

**20
26**

BIOPARTITIONING AND PURIFICATION CONFERENCE

6th – 9th September 2026,
Bled, Slovenia

BOOK OF ABSTRACTS



Publisher:

Department of Catalysis and Chemical Reaction Engineering
National Institute of Chemistry, Ljubljana, Slovenia
Ljubljana, September 2026

Editors:

Filipa A. Vicente, Brigita Hočevar, Anja Verbič, Assya Bellaadem

Organizing Committee:

Filipa A. Vicente, Gasan Osojnik, Ana Rotter, Annamaria Vujanović, Ernesta
Grigalionyte-Bembič, Maja Čič, Brigita Hočevar, Anja Verbič, Assya Bellaadem

Issued by:

National Institute of Chemistry

Place and year of publication:

Ljubljana, 2026

Price:

Free copy

Access method (URL):

[book_of_abstracts_BPP2026.pdf](#)

Katalogni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani

Table of Contents

PRESENTATION OVERVIEW	1
PLENARY LECTURES	5
PL01	6
PL02	7
PL03	8
PL04	9
KEYNOTE LECTURES	10
K01	11
K02	12
K03	13
ORAL PRESENTATIONS	14
DOWNSTREAM PROCESSING	15
O01	16
O02	17
O03	18
O04	19
O05	20
O06	21
O07	22
O08	23
O09	24
O10	25
O11	26
PROCESS INTEGRATION & OPTIMIZATION	27
O12	28
O13	29
O14	30
O15	31
O16	32
O17	33
BIOMASS PROCESSING	34

O18	35
O19	36
O20	37
O21	38
O22	39
O23	40
O24	41
O25	42
SAFE-AND-SUSTAINABLE-BY-DESIGN WORKSHOP	43
REGULATORY AND SUSTAINABILITY CONSIDERATIONS	43
O26	44
O27	45
O28	46
O29	47
O30	48
O31	49
BIOPRODUCTS ENGINEERING FOR BIOPROCESSING ENHANCEMENT	50
O32	51
O33	52
O34	52
O35	53
O36	55
O37	56
POSTER PRESENTATIONS	57
DOWNSTREAM PROCESSING	58
P01	59
P02	60
P03	61
P04	62
P05	63
P06	64
P07	65
P08	66
P09	67

P10.....	67
P11.....	69
P12.....	70
P13.....	71
P14.....	72
P15.....	73
P16.....	74
P17.....	75
P18.....	76
PROCESS INTEGRATION & OPTIMIZATION	77
P19.....	78
P20.....	79
P21.....	80
P22.....	81
P23.....	82
P24.....	83
P25.....	84
P26.....	85
P27.....	86
BIOMASS PROCESSING.....	87
P28.....	88
P29:.....	89
P30.....	90
P31.....	91
P32.....	92
P33.....	93
P34.....	94
P35.....	95
P36.....	96
P37.....	97
P38.....	98
P39.....	99
P40.....	100
P41.....	101

P42.....	102
REGULATORY AND SUSTAINABILITY CONSIDERATIONS	103
P43.....	104
P44:.....	105
P45.....	106
BIOPRODUCTS ENGINEERING FOR BIOPROCESSINGENHANCEMENT	107
P46.....	108
P47.....	109
P48.....	110
P49.....	111
P50.....	112
GOLD SPONSORS	113
SILVER SPONSORS.....	113
SUPPORTERS	114

PRESENTATION OVERVIEW

Code	Abstract title	Presenter
PL01	BETWEEN TWO PHASES: ADVANCES IN BIOPRODUCT SEPARATION AND PURIFICATION	Raquel Aires-Barros
PL02	AQUEOUS BIPHASIC SYSTEMS: FROM CELLULAR MICROENVIRONMENTS TO CLINICAL CHEMISTRY	John Frampton
PL03	ADVANCES AND APPLICATIONS OF MULTIMODAL CHROMATOGRAPHY IN BIOPHARMACEUTICAL PURIFICATION	Leif Bülow
PL04	SAFE AND SUSTAINABLE BY DESIGN FOR BIO-BASED INNOVATION: INTEGRATING SCIENTIFIC EVIDENCE, DIGITAL TECHNOLOGIES AND HUMAN-AI COLLABORATION	Barry Hardy
K01	SUSTAINABLE EXTRACTION BREAKTHROUGHS: UNLOCKING THE BIOACTIVE POTENTIAL OF FOOD INDUSTRY BY-PRODUCTS	Stela Jokić
K02	ENGINEERED LIVING MATERIALS AS INTEGRATED BIOPROCESSING SYSTEMS: FROM LIVING CELLS TO RESILIENT CITIES	Anna Sandak
K03	ALTERNATIVE PROTEIN PRODUCTION FROM FOOD BIOMASS: A BIOPROCESSING APPROACH TO SUSTAINABLE PROTEIN RECOVERY	Mecit Halil Öztöp
O01	LINKING WATER STRUCTURE TO PHYSICOCHEMICAL PROPERTIES IN AQUEOUS SOLUTIONS BY ATR-FTIR SPECTROSCOPY	Amber Titus
O02	ISOLATION AND PURIFICATION OF LECTIN FROM GREEN LENTIL (LENS CULINARIS) SEEDS: AN ACCESSIBLE STRATEGY FOR BIOSENSING APPLICATIONS	Marija Kostić
O03	BENEFITS AND EASE-OF-USE OF PREPACKED COLUMNS FOR THE PURIFICATION OF THERAPEUTIC ANTIBODIES IN A GMP ENVIRONMENT	Elena Kumm
O04	ANTIGEN BINDING FRAGMENTS PURIFICATION FROM ANTIBODIES ENZYMATIC DIGESTION MIXTURES USING IONIC LIQUID-BASED AQUEOUS BIPHASIC SYSTEMS	Ana Miguel Loureiro
O05	METAL-BINDING FUSION TAGS AS A PLATFORM FOR THE RECOMBINANT PRODUCTION OF FUNCTIONAL ANTIMICROBIAL PEPTIDES	Xristo Zarate
O06	DEVELOPMENT OF A SCALABLE DOWNSTREAM PROCESSING PLATFORM FOR THE PURIFICATION OF LEMON-DERIVED EXTRACELLULAR VESICLES	A. Rita Silva-Santos
O07	SCALABLE PURIFICATION OF SSDNA SCAFFOLDS FOR DNA-ORIGAMI NANOSTRUCTURES	Sebastião Santos Pereira
O08	PH DENATURATION OF DSRNA: A NOVEL PARADIGM FOR AFFINITY PURIFICATION OF RNA THERAPEUTICS	Rok Sekirnik
O09	ENRICHMENT OF A SINGLE MRNA CONSTRUCT FROM COMPLEX BIOLOGICAL SAMPLES USING MONOLITH AFFINITY CHROMATOGRAPHY	Miklavčič Rok
O10	INCREASING THE DYNAMIC CAPACITY OF PROTEIN A CHROMATOGRAPHY RESINS	James M. Van Alstine
O11	ANALYTICAL AND OTHER UNUSUAL APPLICATIONS OF PROTEIN A AND ITS ANALOGS	Richard C. Willson
O12	MACHINE LEARNING AND COSMO-RS FOR THE DESIGN OF SUSTAINABLE ANTHOCYANIN EXTRACTION USING EUTECTIC SOLVENTS	Filipe H. B. Sosa
O13	THERMOREVERSIBLE AQUEOUS BIPHASIC SYSTEMS COMBINED WITH IONIC LIQUIDS FOR INTEGRATION OF MRNA PRODUCTION AND CLARIFICATION	Maria I. Sousa
O14	AQUEOUS BIPHASIC SYSTEM-INSPIRED STRATEGIES FOR BIOANALYTICAL APPLICATIONS: FROM LIQUID-LIQUID TO SOLID-PHASE PLATFORMS IN HUMAN SERUM PRETREATMENT	Maria S. M. Mendes
O15	THERMOSENSITIVE AQUEOUS TWO-PHASE SYSTEMS AS A PLATFORM FOR INTEGRATED PRODUCTION AND RECOVERY OF EPS FROM NEOCHLORIS OLEOABUNDANS	Alma Gómez-Loredo
O16	INTEGRATED EXTRACTION-DYEING STRATEGIES USING BIO-BASED DYES AND ADDITIVES FOR SUSTAINABLE TEXTILE PRODUCTION	Alexandre M. S. Jorge
O17	OPTIMIZING PROTEIN EXTRACTION AND PURIFICATION FROM TENEBRIO MOLITOR AT AN INDUSTRY SETTING AND SCALE	Pedro Moleiro
O18	OPTIMIZATION OF PROTEIN EXTRACTION FROM OLIVE POMACE USING DEEP EUTECTIC SOLVENTS	Elif Gökçen Ateş
O19	A SUSTAINABLE APPROACH FOR RECOVERING MICROBIAL INDIGOIDINE USING AN ETHANOL-BASED SOLVENT SYSTEM COMBINED WITH MECHANICAL CELL DISRUPTION	Valéria de C. Santos-Ebinuma
O20	KERATIN PURIFICATION AND ITS USE IN BIOMATERIALS PRODUCTION	João A. P. Coutinho
O21	SYNERGISTIC ANTIOXIDANT PERFORMANCE OF LIGNIN EXTRACTED WITH DEEP EUTECTIC SOLVENT FROM OLIVE MILL SOLID WASTE IN POLYVINYL ALCOHOL FILMS AND NANOFIBERS	Kamil Urgan

