵ -pLys), a homopolypeptide with 25-35 subunits of L-lysine amino acids, was first identified as an extracellular material produced by *Streptomyces albus*.^[1] It is a highly water-soluble homopolypeptide, with applications as food preservative, emulsifying, anti-obesity and anticancer agent, and in the preparation of absorbable hydrogels, biodegradable fibers, etc. Given its large applicability, it is relevant to find suitable solvents for its extraction and purification from the original medium in which it is produced. Over the past decades, ionic liquids (ILs) have been proposed as emerging green solvents for the extraction of value-added biomolecules. ILs can also be used in the formation of aqueous biphasic systems (ABS), as originally proposed in 2003 by Rogers and co-workers.^[2] In this work, IL- ϵ -pLys-based ABS were explored making use of phosphonium- and ammonium-based ILs. The respective phase diagrams and tie-lines were determined at 298K and 323K. These systems were further investigated for the recovery of ϵ -pLys from the natural culture broth, providing high extraction efficiency and high purity. The purified ϵ -pLys obtained from culture broth was characterized by FT-IR, CD, SDS-PAGE and HPLC. The use of IL-based ABS to directly recover ϵ -pLys represents a step forward in the biorefinery concept envisaging the sustainable conversion of biomass into a broad spectrum of biomaterials.

References

¹Shima et al., *Agric. Biol. Chem.*, 1977, 41, 1807–1809.

²Gutowski et al., *J. Am. Chem. Soc.*, 2003, 125, 6632–6633.

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. KP thanks CSIR for financial support and RAS acknowledges DST for INSPIRE fellowship.

Fractionation of value-added compounds from honey using ionic-liquid-based systems

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In the past years it has been faced a strong demand for the development of sustainable and integrated fractionation processes from biomass samples aiming at increasing their value. Given their designer ability, ionic-liquid-based aqueous biphasic systems (IL-based ABS) have emerged as appealing alternatives to the currently used extraction processes ¹. In addition to the separation of value-added products between the coexisting phases of ABS, IL-TPP (three phase partitioning systems) can also be created ², with the formation of a third and intermediate interphase that usually corresponds to precipitated proteins. In the present work novel bio-based ABS were formed by mixing analogues of glycine-betaine ILs (AGB-ILs) and honey samples in aqueous media, allowing the fractionation of antioxidants, carbohydrates and proteins in one-step. In particular, Major Royal Jelly Proteins (MRJPs), with well-known benefits and of high interest for the pharmaceutical, food and cosmetic industries³, can be recovered at the ABS interphase, with recovery yields ranging between 83.8 and 97.3%, and with MRJP1 purified at a high level (80.02 -90.08%). The remarkable results obtained demonstrate the feasibility of forming ABS by the direct application of ILs to complex and natural matrices, and how these systems act as potential platforms to recover value-added compounds from biomass.

References

1 Gutowski, K.E., Broker, G. A., Willauer, H. D., Huddleston J. G., Swatoski, R. P., Holbrey, J. D., Rogers, R. D. *Journal of the American Chemistry Society*, 2003; 125(22):6632–3.

² Alvarez-Guerra E., Ventura, S. P. M., Coutinho, J. A. P., Irabien, A.; *Fluid Phase Equilibria*, 2014; 371: 67–74.

³ Bilikova K., Kristof Krakova T., Yamaguchi Y. *Arh Hig Rada Toksikol.* 2015; 66(4):259-67.

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. S. N. Pedro acknowledges the PhD grant SFRH/BD/132584/2017. M. G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ECR-2013-StG-337753.

New nanomaterials from protein nanofibers: from fibrillation processes to innovative applications

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Protein nanofibers, also known as amyloid fibrils, are gaining much attention due to their peculiar morphology, mechanical strength and functionalities, and are emerging as innovative building nanoblocks for the development of functional nanomaterials as, for instance, biosensors, bioactive membranes and tissue engineering scaffolds.