Code	Abstract title	Presenter
O22	ACTIVATED CARBON FROM BREWERY WASTE FOR ENHANCED BORON REMOVAL	Pinar Degirmencioglu
O23	VALORISATION OF COTTON TEXTILE WASTE VIA ACID HYDROLYSIS AND LIQUID-LIQUID EXTRACTION FOR INTEGRATED BIOREFINERY APPLICATIONS	Joaquim F. Pinto
O24	FUELS FROM FOREST: SLURRY-PHASE UPGRADING OF LIGNOCELLULOSIC PYROLYSIS BIO-OILS	Sari Rautiainen
O25	TUNABLE HYDROGEL ARCHITECTURES FOR ADVANCED ELECTROCHEMICAL ENERGY SYSTEMS: TOWARD ADAPTIVE MEMBRANES AND ELECTRODE INTERFACES FOR GREEN HYDROGEN TECHNOLOGIES	Ilja Gasan Osojnik Črnivec
O26	SSBD IN PRACTICE WITHIN THREE INDUSTRIAL VALUE CHAINS - FIRST LESSONS FROM THE PLANETS APPROACH TO INNOVATION OF SAFER AND MORE SUSTAINABLE PLASTICIZERS, SURFACTANTS, AND FLAME RETARDANTS	Martin Himly
O27	SAFE AND SUSTAINABLE BY DESIGN IMPLEMENTATION IN THE TOXBOX TOXICITY TESTING PLATFORM	Guillermo Ormazabal
O28	IMPLEMENTING A SAFE-AND-SUSTAINABLE-BY-DESIGN (SSBD) FRAMEWORK FOR NANOCELLULOSE-ENHANCED COSMETIC FORMULATIONS	Uroš Novak
O29	LINKING CHEMICAL STRUCTURE TO SPHEROID PHENOTYPES: AN INTERPRETABLE QSAR APPROACH FOR HEPATOTOXICITY IN THE CONTEXT OF SSBD	Milica Nikolić
O30	LIFE CYCLE ASSESSMENT OF BLUE BIOREFINERIES: FROM CRUSTACEAN WASTE VALORISATION TO FUNCTIONAL CHITOSAN APPLICATIONS	Jan Puhar
O31	ORGANIZATIONAL PURCHASING BEHAVIOUR OF BIOPROCESSING TECHNOLOGIES IN A POST-PANDEMIC ENVIRONMENT: A QUALITATIVE RESEARCH APPROACH	Marcelo Fernandez Lahore
O32	SIMULTANEOUS EXTRACTION AND SEPARATION OF ANTHOCYANINS AND CARBOHYDRATES FROM BERRY POMACE IN AQUEOUS TWO-PHASE SYSTEMS	Marija Lemić
O33	EXTRACTION OF PROTEINS FROM ACHETA DOMESTICUS USING SUSTAINABLE STRATEGIES FOR THE DEVELOPMENT OF NEW FOOD PRODUCTS	Daniela B. Silva
O34	MARINE-DERIVED HYDROGELS AND THE CIRCULAR BIOECONOMY	Katja Klun
O35	AQUEOUS SOLUBILITY ENHANCEMENT OF PORPHYRINS THROUGH HYDROTROPY AND COMPUTATIONAL CHEMISTRY	Ricardo G. Rocha
O36	UNABILITY OF SWELLING, AEROPHOBIC AND VISCOELASTIC PROPERTIES OF BIO-BASED CHITOSAN/DIALDEHYDE STARCH HYDROGELS FOR ELECTROCHEMICAL APPLICATIONS	Vojtěch Škopek
O37	ATALYTIC CONVERSION OF AMINO ACIDS TO HIGH-VALUE NITROGEN CHEMICALS	Rok Pogorevc
P01	THE ELUTION DILEMMA IN PROTEIN A CHROMATOGRAPHY	Mikael Andersson Schönn
P02	SUSTAINABLE EXTRACTION OF CURCUMINOIDS USING MICELLAR TWO-PHASE SYSTEMS	Assya Bellaadem
P03	SALT EFFECTS ON HYDROGEN BONDING AND VISCOSITY IN AQUEOUS TWO-PHASE SYSTEMS	Lyndsey E. Corrigan
P04	RECOVERY AND STABILIZATION OF RECOMBINANT NUCLEIC-ACID BIOPHARMACEUTICALS WITH IONIC LIQUIDS	João L. Fernandes
P05	SELECTIVE LEACHING OF LITHIUM IRON PHOSPHATE BATTERY WASTE USING DEEP EUTECTIC SOLVENTS FOR SUSTAINABLE BATTERY RECYCLING	Marko Gabrovšek
P06	NMR INVESTIGATION OF WATER-INDUCED STRUCTURAL TRANSITIONS IN DEEP EUTECTIC SOLVENT FOR STARCH DISSOLUTION	Marko Gabrovšek
P07	METAL-SUPPORT EFFECTS IN RE CATALYSTS FOR BIO-BASED ADIPIC ACID DERIVATIVES	Brigita Hočevar
P08	PRECIPITATION AND ATPS OF VIRUS LIKE PARTICLE	Jürgen Hubbuch
P09	VALORIZATION OF ONION PEEL WASTE: EUTECTIC SOLVENT EXTRACTION OF QUERCETIN-ENRICHED EXTRACTS FOR COTTON DYEING	Alexandre M. S. Jorge
P10	NATURAL DEEP EUTECTIC SOLVENTS FOR STEROID HORMONE EXTRACTION FROM WATER: CHARACTERIZATION AND BIO-UPCYCLING POTENTIAL	Robert Karcz
P11	TUNABLE BIO-BASED SOLVENT MIXTURES FOR ENHANCED ASTAXANTHIN SEPARATION FROM MICROBIAL BIOMASS	Adalberto Pessoa Jr
P12	COMPUTATIONAL AND EXPERIMENTAL SCREENING OF SOLVENTS FOR HYDROXYTYROSOL EXTRACTION FROM OLIVE-DERIVED EXTRACTS	Joaquim F. Pinto
P13	LIGNIN-BASED MEMBRANES FOR SUSTAINABLE SEPARATION PROCESSES	Filipe H. B. Sosa
P14	REMOVAL OF dsRNA FROM IN VITRO TRANSCRIBED mRNA USING MULTI-STAGE AQUEOUS BIPHASIC SYSTEMS	Maria I. Sousa
P15	SALT EFFECTS ON BOUND AND APO ALPHA-1-ACID GLYCOPROTEIN PARTITIONING IN AQUEOUS TWO-PHASE SYSTEMS	Amber R. Titus
P16	ALKALINE AND AUTOCLAVE-ASSISTED PROTEIN EXTRACTION FROM OLIVE POMACE: RECOVERY EFFICIENCY	Gokcem Tonyali Karstli
P17	DEVELOPMENT OF A CURCUMIN-BASED DYEING PLATFORM USING AQUEOUS MICELLAR SYSTEMS	Filipa A. Vicente

Code	Abstract title	Presenter
P18	EVALUATION OF NADES-MODIFIED BREWERS' SPENT GRAIN FOR THE REMOVAL OF SYNTHETIC DYES FROM AQUEOUS SOLUTIONS	Filipa A. Vicente
P19	ANTHOCYANIN EXTRACTION FROM BILBERRY POMACE (<i>Vaccinium myrtillus</i> L.) USING DEEP EUTECTIC SOLVENTS: SOLVENT SCREENING AND PROCESS OPTIMISATION	Maja Čič
P20	DESIGN OF WATER-DRIVEN FRACTIONATION OF ORGANOSOLV LIGNIN TOWARD TAILORED MOLECULAR WEIGHT AND FUNCTIONALITY	Edita Jasiukaitytė-Grojzdek
P21	TAILORED DISTRIBUTION, STRUCTURE, AND FUNCTIONAL PROPERTIES OF OLIGOMERIC FRAGMENTS VIA RUTHENIUM-CATALYZED DEPOLYMERIZATION OF TECHNICAL LIGNIN	Edita Jasiukaitytė-Grojzdek
P22	APPLICATION AND IMPACT OF NOVEL EXTRACTION TECHNOLOGIES FOR XYLOGLUCAN FROM NASTURTIUM SEEDS (<i>TROPAEOLUM MAJUS</i>)	Simon Körber
P23	HYDROGEN PEROXIDE GENERATION FROM PERSULFATE: EXPERIMENTAL INSIGHTS AND THEORETICAL MODELLING	Katja Makovšek
P24	ILIMITED PROJECT: UNDERSTANDING THE WATER LIQUID SORBENTS FOR HIGH TEMPERATURE APPLICATIONS	Anže Prašnikar
P25	DESIGNING SAFE AND SUSTAINABLE PROTEIN HYDROLYSIS FROM DAIRY WASTE USING DEEP EUTECTIC SOLVENTS FOR INTEGRATED PROCESSING AND SEPARATION	Pardis Saadatmand
P26	OPTIMIZATION OF THE PURIFICATION OF C-PHYCOCYANIN FROM <i>LIMNOSPIRA PLATENSIS</i> BIOMASS USING A QUALITY BY DESIGN APPROACH	Valéria de Carvalho Santos-Ebinuma
P27	CIRCULAR APPROACH FOR THE DEVELOPMENT OF SUSTAINABLE FUNCTIONAL COATINGS FROM BIOMASS-DERIVED POLYMERS	Anja Verbič
P28	INTEGRATING IN SILICO SCREENING AND GREEN EXTRACTION FOR LIMONENE RECOVERY FROM CITRUS WASTE	Monika Arnič
P29	COLLAGEN TYPE I EXTRACTION FROM EGGSHLL MEMBRANES USING EUTECTIC SOLVENTS AT AMBIENT TEMPERATURE	João A. P. Coutinho
P30	LIGNIN-BASED CANS WITH SELF-HEALING PROPERTIES AS SUSTAINABLE ALTERNATIVES TO CONVENTIONAL EPOXY THERMOSETS	Iva Edrovska
P31	THE THERMOMECHANICAL PROPERTIES OF LIGNIN-EPOXY	Meta Florjančič
P32	TAILORING RHENIUM-CATALYSED DEOXYDEHYDRATION OF GLYCERIC ACID TOWARDS BIO-BASED ACRYLATES	Maja Gabrič
P33	IONIC LIQUID-ISOLATED LIGNIN PELLETS AS ADVANCED CLEAN SOLID FUELS	Marcell Gyurkač
P34	COUPLING SUPERCRITICAL FLUIDS WITH BIOBASED IONIC LIQUIDS FOR THE GREEN ISOLATION OF PIGMENTS FROM BROWN MACROALGAE	Marcell Gyurkač
P35	CONVERSION OF WOOD CELLULOSE PULP RESIDUE INTO 5-HMF AND ENERGY-DENSE HYDROCHAR VIA WET TORREFACTION UNDER NITROGEN ATMOSPHERE	Andrii Kostyniuk
P36	CHEMOCATALYTIC WET TORREFACTION OF BIOMASS WASTE OVER ZEOLITE CATALYSTS FOR THE PRODUCTION OF BIO-ETHANOL, LEVULINIC ACID, AND HIGH-QUALITY HYDROCHAR	Andrii Kostyniuk
P37	VALORISATION OF RESIDUAL MICROALGAL BIOMASS BY LIQUEFACTION AND CATALYTIC UPGRADING	Dana Marinič
P38	TERPENE-BASED SOLVENTS FOR ARTEMISININ EXTRACTION	Mónia A. R. Martins
P39	FROM SOLID LIGNIN TO LIQUID FEEDSTOCK: SUSTAINABLE SOLUBILIZATION WITH GREENER SOLVENTS	Irina Modrušan
P40	HETEROGENEOUS CATALYSIS FOR BIOMASS CONVERSION	Matej Rukše
P41	CASCADE RECOVERY OF ASTAXANTHIN AND CHITIN FROM SHRIMP SHELLS USING DEEP EUTECTIC SOLVENTS	Sunčica Srzentič
P42	GREEN EXTRACTION OF PROTEINS FROM MICROALGAE USING DEEP EUTECTIC SOLVENTS AS AN ALTERNATIVE TO CONVENTIONAL EXTRACTION METHODS	Anja Verbič
P43	CARBNETS PILLARS III AND IV: ADVANCED CARBON-SMART MATERIALS, WASTE HEAT RECOVERY, AND INDUSTRIAL WATER CIRCULARITY	Leon Kaluža
P44	INTEGRATED LIFE CYCLE AND COST-BENEFIT ASSESSMENT OF POWDER AND CONVENTIONAL SHAMPOO SYSTEMS	Jan Puhar
P45	PRELIMINARY TECHNO-ECONOMICAL ANALYSIS AND SOCIAL LIFE CYCLE ASSESSMENT FRAMEWORK FOR THE TOXBOX CHIP SYSTEM	Anja Verbič
P46	EXPLORING THE ENZYMATIC DEGRADATION POTENTIAL OF BENZOXAZINE-BASED VITRIMERS	Miša Mojca Cajnko
P47	ECO-FRIENDLY METHODS FOR CAPTURING CO ₂ WITH DEEP EUTECTIC SOLVENTS AND UTILIZING MICROALGAE FOR BIO FIXATION	Muhammad Irshad
P48	ATR-FTIR CHEMOMETRICS REVEALS BATCH AND SOURCE VARIABILITY IN BIOMASS-DERIVED POLYSACCHARIDES FOR HYDROGEL MEMBRANE DEVELOPMENT	Vuk Martinović

Code	Abstract title	Presenter
P49	BEYOND SEPARATION: ATPS-BASED PLATFORMS FOR NEXT-GENERATION HEALTH APPLICATIONS	Marco Rito-Palomares
P50	BIOACTIVE AND METABOLOMIC POTENTIAL OF THE ENDEMIC BROWN MACROALGA FUCUS VIRSIDES	Drago Šubarić

PLENARY LECTURES

PL01: BETWEEN TWO PHASES: ADVANCES IN BIOPRODUCT SEPARATION AND PURIFICATION

Raquel Aires-Barros¹

¹Instituto Superior Técnico, Lisbon Portugal

Email: rabarros@tecnico.ulisboa.pt

The separation and purification of bioproducts remain key challenges in bioprocess development, influencing product quality, yield, cost and sustainability. This work has focused on the development and application of aqueous two-phase systems as mild, selective and sustainable platforms for the recovery and purification of biological products.

Starting with the partitioning of proteins and enzymes, it evolved towards high-value bioproducts, including recombinant proteins, antibodies, plasmid DNA, viral particles and cells. A central contribution has been to show that aqueous two-phase systems can go beyond conventional liquid-liquid extraction, serving as versatile tools for capture, concentration, purification and integration in downstream processing.

This research has also advanced the understanding of the parameters governing biomolecule partitioning, supporting the rational design of systems with improved selectivity, yield and purity, while preserving biological activity and reducing process complexity.

More recently, the work has moved towards the miniaturisation and integration of aqueous two-phase systems in microfluidic platforms. These lab-on-a-chip approaches enable faster process development, reduced sample and reagent consumption, improved screening and the integration of multiple bioprocessing steps.

This lecture will reflect on the evolution of aqueous two-phase systems from partitioning fundamentals to applied bioproduct purification and microfluidic bioprocessing, highlighting their contribution to more efficient, sustainable and intensified downstream processes for biotechnology, biopharmaceutical production and the bioeconomy.

PL02: AQUEOUS BIPHASIC SYSTEMS: FROM CELLULAR MICROENVIRONMENTS TO CLINICAL CHEMISTRY

John Frampton^{1,2}

¹Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax NS B3H 4R2, Canada

²School of Biomedical Engineering, Dalhousie University, Halifax NS B3H 4R2, Canada

e-mail: John.Frampton@dal.ca

Traditionally associated with bioseparations, aqueous biphasic systems have more recently emerged as multifunctional systems for bioengineering, owing to their tunable partitioning behavior, cytocompatibility, and compatibility with advanced liquid handling strategies. Contemporary studies have expanded the utility of these systems beyond extraction toward applications in spatially controlled cell and reagent confinement, microtissue production, bioprinting, and tools for clinical chemistry. This talk will cover the state of the art in these emerging applications areas, highlighting some of the physicochemical principles underlying phase separation and reagent confinement from the picoliter to the milliliter scale. Emerging opportunities and current limitations in the applications of these systems will also be considered.

PL03: ADVANCES AND APPLICATIONS OF MULTIMODAL CHROMATOGRAPHY IN BIOPHARMACEUTICAL PURIFICATION

Leif Bülow¹

¹Center for Molecular Protein Sciences, Department of Chemistry, Lund University, Lund, SWEDEN

e-mail: Leif.Bulow@chem.lu.se

Multimodal chromatography (MMC) has emerged as a powerful and versatile technique in downstream bioprocessing, combining multiple interaction modes—such as ion exchange, hydrophobic interaction, and hydrogen bonding—within a single chromatographic ligand. This hybrid functionality enables enhanced selectivity and binding capacity compared to traditional single-mode chromatography. MMC resins are particularly effective in the purification of complex biological mixtures, including monoclonal antibodies, recombinant proteins, and viral vectors, where conventional methods often require multiple sequential steps.

Recent developments in ligand design and matrix engineering have improved process robustness, allowing MMC to operate efficiently across a wide range of pH and salt conditions. This flexibility reduces the need for extensive buffer optimization and intermediate processing steps, thereby streamlining purification workflows and lowering production costs. Furthermore, MMC has demonstrated strong impurity clearance capabilities, including removal of host cell proteins, DNA, aggregates, and endotoxins, making it highly suitable for high-purity biopharmaceutical manufacturing.

Despite these advantages, challenges remain in fully understanding the complex binding mechanisms and optimizing scale-up performance. Ongoing research focuses on mechanistic modeling, process integration, and the development of next-generation resins with tailored selectivity. Overall, multimodal chromatography represents a significant advancement in separation science, offering improved efficiency, scalability, and economic viability for modern bioprocessing applications.

PL04: SAFE AND SUSTAINABLE BY DESIGN FOR BIO-BASED INNOVATION: INTEGRATING SCIENTIFIC EVIDENCE, DIGITAL TECHNOLOGIES AND HUMAN-AI COLLABORATION

Barry Hardy¹

¹Edelweiss Connect, Basel, Switzerland

e-mail: barry@edelweissconnect.com

The transition towards a sustainable bioeconomy requires more than the replacement of fossil-based materials with biological alternatives. It requires new ways of designing, evaluating, and governing innovation to ensure that products and processes are safe, sustainable, economically viable, and socially acceptable throughout their life cycle.

Safe and Sustainable by Design (SSbD) has emerged as a promising framework for achieving these goals. Yet one of the greatest challenges facing researchers, industry, and policymakers is how to transform rapidly growing volumes of scientific data into trusted knowledge and actionable decisions. The future of SSbD will therefore depend not only on advances in experimental science and predictive modelling, but also on our ability to connect evidence, manage uncertainty, and support transparent decision-making.

This lecture will explore how evidence-based SSbD workflows are evolving to address these challenges. Using examples from bio-based materials, advanced materials, and industrial biotechnology, it will illustrate how New Approach Methodologies (NAMs), predictive models, sustainability assessment, mechanistic knowledge, and digital infrastructures can be integrated into practical decision-support systems. Particular attention will be given to emerging approaches that combine human expertise with artificial intelligence to create transparent, explainable, and auditable evidence workflows.

The presentation will argue that the future of SSbD lies not in replacing human judgement with automation, but in creating effective partnerships between human and artificial intelligence that enable better decisions, faster innovation, and greater trust. Such approaches offer the potential to accelerate the development of safer and more sustainable products while supporting the broader transition towards a resilient and circular bioeconomy.

KEYNOTE LECTURES

K01: SUSTAINABLE EXTRACTION BREAKTHROUGHS: UNLOCKING THE BIOACTIVE POTENTIAL OF FOOD INDUSTRY BY-PRODUCTS

Stela Jokić¹

¹Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 18, 31000 Osijek, Croatia

e-mail: stela.jokic@ptfos.hr

The escalating volumes of by-products generated throughout the food processing chain represent one of the most pressing challenges facing the agri-food sector today. Beyond the substantial environmental burden associated with inadequate by-product management, the failure to harness the intrinsic value of these materials constitutes a significant economic loss and undermines the principles of sustainable resource utilisation. Conventional extraction technologies, reliant on hazardous organic solvents, elevated temperatures, and prolonged processing times, have proven inadequate in fully unlocking the bioactive potential embedded within these residual matrices — while simultaneously posing risks to extract quality, human health, and the environment.

This lecture presents a comprehensive overview of cutting-edge sustainable extraction strategies specifically developed and optimised for the valorisation of food industry by-products. The discussed approaches include supercritical CO₂ extraction, subcritical water and ultrasound-assisted extraction (UAE) — all characterised by reduced solvent consumption, lower energy input, and superior selectivity for target bioactive compounds.

Overall, the waste generated during food processing can be effectively utilised by applying different green extraction techniques in the development of value-added products, thus contributing to a complete circular economy — something that is truly imperative today. By connecting fundamental research with practical industrial application, this work highlights the potential of sustainable extraction approaches to improve resource efficiency, lower environmental impact, and respond to the growing demand for clean-label, sustainably sourced bioactive ingredients.

ACKNOWLEDGEMENTS

This research was conducted as part of the project “From food industry by-products to new functional products (NUS-PRO-FUN, 581-UNIOS-94)” funded by National Recovery and Resilience Plan (funded by the European Union, NextGenerationEU).

K02: ENGINEERED LIVING MATERIALS AS INTEGRATED BIOPROCESSING SYSTEMS: FROM LIVING CELLS TO RESILIENT CITIES

Anna Sandak¹

¹InnoRenew CoE, UP IAM & UP FAMNIT, University of Primorska, Titov trg 4, 6000 Koper, Slovenia

e-mail: anna.sandak@innorenew.eu

The convergence of biotechnology, materials science, and bioprocess engineering is opening new pathways for the creation of a new class of sustainable, high-performance materials. By organising living cells within structured matrices, Engineered Living Materials (ELMs) blur the traditional boundaries between upstream production, downstream purification, and final material functionality, enabling systems that grow, self-heal, and dynamically respond to their environment. Many natural systems, from microbial biofilms to biological tissues, already function as highly integrated living structures in which production, transport, and functional performance occur simultaneously. These natural systems provide powerful sources of bioinspiration for the design of materials that combine biological activity with structural functionality. This keynote will explore ELMs from the perspective of integrated bioprocessing processes. The talk will focus on engineered fungal biofilms and microbial consortia that can be organised within materials to form living coatings, printable inks, or composite materials. In these systems, microorganisms grow and produce their own structural matrices, creating functional materials directly during cultivation. By controlling growth conditions, formulation, and spatial organisation, living cells can be retained within the material, where they continue to perform useful functions such as sensing, regeneration, or bioremediation. This approach reduces the need for extensive downstream processing while enabling materials that remain active over time.

Beyond laboratory-scale design, the talk will address key challenges associated with scaling and deploying ELMs for the built environment. These include manufacturability, robustness and reproducibility, integration within existing bioprocess workflows, and the implications for techno-economic analysis and long-term performance. Regulatory classification, safety considerations, and public perception will also be discussed, as ELMs challenge established distinctions between materials and biological systems. By connecting bioinspired design, advances in bioseparation, process integration, and material design, this contribution aims to stimulate discussion within the BPP community on how engineered living materials can redefine sustainable manufacturing - from cells to cities.

ACKNOWLEDGMENTS

Funded by the European Union (ERC, ARCHI-SKIN, #101044468). Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the European Research Council. This research has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101185862. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or European Innovation Council and SMEs Executive Agency (EISMEA). Neither the European Union nor the granting authority can be held responsible for them. The author acknowledges the support of the Slovenian Research and Innovation Agency (project N2-0380).

K03: ALTERNATIVE PROTEIN PRODUCTION FROM FOOD BIOMASS: A BIOPROCESSING APPROACH TO SUSTAINABLE PROTEIN RECOVERY

Mecit Halil Öztop

Department of Food Engineering, Middle East Technical University, 06800 Ankara, Turkey

e-mail: mecit@metu.edu.tr

The growing demand for sustainable protein sources has driven significant interest in valorizing food biomass and agro-industrial by-products. This work investigated multiple strategies for alternative protein production from underutilized plant-based feedstocks, encompassing both single-cell protein (SCP) fermentation and direct protein extraction approaches.

Tomato residues, including leaves, stems, and pomace, were employed as low-cost, abundant biomass. Following appropriate pretreatment and enzymatic hydrolysis to eliminate antinutritional compounds and release fermentable sugars, these materials served as substrates for *Saccharomyces cerevisiae* bioreactor fermentations, yielding SCP with favorable protein content and a complete essential amino acid profile. Fermentation condition optimization led to substantial improvements in biomass protein yield relative to unprocessed residues. Tomato pomace was further valorized through a systematic experimental design approach, with solid load, inoculum size, and fermentation duration identified as critical process parameters.

Direct protein extraction from tomato pomace via conventional alkaline, microwave-assisted, and ultrasound-assisted methods was also explored. Microwave-assisted extraction proved particularly effective, achieving comparable protein content to conventional approaches in markedly reduced processing time, alongside superior antioxidant activity and functional properties. High-pressure homogenization-assisted extraction from the resulting yeast biomass was identified as a promising route to functional proteins suitable for meat analogs, bakery, and fortified beverage applications.

Finally, bleaching pretreatment prior to alkaline extraction of faba bean protein was shown to improve isolate color while preserving solubility and techno-functional performance. Collectively, these findings support the use of diverse food biomass streams as sustainable feedstocks for alternative protein production within a circular bioeconomy framework.

ORAL PRESENTATIONS

DOWNSTREAM PROCESSING

O01: LINKING WATER STRUCTURE TO PHYSICOCHEMICAL PROPERTIES IN AQUEOUS SOLUTIONS BY ATR-FTIR SPECTROSCOPY

Luisa Ferreira,¹ Amber Titus,¹ Pedro Madeira,² Vladimir N. Uversky,³ Boris Y. Zaslavsky⁴

¹Cleveland Diagnostics, 3615 Superior Ave., Cleveland, OH 44114, USA

²BiotecFoz, R. Alfredo Allen, 4200-135 Porto, Portugal

³Department of Molecular Medicine and Byrd Alzheimer's Research Institute, Morsani College of Medicine, University of South Florida, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, USA

⁴Analiza, 3615 Superior Ave., Cleveland, OH 44114, USA

e-mail: bz@analiza.com

Aqueous two-phase systems are unique in regard to their properties and applications due to the fact that they are formed in aqueous media. It has been shown in our previous studies that the water properties and structure in the phases are different. Here we established how to characterize water structure and mechanism of action of hydrotropes, compounds capable to enhance water solubility of poorly soluble substances.

Analysis of the data obtained in the studies of aqueous solutions of different compounds from inorganic salts and osmolytes to polymers and proteins by Kamlet-Taft solvatochromic comparison approach show that water activity in all solutions may be expressed in terms of solvent dipolarity/polarizability and hydrogen bond donor acidity. The data obtained with ATR-FTIR spectroscopy reported earlier show that water OH-stretching band is described by a set of four Gaussian components with fixed peak positions attributed to distinct water subpopulations or clusters attributed to different H-bonds arrangement. Analysis of these data shows that water activity and different physicochemical properties of aqueous solutions can be described by the relative contributions of two ATR-FTIR components. This analysis suggests that the relative contributions of ATR-FTIR components can be used as surrogates of the water structure in aqueous solutions.

Analysis of properties of aqueous solutions of different hydrotropes are also presented and new insights into mechanism of hydrotropes action are suggested.