However, one of the main limitations for the exploitation of protein nanofibers in the design of new materials is their high production time, since fibrillation is a time-consuming process that can take hours, days, and even weeks. In this study, the use of alternative solvents, such as ionic liquids (ILs) and deep eutectic solvents (DES), as fibrillation promoters was investigated, with considerable reduction in the fibrillation time to a few (2-3) hours with conversion ratios over 80%, using lysozyme as model protein.¹⁻²

The potential of the obtained lysozyme nanofibers was then evaluated by preparing pullulan-based nanocomposite films containing lysozyme nanofibers, resulting in highly homogenous and transparent films with improved mechanical performance. The incorporation of lysozyme nanofibers imparted the films with bioactive functionalities, namely antioxidant and antibacterial activities, supporting the use of these films as, for example, eco-friendly edible films for active packaging.³ Lysozyme nanofibers were also blended with nanocellulose fibers to produce a sustainable sorbent film to be used in the removal of mercury (II) from natural waters. The presence of lysozyme nanofibers demonstrated to increase expressively the mercury (II) removal with efficiencies higher than 80-90 %, using realistic concentrations of mercury (II) under the limit established by the European Union regulations. In this communication an overview of these works will be presented, and the most relevant achievements highlighted.

References

¹Silva *et al.*, *Colloids and Surfaces B: Biointerfaces*, 2016, 147, 36-44

²Silva *et al.*, *International Journal of Biological Macromolecules*, 2018, DOI: 10.1016/j.ijbiomac.2018.03.150

³Silva *et al.*, *Food Hydrocolloids*, 2018, 77, 921-930

Integrated process to extract and purify violacein from *Yarrowia lipolytica* cells

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Violacein is a naturally-occurring indole derivative pigment, noteworthy owing to its promising biological activities, namely as an antibacterial, antiviral, antioxidant and antitumoral compound. Since violacein is expressed intracellularly, downstream processes are of utmost relevance. Efficient and industrially viable recovery and purification of the pigment has to be assured. Cell disruption is the first step, recurrently performed by mechanical or organic solvent dependent methods. Violacein was produced by genetically engineered strains of *Yarrowia lipolytica*. This work covers an alternative approach employing alternative solvents aiming the most efficient release of the pigment.¹ In this sense, several surface-active compounds were investigated regarding their extractive performance. The best alternative solvents were selected and showed to be 53% more efficient releasing the violacein from the cells when compared with the conventional solvent, ethanol. Optimization of the operational conditions was also performed, such as considering the solid-liquid ratio, extraction time and amount of the alternative solvent. Finally, the performance of different aqueous biphasic systems (ABS) was tested in order to fractionate the pigment from the main contaminants present on the extract obtained from extraction step.

References

¹ Passos et al., *Green Chem.*, 2014, 16, 4786-4815.

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors are grateful for the financial support of the Portuguese Foundation for Science and Technology (FCT) for the doctoral grant of SFRH/BD/122220/2016 of M. Martins and the IF/00402/2015 of S.P.M. Ventura. Pauline Trébulle was supported through the “IDI 2016” project funded by the IDEX-Saclay, ANR-11-IDEX-0003-02.

Extraction of intracellular carotenoids from yeast *Rhodotorula glutinis* CCT-2686 using protic ionic liquids as cell disrupting agents

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Carotenoids are produced by many plants and microorganisms and have several applications in the pharmaceutical, cosmetic and food industries due to their antioxidant, anticancer and anti-inflammatory potential [1]. The microbial production of carotenoids is intracellular. Therefore, the study of the most appropriate methodologies for cell disruption is required [2]. Traditionally, the *Rhodotorula* yeasts cell disrupting occurs by the use of organic solvents (petroleum ether, DMSO, acetone, chloroform and hexane) [3], and the highest carotenoids release yield was attained with DMSO. However, since the use of DMSO involves some health and environmental concerns, the search for more biocompatible and less toxic solvents is still required. In this work, the capability of 12 ammonium-based protic ionic liquids (PILs) to disrupt the *Rhodotorula glutinis* CCT-2686 cells, and carotenoids releasing, was investigated. The PILs under study comprise a range of ionic liquids of variable hydrophobicity, which were synthesized through the combination of different amines and carboxylic acids. The results have shown that aqueous solutions (at 90% v/v) of all PILs can release the intracellular carotenoids from a cell suspension of 0.2 g/mL. The hexanoate-based ILs were able to recover almost 3-fold more of the carotenoids than the common organic solvent (DMSO). In general, the higher the relative hydrophobicity of the PIL the higher is the carotenoids extraction yield. This study shows that the use of PILs as cell disrupting agents can be a simple, efficient and feasible method to recover intracellular carotenoids, allowing not only to improve the extraction yields but also to improve the sustainability of the traditional cell disrupting methods using organic solvents.