O02: ISOLATION AND PURIFICATION OF LECTIN FROM GREEN LENTIL (*Lens culinaris*) SEEDS: AN ACCESSIBLE STRATEGY FOR BIOSENSING APPLICATIONS

Marija Kostić,¹ Milica Perović,² Elizaveta Pavlova,¹ Alan Fidarov,¹ Branimir Bajac,¹ Mirjana Antov²

¹University of Novi Sad, BioSense Institute, Serbia

²University of Novi Sad, Faculty of Technology, Serbia

e-mail: marija.kostic@biosense.rs

Lectins are a class of carbohydrate-binding proteins widely distributed in nature, characterized by their ability to selectively and reversibly recognize diverse glycans and glycoconjugates without altering their structure.^[1] Due to the key role of protein-carbohydrate interactions in biological processes such as cell-cell communication, cell signaling, and host-pathogen interactions, lectins have been recognized as valuable biorecognition molecules, particularly in biosensing applications.^[1,2] Purification of lectins from crude extracts is commonly based on chromatographic methods, which are time-consuming, costly, and poorly suited for complex sample matrices, often leading to reduced yields.^[3] Therefore, given the importance of lectins, there is a need for selective purification strategies that can efficiently handle complex samples, reduce overall operational costs, and support process integration.^[4]

This study investigates the potential of lectin from green lentil (*Lens culinaris*) seeds, isolated via a simple and cost-effective non-chromatographic protocol, as a bioreceptor for sensing applications. The samples of lectin taken after key steps of applied bioseparation protocol were evaluated in terms of protein content, hemagglutinating activity and protein profile using SDS-PAGE. The final lectin sample was assessed for activity and sugar specificity, and further compared with a commercial *Lens culinaris* lectin standard. The developed protocol enabled selective isolation of lectin from seeds of green lentil, resulting in a 15.5-fold purification and a yield of ~60%. The isolated lectin exhibited hemagglutinating activity and specificity toward glucose and mannose, and showed no change in activity over a wide pH range (3 - 9) and at temperatures up to 70 °C. Protein bands corresponding to the subunits of the lectin standard were detected in the isolated lectin. Finally, the obtained lectin was tested in fluorescence-based assays and in magnetic nanoparticle-conjugated systems, where it demonstrated binding activity toward yeast cells.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grants No. 451-03-33/2026-03/ 200358, 451-03-34/2026-03/ 200134 and 451-03-33/2026-03/ 200134).

REFERENCES

- [1] Katoch R, Tripathi A. Research advances and prospects of legume lectins. *J Biosci.* 2021;46(4).
- [2] Chettri D, Boro M, Sarkar L, Verma AK. Lectins: Biological significance to biotechnological application. *Carbohydr Res.* 2021;506:108367.
- [3] Nakamura-Tsuruta S, Kishimoto Y, Nishimura T, Suda Y. One-step purification of lectins from banana pulp using sugar-immobilized gold nanoparticles. *J Biochem.* 2007;143(6):833-839.
- [4] Nascimento KS, Cunha AI, Nascimento KS, Cavada BS, Azevedo AM, Aires-Barros MR. An overview of lectin purification strategies. *J Mol Recognit.* 2012;25(11):527-541.

O03: BENEFITS AND EASE-OF-USE OF PREPACKED COLUMNS FOR THE PURIFICATION OF THERAPEUTIC ANTIBODIES IN A GMP ENVIRONMENT

Elena Kumm,¹ Carsten-René Arlt,¹ Balu Guduri,¹ Jonas Wege,¹ Patrick Endres,¹ Jeannine Lincoln,² Kevin O'Donnell²

¹Tosoh Bioscience GmbH, Germany

²Tosoh Bioscience LLC, USA

e-mail: elena.kumm@tosoh.com

The purification of therapeutic biomolecules requires efficient, reliable and reproducible processes, that ensure consistent product quality across manufacturing campaigns. When working in a GMP-regulated environment, prepacked columns can support these complex requirements by combining standardized packing quality with simplified handling and qualification.

This presentation highlights the role of prepacked columns in antibody purification, using performance data generated with a representative Protein A capture workflow on TOYOPEARL® Super A. Key aspects such as process consistency, column performance, and operational reliability are discussed in the context of GMP manufacturing. In addition, the presentation demonstrates how prepacked columns facilitate a straightforward and predictable scale-up from small-scale development formats to larger column dimensions suitable for regulated batch production.

O04: ANTIGEN BINDING FRAGMENTS PURIFICATION FROM ANTIBODIES ENZYMATIC DIGESTION MIXTURES USING IONIC LIQUID-BASED AQUEOUS BIPHASIC SYSTEMS

Ana Miguel Loureiro,^{1,2} João A. P. Coutinho,² Emanuel V. Capela,¹ Mara G. Freire¹

¹CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

²RYA-Purification Technologies, Ílhavo, Portugal

e-mail: amsl@ua.pt

Antigen-binding fragments (Fabs) recently emerged as highly targeted therapies with reduced side effects. The reduced dimensions, absence of the crystallizable fragment (Fc), and simpler structures compared to full-length antibodies confer them better tissue penetration, higher specificity, and lower immunogenicity. Despite significant advances in Fab production, downstream processing lacks a standardized methodology, relying on multiple chromatographic techniques that are difficult to scale up, are laborious and time-consuming, and require expensive affinity ligands and hazardous organic solvents,^[1] remaining as the major contributor to their high manufacturing costs. The development of alternative purification platforms is, therefore, crucial to overcome this bottleneck and enable scalable, cost-effective production of high-quality and purity Fabs, required to meet the market demands. Ionic liquids (ILs) are organic salts with negligible volatility and remarkable chemical structure diversity, which can be foreseen as alternative solvents for such purpose. ILs have been successfully applied to the extraction and partial purification of monoclonal antibodies (mAbs) from cell culture supernatants as components of aqueous biphasic systems (ABS)^[2] and as ligands in chromatographic supports.^[3] In this context, we herein propose, for the first time, the application of IL-based strategies for the purification of Fabs produced through papain digestion of antibodies. A wide screening of ILs was studied as ABS constituents and evaluated for their potential to extract and/or purify Fabs from complex digestion mixtures. Coexisting phases were analyzed by SE-HPLC and SDS-PAGE to assess the biopartitioning profile and determine process performance in terms of recovery yields and purity levels. Results demonstrate that IL-based ABS allow a selective recovery of Fabs from complex mixtures while preserving their integrity/stability. Moreover, these findings provide new insights into the bioseparation properties of ILs and their potential in the development of cost-effective and scalable alternatives for Fab downstream processing.

ACKNOWLEDGMENTS

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). Ana M. Loureiro and Emanuel V. Capela acknowledge FCT for the PhD grant 2024.06446.BDANA (DOI 10.5449.2024.06446.BDANA), and for the research contract 2024.08677.CEECIND, respectively.

REFERENCES

- 1] Rodrigo G, Gruvegård M, Van Alstine JM. Antibody Fragments and Their Purification by Protein L Affinity Chromatography. *Antibodies*. 2015 Sep;4(3):259–77. doi:10.3390/antib4030259
- [2] Capela EV, Santiago AE, Rufino AFCS, Tavares APM, Pereira MM, Mohamadou A, et al. Sustainable strategies based on glycine–betaine analogue ionic liquids for the recovery of monoclonal antibodies from cell culture supernatants. *Green Chem*. 2019 Oct 14;21(20):5671–82. doi:10.1039/C9GC02733E
- [3] Capela EV, Bairos J, Pedro AQ, Neves MC, Raquel Aires-Barros M, Azevedo AM, et al. Supported ionic liquids as customizable materials to purify immunoglobulin G. *Sep Purif Technol*. 2023 Jan 15;305:122464. doi:10.1016/j.seppur.2022.122464

O05: METAL-BINDING FUSION TAGS AS A PLATFORM FOR THE RECOMBINANT PRODUCTION OF FUNCTIONAL ANTIMICROBIAL PEPTIDES

Xristo Zarate,¹ Alma Gomez-Loredo,¹ Nestor G. Casillas-Vega²

¹Laboratorio de Expresion y Purificacion de Proteinas, Facultad de Ciencias Quimicas, Universidad Autonoma de Nuevo Leon, Cd. Universitaria, San Nicolas de los Garza, NL 66455, Mexico

²Laboratorio de Patologia Clinica, Hospital Universitario, Facultad de Medicina, Universidad Autonoma de Nuevo Leon, Monterrey, NL 64460, Mexico

e-mail: xristo.zaratekl@uanl.edu.mx

The recombinant production of antimicrobial peptides (AMPs) remains a significant challenge due to host toxicity, proteolytic degradation, low solubility, and poor yields.^[1] While fusion tags are commonly used to overcome these issues, many existing systems either reduce peptide activity or lack flexibility for different peptide types.^[2] In this study, we introduce a family of engineered metal-binding fusion tags, including SmbP, CusF3H+,^[3] and the recently developed SmallTalk,^[4] as a modular and scalable platform for efficiently producing bioactive peptides and proteins in *Escherichia coli*.

These tags enable robust cytoplasmic and periplasmic expression, high-yield purification via immobilized metal affinity chromatography, and precise tag removal. Using this platform, we produced structurally diverse AMPs, including Bin1b, VpDef, LL-37, the hybrid peptide LL-37_Renalexin, and the anionic peptide scygonadin, while preserving their native biological activity. The purified peptides demonstrated potent antimicrobial activity against clinically relevant pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and methicillin-resistant *S. aureus* (MRSA).

SmallTalk, a minimal SmbP-derived fusion tag, retains metal-binding ability while enhancing expression efficiency and versatility, thereby reducing its impact on the peptide's antimicrobial activity. Beyond AMPs, this platform enables the production of other challenging bioactive molecules, including anticancer peptides, the serine protease inhibitor SLPI, and human growth hormone, demonstrating its broad applicability. Overall, these findings confirm metal-binding fusion tags as a reliable protein-engineering strategy for the recombinant production of difficult-to-produce peptides and proteins, paving the way for future advances in peptide therapeutics and microbiome-friendly antimicrobial strategies.

REFERENCES

- [1] Wen Q, Zhang L, Zhao F, Chen Y, Su Y, Zhang X, et al. Production technology and functionality of bioactive peptides. *Curr Pharm Des.* 2023;29(9):652-674. doi:10.2174/1381612829666230201121353
- [2] Li Y. Recombinant production of antimicrobial peptides in *Escherichia coli*: a review. *Protein Expr Purif.* 2011;80(2):260-267. doi:10.1016/j.pep.2011.08.001
- [3] Gomez-Lugo JJ, Santos BD, Perez-Perez DA, Montfort-Gardeazabal JM, McEvoy MM, Zarate X. Expression and purification of recombinant proteins in *Escherichia coli* tagged with the metal-binding proteins SmbP and CusF3H+. In: *Methods Mol Biol.* 2021;2178:329-344. doi:10.1007/978-1-0716-0775-6_22
- [4] Tariq A, Casillas-Vega NG, Gomez-Loredo A, Zarate X. SmallTalk: a novel small-sized fusion tag for peptide expression and purification. *FEBS Open Bio.* 2026;16(3):461-473. doi:10.1002/2211-5463.70147

O06: DEVELOPMENT OF A SCALABLE DOWNSTREAM PROCESSING PLATFORM FOR THE PURIFICATION OF LEMON-DERIVED EXTRACELLULAR VESICLES

A. Rita Silva-Santos,^{1,2} Etienne Roth,^{1,2,3} Ana M. Azevedo^{1,2}

¹iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal

²Associate Laboratory i4HB – Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

³University of Applied Sciences Northwestern Switzerland FHNW, Muttensz, Switzerland

e-mail: ana.rita.santos@tecnico.ulisboa.pt

Extracellular vesicles (EVs) are nanoscale lipid bilayer particles attracting growing interest as therapeutic carriers and bio-derived functional materials. While mammalian EV systems have been widely studied, scalable manufacturing remains limited by complex processing, low yields, and focus on analytical isolation methods. Plant-derived EVs represent a promising alternative feedstock, offering abundant raw material availability, reduced ethical concerns and potentially lower production costs. Among these, lemon-derived EVs (LDEVs) are of particular interest due to their high expected vesicle content and reproducible isolation from juice matrices. However, the translation of laboratory-scale isolation concepts into structured, scalable bioprocesses remains a critical unmet challenge.

This work presents the development and evaluation of an integrated downstream processing platform for LDEV isolation, designed around established biopharmaceutical manufacturing principles. The platform comprised five sequential unit operations applied to freshly squeezed *Citrus limon* juice: low-speed centrifugation for debris removal, tangential flow microfiltration for clarification, tangential flow ultrafiltration and diafiltration for concentration and buffer exchange, and step-elution anion exchange chromatography as a polishing step. Process performance was evaluated through BCA total protein quantification, PicoGreen dsDNA quantification, light scattering and fluorescence nanoparticle tracking analysis (NTA), zeta potential measurement and size exclusion chromatography.

Both membrane steps demonstrated robust, reproducible operation. Anion exchange chromatography yielded a well-resolved two-peak profile, separating an EV-enriched fraction from a dsDNA-containing fraction. The final product contained $2.42 \times 10^{10} \pm 3.11 \times 10^9$ particles per mL of microfiltrate, with a modal diameter of 80 ± 7 nm and an overall particle recovery of 22.8%. Residual HCP and dsDNA levels remained within regulatory thresholds. SEC profiles showed a reproducible void-volume peak consistent with EV elution, and fluorescence NTA using Nile red confirmed the presence of lipid membrane-associated particles.

These results demonstrate the feasibility of an integrated, scalable manufacturing workflow for plant-derived EVs using established bioprocess unit operations, and establish a practical foundation for process optimization, quality-by-design implementation and application to other edible plant EV sources.

O07: SCALABLE PURIFICATION OF SSDNA SCAFFOLDS FOR DNA-ORIGAMI NANOSTRUCTURES

Sebastião Santos Pereira,^{1,2} Joana Silva Mendes,^{1,2} Duarte M. Prazeres,^{1,2} A. Rita Silva-Santos^{1,2}

¹iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico,
Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal

²Associate Laboratory i4HB – Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de
Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

e-mail: sebastiao.pereira@tecnico.ulisboa.pt

DNA-origami is a self-assembled nanostructure that has emerged with great impact in the fields of nanofabrication, drug delivery and bioimaging systems due to its precision and customizability. Its biomanufacturing often relies on the production of a long, single-stranded DNA (ssDNA) molecule, the scaffold, which folds into a designed structure through hybridization with short oligonucleotides. One essential step in ssDNA scaffold preparation is purification. However, scaffolds are usually purified by agarose gel extraction or ethanol precipitation, that are inefficient, cumbersome and non-scalable methods, limiting their applicability for industrial and pharmaceutical applications. Alternatively, we present a scalable filtration-based approach to purify ssDNA scaffolds from bacteriophage lysates.

Centrifugal ultrafiltration was first evaluated as a simple method to separate large scaffold molecules from smaller host-derived impurities, including proteins. Additionally, this method allows concentration and buffer exchange through diafiltration, and can be used as a preparatory step for further processing. Membranes with molecular weight cut-offs ranging from 10 to 100 kDa were tested, yielding high scaffold recovery with effective impurity removal. However, scalability is limited by mechanical constraints inherent to centrifugal systems.

To address this limitation, tangential flow filtration (TFF) was implemented, leveraging the same size-based separation principle while enabling straightforward scale-up through increased membrane area without altering process parameters. This ensures process consistency across scales. Consistent with centrifugal filtration results, TFF enabled efficient recovery of ssDNA scaffolds from bacteriophage lysates with effective impurity removal using 30 and 100 kDa membranes, outperforming standard purification methods.

The purified scaffold was used to assemble a DNA-origami nanostructure with high assembly yield and purity, as shown with agarose gel electrophoresis, and confirmed using electron microscopy.

ACKNOWLEDGEMENTS

This work is financed by national funds from FCT – Fundação para a Ciência e Tecnologia, I.P., in the scope of the project NanoGamiBioPro 2023.11117.PEX. Research Unit Institute for Bioengineering and Biosciences – iBB acknowledges funding from FCT in the scope of projects UID/04565/2025 and LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

O08: pH DENATURATION OF DSRNA: A NOVEL PARADIGM FOR AFFINITY PURIFICATION OF RNA THERAPEUTICS

Nina Mencin,¹ Andreja Krušič,¹ Janja Skok,¹ Evelin Nett,² Mario Perković,² Rok Sekirnik¹

¹Sartorius BIA Separations d.o.o., Mirce 21, SI-5270 Ajdovščina, Slovenia

²TRON – Translational Oncology, Johannes Gutenberg University, Freiligrathstrasse 12, 55131 Mainz, Germany

e-mail: rok.sekirnik@sartorius.com

mRNA has become an enabling technology in therapeutic areas spanning from traditional prophylactic vaccines (e.g. COVID vaccines) to individualized cancer treatment (e.g. neoantigen therapies) and gene therapies. Removal of double-stranded RNA (dsRNA) impurity is a critical part of production for mRNA vaccines and therapeutics, due to its high immunogenicity. dsRNA contaminants are produced during in vitro transcription reaction, and typically removed using ion-pair reverse-phase or cellulose based chromatography. However, removal is challenging because of dsRNA's intrinsically similar physicochemical properties with single stranded RNA. We present a novel approach to removing of dsRNA which targets hydrogen bonding as the physico-chemical stabiliser of dsRNA: breaking these hydrogen bonds increases chromatographic differentiation, and can be achieved with pH modulation.^[1] We demonstrate that incubation of mRNA at pH ≤ 3.5 denatures dsRNA for mRNA/saRNA constructs spanning 1–10 kb, without compromising mRNA integrity.

Acidic treatment is coupled in-line with Oligo dT affinity chromatography which selectively captures polyadenylated mRNA. This significantly reduces dsRNA content while achieving $\geq 90\%$ mRNA recovery. mRNA cellular potency increases 5-fold in A549 cells while type I interferon signaling is markedly reduced.

Targeting hydrogen bonding coupled with affinity chromatography is a new mRNA purification paradigm, which is scalable and GMP-compliant, thus enabling clinical production of the new generation of mRNA-based therapeutics.

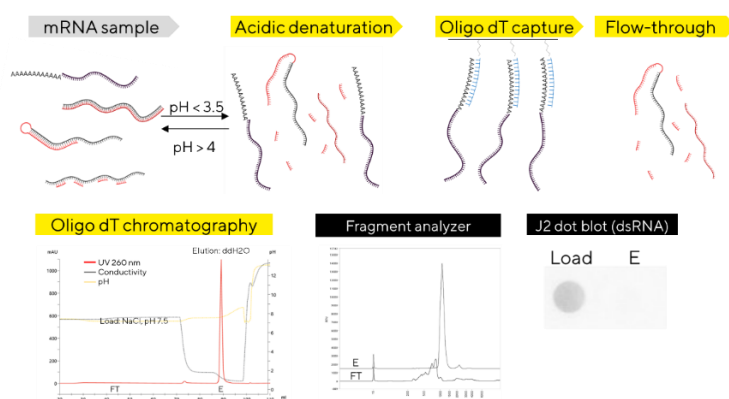


Figure 1: Incubation of mRNA at pH ≤ 3.5 denatures dsRNA structures. Full length mRNA binds to Oligo dT under standard loading conditions, while contaminating RNA strands do not bind. Elution of full-length mRNA from Oligo dT is achieved with ddH₂O. Fragment analyzer confirms the presence of full-length mRNA in elution (E) and RNA fragments in flow-through (FT). J2 dot-blot demonstrates removal of dsRNA from initial mRNA sample.

REFERENCES

[1] Puc, J, Mencin, N, Krušič, A, Nett, E, Perković, M, Sahin, U, Štrancar, A. pH denaturation of dsRNA: A novel approach to mRNA purification, SepPurTech, 2026;390:136864.

O09: ENRICHMENT OF A SINGLE MRNA CONSTRUCT FROM COMPLEX BIOLOGICAL SAMPLES USING MONOLITH AFFINITY CHROMATOGRAPHY

Rok Miklavčič,^{1,2} Polona Megušar,¹ Dejan Slokar,¹ Vianney Bilger,³ Chantal Pichon,³ Katarzyna Pachulska-Wieczorek,⁴ Urh Černigoj¹

¹Sartorius BIA Separations, Slovenia

²University of Ljubljana, Slovenia

³INSERM and University of Orléans, France

⁴Polish Academy of Sciences, Poland

e-mail: rok.miklavcic@sartorius.com

Hybridization affinity chromatography utilizing oligo-deoxythymidilic acid (Oligo dT) probes coupled to a stationary phase is an established technology for purification of messenger RNA (mRNA) produced by in vitro transcription reaction. Due to selective binding mechanism between surface-bound probes and mRNA's poly-adenylic acid (poly A) tail, Oligo dT chromatography efficiently removes process-related impurities in a single chromatographic step.^[1] Purification challenge increases significantly, when the starting sample contains multiple polyadenylated mRNAs, of which only one contains the desired coding sequence, for example when mRNA is bioproduced by engineered (e.g. yeast) cells. Our goal here was to develop a purification strategy for enrichment of a single mRNA construct from yeast lysate containing <0.1% of the target mRNA relative to total RNA. The central strategy was based on development of sequence-specific (not poly A) methods for chromatographic purification of RNA utilizing the hybridization strategy coupled with monolithic columns.

First, in silico structural analyses of the 411 nt long targeted sequence identified accessible sites for hybridization of 20 nt long capture oligonucleotides. Next, hybridization assays with IVT-produced target mRNA encoding luciferase confirmed effective hybridization for three candidate oligonucleotides. Monolith affinity columns prepared with selected oligonucleotides showed selective retention of target mRNA, while a negative control mRNA displayed negligible binding, validating sequence specificity. Finally, affinity chromatography was used in a proof-of-concept experiment to enrich target mRNA from the pool of total yeast RNA, yielding an 18-fold increase in target mRNA content (determined by qPCR), while maintaining its functionality. The developed chromatographic method paves the way for construct-specific mRNA purification strategies, applicable to complex biological samples.

ACKNOWLEDGMENTS

We gratefully acknowledge Yscript research project, which enabled and funded this research. Yscript has received funding from the European Innovation Council (EIC) under grant agreement No 101047214. The EIC receives support from the European Union's Horizon Europe research and innovation program. The content provided in this manuscript reflects the authors' views only.

REFERENCES

[1] Mencin N, Štepec D, Margon A, Vidič J, Dolenc D, Simčič T, et al. Development and scale-up of oligo-dT monolithic chromatographic column for mRNA capture through understanding of base-pairing interactions. *Sep Purif Technol.* 2023;304:122320

O10: INCREASING THE DYNAMIC CAPACITY OF PROTEIN A CHROMATOGRAPHY RESINS

James M. Van Alstine¹

¹JMVA Biotech AB, Stockholm, Sweden 11262

e-mail: jim.vanalstine@jmvabiotech.com

At BPP2024, where I was honored to be given an Albertsson Award, I presented a novel, recombinant approach to increase the dynamic binding capacity of Protein A-based chromatography resins. The method involves simple ligand mutation and is suitable for other ligands. Biacore® sensor (Cytiva) measured bound Ab target mass capacities (RU's) increase 40% (RU120 sec) and 70% (RUmax), with no apparent loss of affinity. The technology has been shown to work with two different Protein A ligands, on both agarose and methacrylate polymer resins, and offer up to a 55% increase in Ab binding per ligand. Initial verification work, presented at BPP2024, was done in collaboration with Professor Leif Bülow and Post-Doctoral Fellow Sandeep Chakane of Lund University [S. Chakane, L. Bülow and J. Van Alstine, manuscript in preparation]. Dr. Chakane is now at Novo Nordisk. US Patent 11,660,550B2, with 47 claims, was granted to JMVA Biotech AB in May 2023. At BPP2026 I will present items not discussed earlier, including: i. The underlying innovation. ii. Molecular mechanisms which allow for improved ligand performance, iii. Future ligand improvements, and iv. How a small company can survive a long patenting process.

O11: ANALYTICAL AND OTHER UNUSUAL APPLICATIONS OF PROTEIN A AND ITS ANALOGS

Vijay M. Maranholkar,¹ Suman Nandy,² Rebecca Shick,² Margarita Ortiz-Martinez,² Suman Rai,¹ Atul Goyal,² Ujwal Patil,¹ Martiela V. Freitas,¹ Binh Vu,² Katerina Kourentzi,² Stacey M. Louie,³ Michael J. Benedik,⁴ Dinler A. Antunes,¹ Mehmet Sen,¹ Richard C. Willson^{1,2,5}

¹Department of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA

²William A. Brookshire Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX 77204, USA

³Department of Civil and Environmental Engineering, University of Houston, Houston, TX 77204, USA

⁴Department of Biology, Texas A&M University, College Station, TX 77843, USA

⁵Institute for Obesity Research, Tecnológico de Monterrey, Monterrey, Nuevo León 64849, Mexico

e-mail: willson@uh.edu

Accurate measurement of antibody and Fc fusion protein concentration during bioprocess operations is essential for optimizing process development and improving manufacturing efficiency. Traditional methods require extensive instrumentation and suffer from limitations in turnaround time and ease of use. We developed a rapid mix-and-read assay that reports IgG concentration by fluorescence intensity, using two types of reporters related to Protein A. We also are pursuing continuous in-line monitoring, which offers the potential to streamline various bioprocess applications, such as optimizing Protein A column loading, tracking bioreactor titers, and promptly detecting ultrafiltration/diafiltration membrane leaks. We developed a real-time, molecule-specific IgG monitoring platform utilizing a fiber-optic biosensor with a flow cell and a replaceable sensor tip functionalized with a fluorophore-labeled protein comprising five Z domains of Protein A. The sensor exhibits concentration-dependent, specific fluorescence enhancement in the presence of human IgG (0.01–0.75 g/L) and maintains consistent performance across multiple runs with 1 g/L and 0.1 g/L IgG. Finally, to broaden the toolkit of binders we also performed comprehensive TBLASTN and Pfam searches, and identified 974 unique protein domains with 20–90% sequence identity to canonical Protein A domains, of which 312 occur in species other than *S. aureus*.