References

- ¹Cardoso, L.A. et al. Biotechnological Production of Carotenoids and Their Applications in Food and Pharmaceutical Products. In *Carotenoids*; InTechOpen: London, UK, 2017.
- ²Almanza A., et al. *Indian J Exp Biol*, 2017, 55, 479–484.
- ³Park P.K., et al. *Sep Purif Technol*, 2007, 53, 148–152

Acknowledgments: This work was developed within the scope of the project Young Researcher Project (FAPESP 2014/16424-7). C. U. Mussagy and J.F.B. Pereira acknowledge the funding from CNPq and CAPES and CAPES-PROEX. C. U. Mussagy also acknowledges the support from Ministry of Science and Technology, High Education and Technical Vocational Training (MCTESTP- Maputo, Mozambique) through HEST Project-World Bank.

Thermoreversible aqueous biphasic systems composed of hydrophilic zwitterions –an integrated bioreaction-separation process

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Enzymes are relevant alternatives in catalytic processes, mainly due to their high activity, specificity and selectivity, and minimization of raw materials consumption and by-products production. Despite these advantages, the maintenance of the enzymes structural stability and their reusability is yet a critical challenge. Reversible aqueous biphasic systems (ABS) appear as an interesting alternative since several processing steps can be combined into a single operation. In this work it is shown that ABS composed of hydrophilic zwitterions (ZIs) combined with aqueous solutions of polymers display thermoreversible behavior and can act as integrated reaction-separation platforms. Unlike classical liquid-liquid systems, ZI-based ABS allow to work in a wide range of temperatures and compositions which can be tailored to fit the requirements of a given separation process. The biocatalytic reaction involving laccase occurs in homogeneous media, after which small changes in temperature induce the formation of two phases and the complete separation of the enzyme from the products in a single-step. These systems also allow the recover and reuse of the enzyme, along with the ZI-rich phase, contributing towards the development of sustainable biocatalytic processes.[1]

References:

¹Ferreira et al., *Green Chemistry* (2018) DOI: 10.1039/C7GC03880A.

Acknowledgments: This work was developed in the scope of the project CICECO-Aveiro Institute of Materials (Ref. FCT UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when applicable co-financed by FEDER under the PT2020 Partnership Agreement. The authors also acknowledge FCT for the PhD grants SFRH/BD/85248/2012 and SFRH/BD/92200/2013 of H.P. and A.M.F., respectively. A.M.F. and H.P. thank the financial support of Professor H. Ohno during their stay in his laboratory for collaboration work. A.P.M. Tavares acknowledges FCT for the Investigator Programme (IF/01634/2015). M.G.F. acknowledges the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ERC Grant 337753.

(Egg)refinery: Egg fractionation into added-value proteins and functional materials

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A high demand on the development of sustainable and integrated fractionation processes from biomass samples has been observed in the past years. Avian eggs are amongst the richest and most balanced sources of nutrients among all the foods available to mankind. For instance, egg yolk contains low-density lipoproteins, livetins, transferrin, several vitamin-binding proteins, carotenoids, salts, among others, while egg white comprises other valuable proteins, such as ovalbumin, ovomucin, lysozyme, among others. These two portions of eggs are also rich in antibodies, namely IgY, IgA and IgM, which can be used as alternative biopharmaceuticals. These antibodies are particularly relevant over the more traditionally studied mammal antibodies since they can be obtained by a less invasive approach and from a renewable source. In addition to these two nutritive egg portions, the egg shell and the egg shell membrane are usually discarded and considered as waste. However, the egg shell is rich in calcium carbonate (94% of its composition), while the egg shell membrane is rich in proteins (60% of its content; collagen (35%), glucosamine (10%), chondroitin (9%) and hyaluronic acid (5%)). Based on this diversity of value-added compounds present in egg, in this work, an (egg)refinery concept will be presented. Examples on the development of novel processes for the purification of proteins and antibodies from egg white and egg yolk, on the production of protein fibrils directly from egg white, and on the production of graphene-like materials from the egg shell membrane will be given.

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. M. G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ECR-2013-StG-337753.