PROCESS INTEGRATION & OPTIMIZATION

O12: MACHINE LEARNING AND COSMO-RS FOR THE DESIGN OF SUSTAINABLE ANTHOCYANIN EXTRACTION USING EUTECTIC SOLVENTS

Leonardo M. de Souza Mesquita,¹ João A. P. Coutinho,² Filipe H. B. Sosa²

¹Department of Botany, Institute of Bioscience, University of São Paulo, Rua do Matão 277, São Paulo, SP, 05508-090, Brazil

²CICECO, Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

e-mail: filipesosa@ua.pt

The growing demand for natural bioactive compounds in food, pharmaceutical, and cosmetic applications has intensified the need for efficient and sustainable extraction processes from biomass. However, the intrinsic complexity and variability of lignocellulosic and plant-derived matrices, combined with the strong dependence of extraction efficiency on solvent–solute interactions, make the rational design of such processes particularly challenging.^[1] In this context, the integration of predictive thermodynamic models with data-driven approaches has emerged as a promising strategy to accelerate solvent screening and process optimization. In this work, a hybrid modeling framework combining COSMO-RS and machine learning (ML) was developed to predict anthocyanin extraction yields from berry-derived matrices using deep eutectic solvents (DES).^[2] A dataset comprising 299 experimental data points across 15 different biomass sources was used to train and evaluate multiple ML algorithms. Among the tested models, Gradient Boosting provided the best predictive performance ($R^2 = 0.92$). The model integrates COSMO-RS-derived descriptors to account for solvent effects, key process variables, and a simple experimental descriptor representing the anthocyanin content of the raw material obtained using ethanol. The predictive capability of the framework was further assessed using independent data from the literature, as well as new experimental results obtained with different DES and berry matrices, showing good agreement between predicted and measured extraction yields. By reducing the need for extensive experimental screening, this approach provides a fast and efficient route for the design and optimization of green extraction processes targeting bioactive compounds from complex biomass.

ACKNOWLEDGEMENTS

This work was developed within the scope of the project CICECO Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC)a and within the scope of the Young Researcher Project supported by the São Paulo Research Foundation (FAPESP) under grant number 2023/16744-0 and fellowship 2025/01561-3. Filipe H. B. Sosa acknowledges FCT – Fundação para a Ciência e a Tecnologia, I.P., for the researcher contracts CEECIND/07209/2022 under the Scientific Employment Stimulus - Individual Call 2022.

REFERENCES

- [1] Dai Y, van Spronsen J, Witkamp GJ, Verpoorte R, Choi YH. Natural deep eutectic solvents as new potential media for green technology. *Anal Chim Acta*. 2013;766:61-68. doi:10.1016/j.aca.2012.12.019.
- [2] Sosa FHB, Pontes PVA, Takahashi K, Ferreira AM, Lopes C. Prediction of laccase activity in deep eutectic solvents by combining COSMO-RS and machine learning techniques. *Chem Eng Sci*. 2026;320:121086. doi:10.1016/j.ces.2025.121086.

O13: THERMOREVERSIBLE AQUEOUS BIPHASIC SYSTEMS COMBINED WITH IONIC LIQUIDS FOR INTEGRATION OF mRNA PRODUCTION AND CLARIFICATION

Maria I. Sousa,¹ Mara G. Freire,¹ Francisca A. e Silva,¹ Augusto Q. Pedro¹

¹CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal

e-mail: mineissousa@ua.pt

Messenger RNA (mRNA) vaccines have emerged as powerful tools for the prevention and treatment of human diseases. However, their large-scale production remains complex, costly, and limited by inefficient purification processes.^[1] A key challenge is the removal of double-stranded RNA (dsRNA) contaminants generated during *in vitro* transcription (IVT),^[2] which compromises mRNA translation efficiency.

Aqueous biphasic systems (ABS) have gained increasing attention as versatile and scalable platforms for biomolecule separation, offering a cost-effective alternative to conventional downstream processing methodologies.^[3] Ionic liquids (ILs) have emerged as promising additives due to their ability to enhance RNA stability^[4] and improve ABS selectivity. In this work, an innovative approach integrating IVT within a thermoreversible IL-ABS is presented, enabling production and initial clarification of mRNA in a single step. mRNA production, partitioning and integrity were assessed, and levels of double-stranded RNA (dsRNA) contaminant were evaluated. Results demonstrate that IVT can proceed efficiently in the presence of ABS components. The presence of ILs yields mRNA with competitive production levels. Notably, the hydrophilic character of ILs influenced transcription performance, with certain compositions enhancing mRNA yield. Moreover, selected systems demonstrated dsRNA reduction, supporting downstream clarification. Overall, this work introduces a novel, integrated production–clarification strategy based on thermoreversible IL-ABS. By reducing complexity and improving contaminant removal, this approach contributes to the development of more efficient and economically viable mRNA manufacturing processes.

ACKNOWLEDGMENTS

This work was supported by FCT – Fundação para a Ciência e Tecnologia, I.P. by project reference 2022.11476.BD (DOI 10.54499/2022.11476.BD) and developed within the scope of the project CICECO – Aveiro Institute of Materials, UID/50011/2025 & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC). This work is additionally funded by FEDER Funds through COMPETE 2030, under project COMPETE 2030-FEDER-00777000 (Ref. FCT: 16593). Augusto Q. Pedro and Francisca A. e Silva acknowledge FCT respectively, for the research contracts CEEC-IND/02599/2020 (DOI 10.54499/2020.02599.CEECIND/CP1589/CT0023) and CEEC-IND/03076/2018 under the Scientific Stimulus–Individual Call. Maria I. Sousa acknowledges FCT for the PhD grant 2022.11476.BD.

REFERENCES

- [1] Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines: a new era in vaccinology. *Nat Rev Drug Discov.* 2018;17(4):261-279. doi:10.1038/nrd.2017.243.
- [2] Lee K, Kim M, Seo Y, Lee H. Development of mRNA vaccines and their prophylactic and therapeutic applications. *Nano Res.* 2018;11(10):5173-5192. doi:10.1007/s12274-018-2095-8.
- [3] Pereira JFB, Freire MG, Coutinho JAP. Aqueous two-phase systems: Towards novel and more disruptive applications. *Fluid Phase Equilib.* 2020;505:112341. doi:10.1016/j.fluid.2019.112341.
- [4] Pedro AQ, Pereira P, Quental MJ, Carvalho AP, Santos SM, Queiroz JA, et al. Cholinium-based Good's buffers ionic liquids as remarkable stabilizers and recyclable preservation media for recombinant small RNAs. *ACS Sustainable Chem Eng.* 2018;6(12):16645-16656. doi:10.1021/acssuschemeng.8b03900.

O14: AQUEOUS BIPHASIC SYSTEM-INSPIRED STRATEGIES FOR BIOANALYTICAL APPLICATIONS: FROM LIQUID-LIQUID TO SOLID-PHASE PLATFORMS IN HUMAN SERUM PRETREATMENT

Maria S. M. Mendes,¹ Sofia Pedro,¹ Hugo Santos,¹ Inês Cruz,¹ Márcia C. Neves,¹ Mara G. Freire,¹ Francisca A. e Silva¹

¹CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

e-mail: msilvinamm@ua.pt

Biomarkers are essential tools for clinical diagnostics and disease monitoring, yet their reliable detection in human serum remains challenging due to matrix complexity and interference from high-abundance proteins. Conventional pretreatment methods often rely on costly affinity materials and laborious workflows, limiting their sustainability and wider applicability. To overcome these limitations, this work exploits the versatility of aqueous biphasic systems (ABS) to develop liquid-liquid and solid-phase extraction (SPE) platforms for serum cleanup and prostate-specific antigen (PSA) isolation. This versatility was explored using polymers and ionic liquids (ILs) as phase-forming components in conventional ABS and as functional materials in ABS-inspired solid interfaces. PEG 2000/citrate buffer ABS were adapted to SPE using ABEC-2000 resin, allowing direct comparison between liquid-liquid extraction, SPE and protein precipitation. Both ABS and ABEC-based SPE performed better than conventional precipitation for the depletion of high-abundance proteins. However, ABS showed greater compositional flexibility, enabling quantitative PSA recovery in the PEG-rich phase while still removing a substantial fraction of matrix-interfering proteins. Using a similar rationale, ILs were investigated either as phase-forming agents in IL-based ABS or as immobilized ligands in silica-supported IL-based SPE. Under optimized conditions, silica-supported IL-based SPE achieved >92% depletion of high-abundance proteins, while preserving PSA in the liquid phase with 89% extraction. Collectively, these results demonstrate that ABS concepts can be extended beyond classical liquid-liquid extraction to design tunable solid interfaces for selective protein depletion, biomarker recovery and greener sample preparation. These platforms reduce reliance on costly affinity materials and organic-solvent-intensive workflows, supporting the development of more sustainable and clinically relevant bioanalytical methods.

ACKNOWLEDGMENTS

This work was developed within the scope of the project CICECO Aveiro Institute of Materials, UID/50011/2025 (DOI: <https://doi.org/10.54499/UID/50011/2025>) & LA/P/0006/2020 (DOI: <https://doi.org/10.54499/LA/P/0006/2020>), financed by national funds through the FCT/MCTES (PIDDAC). This work was developed within the project ILSurvive, PTDC/EMD-TLM/3253/2020 (DOI: <https://doi.org/10.54499/PTDC/EMD-TLM/3253/2020>), funded by national funds (OE), through FCT/MCTES. M.S.M.M. and I.C. acknowledge FCT for the doctoral grants 2022.11229.BD (DOI: <https://doi.org/10.54499/2022.11229.BD>) and 2024.06707.BD (DOI: <https://doi.org/10.54499/2024.06707.BD>), respectively. F.A.eS. acknowledges FCT for the Scientific Employment Stimulus researcher contract CEECIND/03076/2018/CP1559/CT0024 (DOI: <https://doi.org/10.54499/CEECIND/03076/2018/CP1559/CT0024>).

O15: THERMOSENSITIVE AQUEOUS TWO-PHASE SYSTEMS AS A PLATFORM FOR INTEGRATED PRODUCTION AND RECOVERY OF EPS FROM *Neochloris oleoabundans*

Ana Patricia Garza-Chapa,^{1,2} Carlos Iván Ávila-Velasco,^{1,2} José González-Valdez,³ Alma Gómez-Loredo^{1,2}

¹Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León

²Centro de Investigación en Biotecnología y Nanotecnología, Facultad de Ciencias Químicas, UANL

³Tecnológico de Monterrey, Escuela de Ingeniería y Ciencias

e-mail: alma.gomezlr@uanl.edu.mx

Extractive fermentation (EF) is an in situ strategy that exploits aqueous two-phase systems (ATPS) for the production and primary recovery of biomolecules of interest.^[1] This approach enables the partitioning of target compounds into one phase while retaining biomass and contaminants in the opposite phase, thereby reducing downstream processing steps.^[2] In this study, thermoresponsive ethylene oxide–propylene oxide (EOPO) copolymers were evaluated for the EF of exopolysaccharides (EPS) produced by the microalga *Neochloris oleoabundans*. ATPS were prepared using EOPO polymers with molecular weights of 3,900 and 12,000 g/mol at concentrations of 10% and 15% (w/v), in culture volumes of 100 mL (Condition A) and 1.5 L (Condition B). Axenic cultures of *N. oleoabundans* (UTEX 1185) were grown in liquid medium^[3] under controlled conditions of temperature (25–29 ± 2 °C), light intensity (40–90 μmol photons m⁻² s⁻¹), and aeration (1.5 L/min for larger-scale systems). Pre-inoculum cultures were grown to the exponential phase and used to inoculate experimental systems at 10% (v/v). Cultures were maintained for 21 days (Condition A) or 45 days (Condition B), with periodic monitoring of cell growth. After cultivation, thermally induced phase separation was applied to form ATPS and recover EPS. Phase formation occurred at 40 °C for EOPO (3,900 g/mol) and 70 °C for EOPO (12,000 g/mol), requiring 17 minutes for 100 mL systems and 45 minutes for 1.5 L systems. EPS partitioning was achieved in both experimental conditions, however, EPS and biomass were similarly distributed between phases, limiting effective separation. To address this limitation, EF systems were replaced with ATPS generated from 12-day cultures using EOPO (12,000 g/mol) at concentrations of 10–25% (w/v). Under these conditions, although higher temperatures and longer phase formation times were required, EPS partitioning was favored towards the top phase, while biomass was predominantly retained in the bottom phase. These results demonstrate the potential of EOPO-based ATPS for EPS recovery from *N. oleoabundans*. However, further optimization is required to reduce phase separation temperatures and improve process feasibility.

ACKNOWLEDGMENTS

Authors sincerely thank Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) for the financial support given to graduate students A.P.G.-C (grant 791398) and C.I.Á.-V. (grant 752489).