Ultra-low cost ionic liquids for lignocellulose deconstruction of contaminated waste wood

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Ionic liquids (ILs) have proven to be highly tunable ‘designer solvents’ capable of a wide range of exciting chemistries. However, industrial application at large scale is hampered by high solvent cost. This cost is, however, a tunable feature of the solvent itself – provided the ion selection is handled with a careful eye aimed at limiting synthetic complexity. Lowering the solvent cost will increase the attractive opportunities of ILs for bulk processing of lower cost end products – including such applications as biofuels. However, there are serious concerns about the economic viability of their use due to the very high cost of most ionic liquids (> €50/kg). We have overcome this by redesigning the IL based deconstruction process to use low-cost, acidic ILs for lignin dissolution rather than cellulose dissolution, yielding filterable cellulose and a dissolved lignin for precipitation or conversion to high-value chemicals. We have found that processability of the cellulose is high and lignin recoveries near quantitative. We use a range of ‘protic’ ILs, the family typically used in IL industrial processes, because their simple acid-base chemistry results in a simple and cheap synthesis, with a cost (< €1/kg) similar to common organic solvents such as acetone or toluene.

Our process has now been optimised for pretreatment of metal-treated construction wood. Treated wood was successfully pretreated, yielding highly digestible cellulose rich material with a glucose release of up to 76%. We show that, in both cases, 98-99% of the metals could be extracted into the ionic liquid. This presentation will discuss how ionic liquids can be ‘tuned’ to control cost structure of the final solvent, and what implications this will have for the chemical processes involved. The impact of the solvent on large-scale applications, such as biomass pretreatment, will be discussed, with a focus on performance and process considerations such as how the ILs maintain solvent stability under long-term processing conditions, that they can be recovered and continue to exhibit very good performance after multiple reuses.

References

- Brandt-Talbot, A. et al., *Green Chem.*, 2017, 19, 3078-3012.
- Brandt, A. et al., *Green Chem.*, 2015, 17, 5019-5034.
- George, A. et al., *Green Chem.*, 2015, 17, 1728-1734.
- Chen, L. et al., *Green Chem.* 2014, 16, 3098-3106.

Acknowledgments: This work was funded by the Grantham Institute for Climate Change and the Environment, Climate KIC, and the Engineering and Physical Sciences Research Council (EPSRC; EP/K014676/1 and EP/K038648/1).

Insights on the mechanisms of wood delignification mediated by DES and DES aqueous solutions

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Wood is an abundant and renewable lignocellulosic material mainly composed of three main components: cellulose, hemicellulose and lignin. In the current scenario of pulp and paper sector, cellulose is the target macromolecule for the production of pulp and paper, while hemicellulose is practically degraded and lignin is mostly used for energy purposes. This is a poorly valorized approach, which urgently needs improvement through the development of innovative processes for a sustainable wood fractionation in the frame of the biorefinery concept.¹ The application of deep eutectic solvents (DES) in the processing of wood and other types of biomass has been attracting attention as innovative and green technology with a high potential to fractionate wood pulp at lower cost and with reduced environmental impact than conventional technologies.² Furthermore, their capacity to selectively extract lignin and to modify lignin properties for a direct application is promising to achieve higher sustainability.³

The present study was focused on understanding the mechanisms behind lignin extraction from biomass with DES and DES aqueous solutions. In this context, the ability of these systems to cleave covalent bonds present in lignin model compounds was studied. The structure and acidic nature of each DES influenced the cleavage of β -O-4 linkages and the occurrence of further reactions between cleavage products. Furthermore, certain DES, such as lactic acid:choline chloride (LA:ChCl), react with hydroxyl groups of lignin model compounds through esterification reactions and is followed by side chain polymerization of lactic acid. Although consumption of LA:ChCl might carry out during delignification of wood, extracted lignin could have interesting properties for further application.

References

¹Karltorp et al. *Environ. Innov. Soc. Transit.*, 2012, 2, 66-81.

²Alvarez-Vasco et al., *Green Chem.*, 2016, 18, 5133-5141.

³Soares et al. *ACS Sustain. Chem. Eng.*, 2017, 5, 4056-4065.

Acknowledgments: This work was developed in the scope of the project PROVIDES “Deep Eutectic Solvents for Sustainable Paper Production”. The research leading to reported results has received funding directly from the Bio-Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under grant agreement No. 668970. The work was also funded by Fundação para a Ciência e Tecnologia (FCT) through the projects DeepBiorefinery (PTDC/AGR-TEC/1191/2014) and MultiBiorefinery (POCI-01-0145-FEDER-016403).

Fractionation of wood using deep eutectic solvents aqueous solutions

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The biorefinery concept has been emerging as a new business model that involves the integrated exploitation of biomass to produce energy, biofuels, biomaterials and biochemicals following a bioeconomy strategy.¹ To implement this ideology into practice, the pulp and paper industries have been pushing forward the development of biorefinery processes to open up new opportunities for a sustainable valorization of woody biomass.^{2,3} Furthermore, the use of green solvents, such as deep eutectic solvents (DES), for wood fractionation are amongst the main challenges to reach more environmentally friendly and energy efficient processes.⁴ In this context, recent studies have reported a remarkable performance of DES aqueous solutions in the solubilization of macromolecular components of biomass namely lignin⁵ and xylans.⁶ These studies have provided an important contribution to better understand the role of DES on the solubilization of lignin and hemicelluloses, opening new perspectives towards the full exploitation of wood in pulping and biorefinery processes. In the present work, different DES aqueous solutions assisted with mineral acids as catalysts were used to promote *E. globulus* wood delignification. The obtained results show that delignification using examined DES aqueous solutions requires the addition of a mineral acid to enhance lignin removal at mild conditions. This work also demonstrates that DES aqueous solutions are promising solvents for wood delignification at mild conditions allowing the production of pulp and lignin for further valorization.

References

¹Loïc S. and Davide V. *Bio-based and Applied Economics*, 2016, 5(1), 1–4.

²Fernando S. et al., *Energy Fuels*, 2006, 20(4), 1727–1737.

³Jack M. et al., *The Lignin: Technology, applications and Markets – special market analysis study*. RISI 2016.

⁴CEPI / Unfold The Future: The Two Team Project report, 2013.

⁵Belinda S. et al., *ACS Sustainable Chem. Eng.*, 2017, 5(5), 4056–4065.

⁶Eduarda S.M. et al., *ChemSusChem*, 2018, 11, 1–11.

Acknowledgments: This work was developed in the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (Ref. FCT UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors wish to thank FCT and the Navigator Company, for the awarding of a Ph.D. grant (SFRH/BDE/103257/2014) to Belinda Soares. Carmen S. R. Freire acknowledges FCT/MCTES for a research contract under the Program “Investigador FCT 2012”. The Navigator Company were gratefully acknowledge for supplying *Eucalyptus globulus* wood.

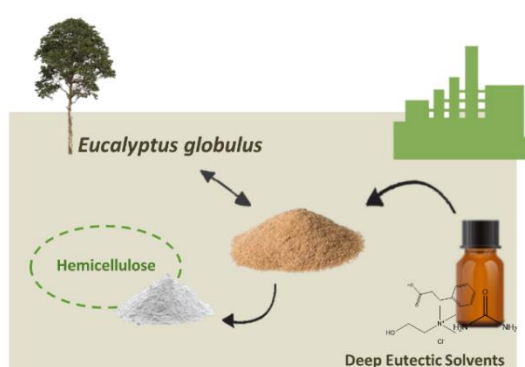
A novel biorefinery inspired process for hemicellulose extraction using Deep Eutectic Solvents

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Focused on reaching a European low carbon bio-economy by 2050, namely in large-scale sectors as the pulp and paper industry, deep eutectic solvents (DES) have emerged as one promising media for biomass fractionation, aiming at enhancing their value while leading to the reduction of the CO₂ emissions¹. Although a considerable number of studies of biomass fractionation with DES have been recently published, they mainly address the extractives fractions (e.g. phenols² and lignin³), while the extraction and processing of most abundant fractions, i.e. polysaccharides, has been poorly investigated. Moreover, an understanding of the mechanisms underlying the solubilisation of biomass components, that would enable a rational selection of DES and process conditions for specific applications, is still needed⁴. In this vein, the present study describes a process developed for the efficient extraction of xylans from wood, covering selection of DES, optimization of extraction conditions, characterization of the isolated xylan, DES recovery and application of the process to *eucalyptus* wood.



References

¹CEPI/Unfold The Future, The Two Team Project report, (2013).

²Pang et al. *Green Chem.* 2012;14(9):2398 – 2401

³Hiltunen et al. *Sci Rep.* 2016;6:1–7.

⁴Dai et al. *Anal Chim Acta.* 2013;766:61–8.