REFERENCES

- [1] Sinha J, Dey PK, Panda T. Aqueous two-phase: the system of choice for extractive fermentation. *Appl Microbiol Biotechnol.* 2000;54:476-486. doi:10.1007/s002530000342.
- [2] Yang ST, Lu C. Extraction-fermentation hybrid (extractive fermentation). In: Yang ST, editor. *Separation and purification technologies in biorefineries*. Chichester: Wiley; 2013. p. 409-437. doi:10.1002/9781118493441.ch15.
- [3] Salim S, Bosma R, Vermuë MH, Wijffels RH. Harvesting of microalgae by bio-flocculation. *J Appl Phycol.* 2011;23:849-855. doi:10.1007/s10811-010-9591-

O16: INTEGRATED EXTRACTION–DYEING STRATEGIES USING BIO-BASED DYES AND ADDITIVES FOR SUSTAINABLE TEXTILE PRODUCTION

Alexandre M. S. Jorge,¹ Timo C. D. Kolb,¹ Jorge F. B. Pereira¹

¹CERES, Department of Chemical Engineering, Faculty of Sciences and Technology, University of Coimbra, Coimbra Portugal

e-mail: alexandrej@eq.uc.pt

The textile industry is a major contributor to global water pollution due to the extensive use of synthetic dyes and hazardous chemicals in dyeing and finishing processes. Replacing these compounds with bio-based alternatives is a key focus of sustainable textile innovation; however, challenges related to extraction costs, dye stability, and textile performance still limit their industrial adoption. This work investigates the use of natural dyes for sustainable wool dyeing and finishing.

Chlorophyll extracts were obtained using an ethanol/sodium hydroxide/water biphasic system. Similar chlorophyll uptake was observed for both unmordanted and copper-mordanted wool fibers, with total uptake efficiencies of up to 87% on unmordanted wool after two dyeing cycles. Importantly, chlorophyll derivatives promoted a self-mordanting effect, enabling dye uptake comparable to that of copper-mordanted wool without the need for external metal mordants. These findings also supported the development of two integrated extraction-dyeing platforms for chlorophyll extraction, concentration, and direct application to wool dyeing.

Phycocyanin-rich extracts were also investigated for wool dyeing using bio-based strategies. Acetic acid, citric acid, hydrochloric acid, gallic acid, and tannic acid were evaluated to improve dye uptake and wool fabric coloration. Among these additives, tannic acid showed the best performance, achieving a K/S value of approximately 2.25 and phycocyanin exhaustion of nearly 84% at an optimal concentration of 10% owf (i.e., of weight of fiber). Enzymatic crosslinking using transglutaminase was explored as a sustainable fixation approach, promoting interactions between phycocyanin and wool proteins; however, lower coloration intensities were obtained, with a maximum K/S value of 0.55 after 4 h. Chemical crosslinking with genipin produced the most intense coloration, reaching K/S values of 6.26 and significantly improving phycocyanin fixation compared to untreated controls. Overall, these findings demonstrate the potential of bio-based dyes and additives for sustainable textile production and support the development of circular, environmentally responsible dyeing processes.

ACKNOWLEDGMENTS

CERES acknowledges financial support from FCT through the projects UID/00102/2025 (<https://doi.org/10.54499/UID/00102/2025>), UID/PRR/00102/2025 under the Recovery and Resilience Program (PRR) of the Portuguese Republic (<https://doi.org/10.54499/UID/PRR/00102/2025>) and Equipar+2 (<https://doi.org/10.54499/UID/PRR2/00102/2025>). The authors acknowledge FCT for funding HIPERBIOTEX (COMPETE2030-FEDER-00708800) and Calouste Gulbenkian Foundation for funding DyeLoop – Circular Technologies for Textile Dyeing. The authors also acknowledge ATB – Acabamentos Têxteis de Barcelos for supplying the textile materials.

O17: OPTIMIZING PROTEIN EXTRACTION AND PURIFICATION FROM *TENEbrio MOLITOR* AT AN INDUSTRY SETTING AND SCALE

Pedro Moleiro,¹ Luís Branco,² Marisa Santos³, Mara G. Freire¹

¹Departamento de Química, CICECO, Universidade de Aveiro, 3810-193 Aveiro, Portugal

²Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

³Thunder Foods, Lda., 2005-332 Santarém, Portugal

e-mail: pdm@ua.pt

The increasing global population has intensified the demand for sustainable protein sources.^[1] Insects, mainly *Tenebrio molitor* (TM) are promising candidates, with high protein content, nutrient richness, low environmental impact, and cost-effective production compared to poultry/cattle.^[2] However, consumer acceptance remains a challenge in Western countries. It is known that protein extraction and formulation into new food products enhances end-user acceptance. Due to this, the optimization of the extraction and purification in an industrial scale-up was carried out to make the consumption of insects more practical. Design of Experiments (DOE) was applied to optimize parameters and maximize protein extraction yields using aqueous solutions. Insect powder was mixed with solvent, centrifuged, and the supernatant collected for analysis. Proteins were recovered via isoelectric precipitation, followed by freeze-drying to obtain purified protein powder. Optimized parameters were defined (temperature of extraction, duration, and the concentration of the aqueous solution) and yielded extraction efficiencies of over 85 % for *T. molitor*. The final protein powder contained 70-82% extracted proteins. These findings highlight the potential of integrating insect proteins into novel food products as a sustainable strategy for global protein demands, proving the process feasibility at a larger industrial scale.

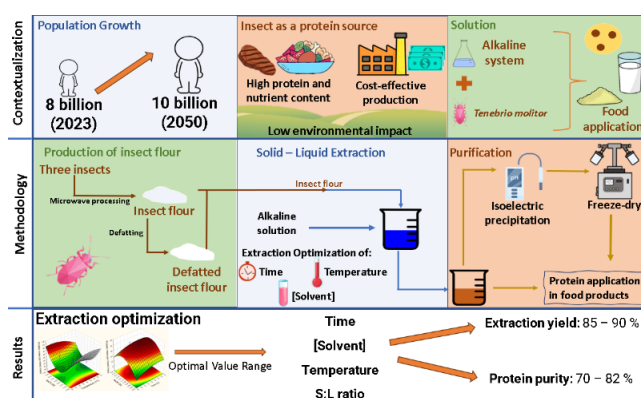


Figure 1: Graphical abstract.

ACKNOWLEDGMENTS

PM acknowledge FCT for the doctoral grant 2024.04187.BDANA.

REFERENCES

- [1] Dreyer M, Hörtenhuber S, Zollitsch W, Jäger H, Schaden LM, Gronauer A, et al. Int J Life Cycle Assess. 2021;26:2232-2247. doi:10.1007/s11367-021-01980-4.
- [2] Laroche M, Perreault V, Marciniak A, Gravel A, Chamberland J, Doyen A. Foods. 2019;8(11):572. doi:10.3390/foods8110572.

BIOMASS PROCESSING

O18: OPTIMIZATION OF PROTEIN EXTRACTION FROM OLIVE POMACE USING DEEP EUTECTIC SOLVENTS

Elif Gökçen Ateş¹

¹Çankırı Karatekin University, Uluyazı, Çankırı, Türkiye

e-mail: elifgokcen@karatekin.edu.tr

Olive pomace is an abundant by-product of olive oil production and still contains a considerable amount of protein that is usually not recovered. In this study, deep eutectic solvents (DES) were explored as a greener alternative to conventional alkaline extraction for recovering proteins from olive pomace. The aim was to improve protein yield while also obtaining fractions with suitable structural and functional properties for potential food applications.

Choline chloride was combined with malic acid, citric acid and sorbitol as hydrogen bond donors (HBD) to formulate the DES systems. A Box–Behnken design was used to optimize four process variables: ChCl:HBD molar ratio (1:1, 1:1.5, 1:3), solid to solvent ratio (1:5, 3:20 and 1:10), water content (25, 40, 55% w/w) and extraction time (30, 60, 90 min) at 40°C, with soluble protein yield as the main response. A conventional alkaline extraction at pH 11–2.5, conducted under comparable solid-to-liquid ratio, temperature and time, was used as a reference method. Extraction yield, total protein content determined by Kjeldahl analysis, soluble protein content, emulsion stability and FTIR spectroscopy were used to evaluate the structural and functional properties of the recovered protein fractions.

The response surface analysis showed that DES composition and ratio were the most influential factors on protein solubilization, whereas extraction time had a smaller but positive effect. Among the tested HBDs the citric acid–choline chloride DES provided the highest soluble protein yield, higher soluble protein content at pH 7 and better emulsion stability than the alkaline control. FTIR and TD-NMR results indicated that proteins extracted with DES retained their characteristic protein bands and exhibited altered water–protein interactions compared with the alkaline extracts.

ACKNOWLEDGEMENTS

The financial support was provided by TÜBİTAK under Türkiye–Malta Bilateral Cooperation Program (project no: 124N564).

O19: A SUSTAINABLE APPROACH FOR RECOVERING MICROBIAL INDIGOIDINE USING AN ETHANOL-BASED SOLVENT SYSTEM COMBINED WITH MECHANICAL CELL DISRUPTION

Pedro G. Pereira Silva,¹ Maria E. Jacob Ananias,¹ Amanda B. Cunha Silva,¹ Laura A. S. Amorim,¹ Danielle B. Pedrolí,¹ Valéria de C. Santos-Ebinuma¹

¹Department of Bioprocess Engineering and Biotechnology, School of Pharmaceutical Sciences, São Paulo State University, 14801902, Araraquara, SP, Brazil

e-mail: valeria.ebinuma@unesp.br

Color plays a critical role in different industrial segments. Consequently, driven by the increasing demand for natural colorants, indigoidine has emerged as a promising blue bacterial colorant with different potential applications. However, efficient recovery from bacterial cells remains a challenge due to intracellular accumulation.^[1] Thus, this study evaluated the colorant recovery yield of combining sustainable solvent systems with mechanical disruption using glass beads. The bacterial strain was reactivated and inoculated in a minimal medium (6% sucrose, pH 7.0) at 37 °C and 220 rpm for 16 h.^[2] The main culture was performed in a stirred-tank bioreactor (250 mL medium, 37 °C, 400 rpm, 1 vvm) for 115 h. Cells were harvested by centrifugation, washed, and stored frozen. For extraction, 2 g of wet biomass were washed with water and methanol. The extraction cycle employed 4 mL of ethanol (50% v/v) combined with 2 g of glass beads of different diameters (2, 3 and 4 mm). The mixture was subjected to vortex agitation for 3 min, and this cycle was repeated four times. Indigoidine recovery yield was estimate based on assay using DMSO without glass beads as 100%.^[3] The incorporation of glass beads in the solvent system presented interesting results. The 3 mm protocol achieved the highest recovery yield (63.08%), while 2 and 4 mm protocol reached 39.86 and 55.46%, respectively. The ethanol-based solvent system combined with mechanical disruption proved to be a sustainable and effective strategy, achieving promising recovery yields. This environmentally friendly approach aligns with the UN Sustainable Development Goals (SDGs) and supports the potential industrial application of this natural blue bacterial colorant.

ACKNOWLEDGMENTS

This study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES - Finance Code 001). This study was also supported by the São Paulo Research Foundation – Brazil (FAPESP – Grant n° 2021/06686-8, 2023/01368-3 and 2024/08205-5). Valeria C. Santos-Ebinuma thanks the National Council for Scientific and Technological Development – Brazil (CNPq – Proc. 306475/2025-1). Pedro Garcia Pereira Silva thanks the research fellow provided by FAPESP (Grant n° 2025/21726-7). Maria E. J. Ananias thanks the research fellow provided by FAPESP (Grant n° 2025/25777-5).