Acknowledgements: This work was developed in the scope of the project CICECO-Aveiro Institute of Materials (Ref. FCT UID/CTM/50011/2013), financed by national funds through the FCT/MEC and co-financed by FEDER under the PT2020 Partnership Agreement. The research leading to reported results has received funding from Fundação para a Ciência e Tecnologia FCT through the projects DeepBiorefinery (PTDC/AGR-TEC/1191/2014) and MultiBiorefinery (POCI-01-0145-FEDER-016403) and through C. Freire Researcher contract (IF/01407/2012), and from the European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013)/ERC grant agreement no. 337753. E. S. Morais is thankful to FCT for the financial support from the doctoral grant (SFRH/BD/129341/2017).

Technological development towards the industrial marine biomass valorization

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Biomass is a natural source of bioactive compounds with high industrial and commercial interest. Included in the biomass sources are the marine raw materials, namely micro and macroalgae, which will be focused here as an example.

Macroalgae (or seaweeds) are rich in chemicals of economic/industrial interest, however most are poorly explored or totally neglected. Phycobiliproteins, carotenoids and chlorophylls, are the principal pigments found in red, brown and green macroalgae, respectively, with a large range of applications, from food industry to pharmaceuticals, biomedical research and cosmetics to the energy field. However, the current extraction and purification (downstream) processes applied in the recovery of pigments and other bioactive compounds from marine biomass need much improvement to make many reported processes economically viable, and also to enabling them to use fresh algae, since the water composing this biomass is one of the major limitations in the purification process.

In our group, we are focused in the development and optimization of different tools and processes integrating the extraction and purification steps in continuous flow. Actually, the processes under development are transversal to various biomasses, from those with marine origin to the forestry biomass and passing through the use of residues. The use of alternative solvents, namely ionic liquids¹ and surfactants², will be discussed, as well as the development and implementation of aqueous biphasic systems as purification methodologies. Enriched extracts/products based in the pigments and other added-value bioactive compounds from marine origin are being created. These extracts are tailor-made considering the purity levels required and the final applications focused.

References

¹Martins et al., *Green Chem.* 2016, 18, 4287-4296

²Vieira et al., *Sep. Purif. Tech.* 2017, 172, 268–276

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. S.P.M. Ventura acknowledges FCT for the IF contract ref: IF/00402/2015.

Phycobiliproteins purification by induced precipitation

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In the past few years, there has been an increased demand to use cheap and abundant marine biomass in order to get compounds of added-value for industry. Phycobiliproteins are intensely fluorescent proteins that function as “helper” components in the photosynthetic apparatus in macroalgae and cyanobacteria, being mainly recognized by their high solubility in water and very high photoluminescence efficiency. Recently, phycobiliproteins were point out as an alternative strategy as optical active centers to be applied on luminescent solar concentrators, since the protein complexes have the ability to capture natural light and convert photons into a flow of electrons leading to the energy production.¹

One of the major drawbacks associated with natural molecules is the demand for an effective, more sustainable, as less costly as possible extraction and purification method, and simultaneously, capable of to maintain their properties and main activities. Conventionally, after the solid-liquid extraction, a pre-purification step, using ammonium sulfate as the precipitating agent followed by a more sensitive approach using chromatographic techniques, is applied to treat the crude extract.² In this context, this work focus on the development of a simple and low cost alternative methodology to the phycobiliproteins purification based on the selective precipitation and purification of phycobiliproteins in a more selective way using aqueous solutions of different polymers.

References

¹ Cubicciotti, U.S. Patent 7,522,162 B2, 2009.

² Liu et al., *J. Biotechnol.*, 2005, 116, 91–100.

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors thank FCT for the doctoral grant SFRH/BD/122220/2016 of M. Martins. S.P.M. Ventura acknowledges FCT for the IF contract ref: IF/00402/2015. The authors thank financial support FCT considering the project FAPESP/19793/2014.

Phycobiliproteins Purification by Aqueous Micellar Two-phase Systems: A Continuous Tubular Separation Unit Tale

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Seaweed is currently in the focus of the blue biorefinery research owing to its natural, abundant and cheap character while being rich in high-added value components, such as phycobiliproteins.¹ Phycobiliproteins, namely R-phycoerythrin, are proteins acting as auxiliary photosynthetic pigments in red macroalgae that stand out as a great replacement of synthetic components in solar cells². Additionally, R-phycoerythrin displays many biological properties, for instance anti-inflammatory and antioxidant, making it highly relevant on pharmaceutical and cosmetic formulations.² Regardless R-phycoerythrin potential for new fields of application, R-phycoerythrin is mostly used on agar production¹, as a fluorescence-based marker for numerous molecules in cell biology and immunology and as a natural food colorant.³ Thus, R-phycoerythrin stands out as an extremely valuable protein to be extract and purified. The most common industrial methodologies describe their purification from cyanobacteria and red macroalgae using distilled water leaching, staged precipitation with ammonium sulfate and ionic exchange chromatography;⁴ a multiple step and time-consuming process. In this sense, this work aims at developing a continuous purification process of phycobiliproteins, in particular R-phycoerythrin, using the environmental friendly aqueous two-phase systems (ATPS). Here, aqueous micellar two-phase systems (AMTPS) will be used, owing to their biocompatible character, simplicity and enhanced extractive performance⁵, aiming at developing a continuous liquid-liquid equilibrium separation unit operating at constant temperature and laminar flow, and able to achieve high selectivities. The dimensioning of the separation unit, namely the streams flow, time of residence and flow regime are among the main parameters to evaluate and optimize.

References

- ¹Baghel et al., *Bioresour. Technol.*, 2014, 159, 280–285.
- ²Ihssen et al., *Curr. Protein Pept. Sci.*, 2014, 15, 374–84.
- ³Sonani et al., *World J. Biol. Chem.*, 2016, 7, 100–9.
- ⁴Sekar et al., *J. Appl. Phycol.*, 2008, 20, 113–136
- ⁵Vicente et al., *Sep. Purif. Technol.*, 2014, 135, 259–267.

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors are also grateful for the national fund through the Portuguese Foundation for Science and Technology (FCT) for the doctoral grant SFRH/BD/101683/2014 of F.A. Vicente. S.P.M. Ventura and Pedro J. Carvalho acknowledge FCT for the contracts under the Investigador FCT 2015, contract IF/00402/2015 and IF/00758/2015, respectively.

Extraction of collagen from codfish skin

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Marine fish industries discard huge amounts of fish waste every year, which in turn impose problems of environmental pollution and loss of economic value. To avoid such issues, by-products generated by seafood processing industries were started to be utilized as natural sources of an industrially interesting plethora of bioactive compounds. Collagen recurrently used in human health-related applications is one interesting example.¹ This compound is actually present as one of the bioactive compounds composing fish, in general. Codfish is such an example, where collagen I could be found in significant amounts in skin and bones. As well recognized, Portugal is one of the countries most passionate by fish, and in particular, by codfish.

In this work, the development of an efficient process of extraction to isolate collagen I from the codfish skins (of Baltic cod, *Gadus morhua* from Pascoal Company) is envisaged. Standard methods were firstly adopted to quantify collagen I, namely UV-Vis, SDS-PAGE, and HPLC. Then, the extractions were conventionally performed at 4°C, and for 72 hr using a solid-liquid ratio of 1:10 (w/v), by applying a large plethora of aqueous acidic solutions. After the understanding of the main mechanisms behind the collagen extraction from codfish skin performed by different acids, various deep eutectic solvents (DESs) based in the same acids were tested. In the end, it was expected to develop an efficient but selective process of extraction to obtain purer collagen from codfish skin.

References

¹Nagai et al., Food Chem. 2002, 78, 173–177.

Acknowledgments: This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. S.P.M. Ventura acknowledges FCT for the IF contract ref: IF/00402/2015. The authors thank FCT for the doctoral grant SFRH/BD/122220/2016 of M. Martins. The authors acknowledge the FCT funding through the project “Multi-purpose strategies for broadband agro-forest and fisheries by-products valorisation: a step forward for a truly integrated biorefinery (PAC – *Programa de Atividades Conjuntas*) ref: SAICTPAC/0040/2015 (POCI-01-0145-FEDER 016403).

Acknowledgments

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Co-financiado por:



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This workshop was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The FCT funding through the project “Multipurpose strategies for broadband agro-forest and fisheries byproducts valorisation: a step forward for a truly integrated biorefinery (PAC – Programa de atividades Conjuntas), Ref: SAICTPAC/0040/2015, is also acknowledged.