REFERENCES

- [1] Chen Z, Liu W, Zhang S, Song Y, Zhang Z, Yao G, et al. Harnessing soybean okara for enhanced microbial production of indigoidine. *Bioresour Technol.* 2025;434:132808. doi: 10.1016/j.biortech.2025.132808
- [2] Li Z, Lu R, Hu H, Chen R, Zhou D, Zhu Y, et al. Pathway optimization and membrane engineering for highly efficient production of indigoidine in engineered *Escherichia coli*. *Bioresour Technol.* 2026;440:133415. doi: 10.1016/j.biortech.2025.133415
- [3] Oka LKA, Barbosa EH, Silva PGP, Santos-Ebinuma VC. Production and extraction of a natural blue colorant from *Streptomyces lavendulae*: insights into indigoidine stability and its potential use in sustainable dyeing. *Bioresour Technol Rep.* 2026;34:102715. doi: 10.1016/j.biteb.2026.102715

O20: KERATIN PURIFICATION AND ITS USE IN BIOMATERIALS PRODUCTION

João A. P. Coutinho,¹ Mara G. Freire¹

¹CICECO - Aveiro Institute of Materials, Dep. of Chemistry, University of Aveiro, Aveiro, Portugal

e-mail: jcoutinho@ua.pt

Wastes from chicken production has been object of study by us, as it is an unsuspected wealthy source of valuable compounds when subjected to treatment with ionic liquids. On a first approach we addressed the eggs. At Aveiro, the excess egg white is a by-product hard to valorize. We have shown how to extract the albumin from the egg white^[1] and how we learnt to fibrillate proteins from this rich and cheap source^[2] using a technique that was later successfully applied to human platelet lysates allowing to produce nanofibrils that were used to produce free-standing membranes for cell self-aggregation.^[3] The egg yolk, rich in IgY antibodies, became the object of a purification process for its recovery to be used as biopharmaceuticals.^[4]

Recently we became interested in the keratin extraction from chicken feathers. Millions of tonnes of this unpleasant waste are generated yearly. Feathers are rich in keratin (90 wt %), a fibrous protein with higher stability and lower solubility than most proteins. The conversion of an unavoidable biowaste into value-added products was our purpose. The processing conditions (keratin concentration, plasticizer, and IL type) were optimized to achieve the biomaterials best properties. To achieve a sustainable process, IL recovery and reuse were also evaluated. Our results show that chicken feathers can be efficiently valorized by producing keratin-based biomaterials with various applications.^[5,6]

REFERENCES

- [1] Pereira MM, Cruz RAP, Almeida MR, Lima AS, Coutinho JAP, Freire MG. [Article title]. *Process Biochem.* 2016;51(6):781-791. doi:10.1016/j.procbio.2016.03.002.
- [2] Bharmoria P, Mondal D, Pereira MM, Neves MC, Almeida MR, Gomes MC, et al. [Article title]. *Commun Mater.* 2020;1:34. doi:10.1038/s43246-020-0035-0.
- [3] Monteiro CF, Gomes MC, Bharmoria P, Freire MG, Coutinho JAP, Custódio CA, et al. [Article title]. *ACS Nano.* 2024;18(24):15815-15830. doi:10.1021/acsnano.4c02790.
- [4] Almeida MR, Ferreira F, Domingues P, Coutinho JAP, Freire MG. [Article title]. *Sep Purif Technol.* 2022;299:121697. doi:10.1016/j.seppur.2022.121697.
- [5] Polesca C, Passos H, Neves BM, Coutinho JAP, Freire MG. [Article title]. *Green Chem.* 2023;25:1424-1434. doi:10.1039/D2GC04477C.
- [6] Polesca C, Al Ghatta A, Passos H, Coutinho JAP, Hallett JP, Freire MG. [Article title]. *Green Chem.* 2023;25:3995-4003. doi:10.1039/D3GC00850A.

O21: SYNERGISTIC ANTIOXIDANT PERFORMANCE OF LIGNIN EXTRACTED WITH DEEP EUTECTIC SOLVENT FROM OLIVE MILL SOLID WASTE IN POLYVINYL ALCOHOL FILMS AND NANOFIBERS

Kamil Urgan,^{1,2} Bora Maviş³

¹Graduate School of Science and Engineering, Hacettepe University, Beytepe, 06800 Ankara, Turkey

²Department of Food Engineering, Hacettepe University, Beytepe, 06800 Ankara, Turkey

³Department of Mechanical Engineering, Hacettepe University, Ankara 06800, Turkey

e-mail: kamil.urgun@gmail.com

Lignin is known to exhibit synergistic antioxidant effects when used together with small molecule antioxidants. However, whether similar effects occur in polymer blends containing lignin as the only antioxidant component remains largely unexplored. This study investigates the synergistic antioxidant effects of lignin derived from olive mill solid waste (OL) when incorporated as the sole active component in polyvinyl alcohol (PVA) polymer blends. PVA films and electrospun fibers containing 0–11 wt% OL showed antioxidant activity exceeding rule-of-mixtures predictions. The maximum synergy occurred at 4 wt% OL, with enhancements of 480% for films and 971% for fibers, compared to 33% for sodium lignosulfonate. Spectroscopic and thermal analyses revealed that this synergy stems from morphology-dependent intermolecular interactions. These findings represent one of the strongest synergistic antioxidant effects reported for a lignin/polymer system, presenting a sustainable pathway to convert agricultural waste into high-performance materials for active packaging and other advanced applications.

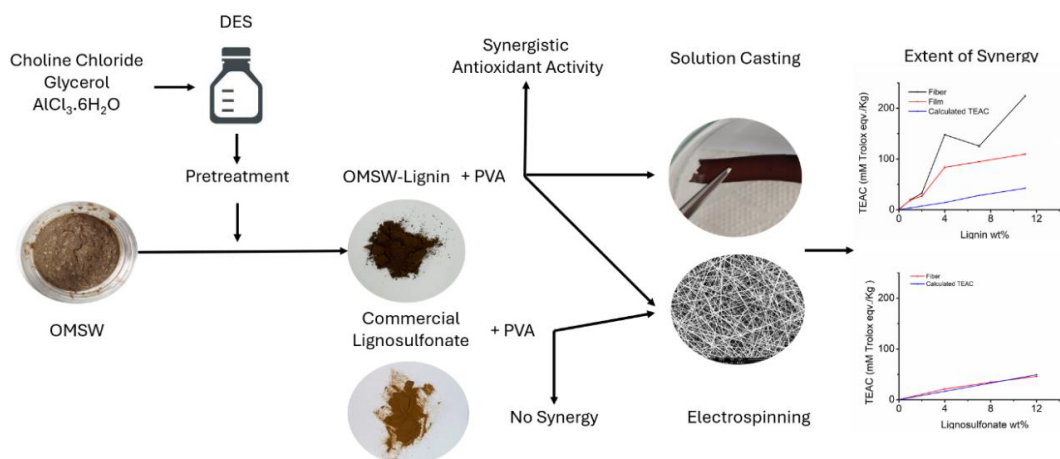


Figure 1: Graphical abstract.

O22: ACTIVATED CARBON FROM BREWERY WASTE FOR ENHANCED BORON REMOVAL

Maryam Wundabsung,¹ Bilge İkizoglu,¹ Esra Bakkaloglu,² Pinar Degirmencioglu¹, Huseyin Arbag,^{1,3} Alpay Sahin,¹ H. Mehmet Tasdemir,¹ D. Dolunay. E. Koyuncu,¹ Mujgan Okur,¹ Janvit Teržan,^{4,5} Maja Čič,^{4,5} Elena Sutormina,^{4,5} Filipa A. Vicente,⁴ Ilja Gasan Osojnik Črnivec^{4,5}

¹Department of Chemical Engineering, Gazi University, 06570 Ankara, Türkiye

²Department of Chemical Engineering, Ondokuz Mayıs University, 55270 Samsun, Türkiye

³Smart Maten Engineering Ltd Co, Ankara, Türkiye

⁴Laboratory for Demonstration of H₂ and CO₂ Technologies, National Institute of Chemistry, SI-1412 Kisovec, Slovenia

⁵Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, SI-1000 Ljubljana, Slovenia

e-mail: harbag@gazi.edu.tr

Sustainable enhanced adsorbent materials were developed from brewery spent grain to facilitate boron removal from aqueous solutions. Activated carbons were synthesized via an FeCl₂-assisted chemical activation at different carbonization temperatures (600, 750, and 900 °C) under argon. The structural properties of the obtained activated carbons were determined using FTIR, XRD, SEM-EDS and N₂ adsorption–desorption analyses. XRD analysis revealed that higher activation temperatures are required to obtain amorphous carbon structure. The diffraction peak at 2θ:26.4° of the graphitic carbon peaks increased with carbonization temperature. In FTIR spectra of activated carbon, O-H (~3400 cm⁻¹), C-H (~2900 cm⁻¹), C=O (~1700 cm⁻¹), and C–O (~900-1100 cm⁻¹) bands were observed. N₂ adsorption–desorption analyses revealed that increasing carbonization temperature significantly improved pore development. Among the prepared materials, the lowest surface area was obtained at the carbonization temperature of 600 °C. As the carbonization temperature increased, the surface area value increased significantly. BET surface area values of the activated carbons synthesized at 750 and 900 °C were well developed, exceeded 800 m²/g and exhibited a mesoporous structure. The adsorption experiments were conducted at pH 2, 1 ppm initial boric acid concentration, and 30 °C in batch system. Boron concentration analyses were determined by carminic acid method. A boron adsorption study was conducted using activated carbon synthesized from brewery spent grains. Boron removal efficiency of the activated carbon carbonized at 900 °C reached up to 35%, indicating further strategies for adsorption enhancement.

ACKNOWLEDGMENTS

The research was funded by the Scientific and Technological Research Council of Turkey Research Fund (TUBITAK-224N420) grant, the Slovenian Research Agency under the SI-TR bilateral B-Bio-Sorb (BI-TR/25-27-006) and P2-0152 projects and the CarbNETS programme, co-funded by the Republic of Slovenia, Ministry of Higher Education, Science and Innovation, and by the European Union under the Cohesion Policy Fund.

O23: VALORISATION OF COTTON TEXTILE WASTE VIA ACID HYDROLYSIS AND LIQUID-LIQUID EXTRACTION FOR INTEGRATED BIOREFINERY APPLICATIONS

Joaquim F. Pinto,¹ Barbara R. Leite,² Lucília Domingues,³ Jorge F. B. Pereira¹

¹University of Coimbra, CERES, Department of Chemical Engineering, 3030-790 Coimbra, Portugal

²RDD Textiles, Rua Professor Celestino Costa, No. 110, 4755-058 Barcelos, Portugal

³CEB - Centre of Biological Engineering, University of Minho, Braga, 4710-057, Portugal

e-mail: joaquimfp99@gmail.com

The textile industry is among the most polluting sectors worldwide, generating substantial amounts of solid waste during manufacturing stages, with less than 12% of textile waste is currently recycled. Textile residues are highly heterogeneous and often contains dyes and finishing additives that hinder biological processing. Pre-treatment methods such as acid, alkaline, or enzymatic hydrolysis aim to release fermentable sugars from cellulose-based textiles; however, their efficiency and scalability remain limited, due to the presence of inhibitors. To develop simpler and more competitive purification strategies for hydrolysates, liquid–liquid extraction (LLE) has emerged as a promising approach for the selective separation of sugars and the removal of process-derived contaminants. Aiming at full process integration, sulfuric and acetic acid hydrolysis were evaluated on 100% cotton post-industrial knitted textile waste streams: untreated raw fabric and finished black fabric. Subsequently, sequential alcohol-based liquid-liquid extraction (LLE) processes were performed to concentrate sugars and remove fermentation inhibitors, such as HMF, residual dyes, and additives. Sulfuric acid hydrolysis predominantly released glucose, yielding higher sugar recovery from the untreated fabric (9.43%) than from the finished black fabric (7.49%). In contrast, acetic acid hydrolysis produced greater overall sugar release from the finished fabric. This suggests that dyes and finishing agents modulate carbohydrate accessibility. Ultimately, integrating textile-waste hydrolysis with LLE purification provides optimal mixed sugar streams for valorisation by engineered *Saccharomyces* strains. These results demonstrate that combining tailored hydrolysis and downstream partitioning is essential for advancing truly circular biorefinery and bioeconomy strategies within the textile sector.

ACKNOWLEDGMENTS

This work was supported by the Fundação Calouste Gulbenkian through the project DyeLoop – Circular Technologies for Textile Dyeing, and by the Fundação para a Ciência e a Tecnologia (FCT) through national funds and by the European Regional Development Fund (FEDER) under the Thematic Programme Innovation and Digital Transition (COMPETE 2030), within the framework of Portugal 2030, and by the European Union, under Project No. 15955 | COMPETE2030-FEDER-00708800.

CERES acknowledges financial support from FCT through the projects UID/00102/2025 (<https://doi.org/10.54499/UID/00102/2025>), UID/PRR/00102/2025 under the Recovery and Resilience Programme (PRR) of the Portuguese Republic (<https://doi.org/10.54499/UID/PRR/00102/2025>) and Equipar+2 (<https://doi.org/10.54499/UID/PRR2/00102/2025>)

The authors also acknowledge RDD-Textiles, Lda. for supplying the textile fibers used in this study.

Joaquim F. Pinto acknowledges FCT for the PhD scholarship 2025.00956.BDANA.

O24: FUELS FROM FOREST: SLURRY-PHASE UPGRADING OF LIGNOCELLULOSIC PYROLYSIS BIO-OILS

Sari Rautiainen,¹ Eveliina Mäkelä,¹ Tyko Viertiö,¹ Silja Käsäkoski,¹ Jessica Ekholm,¹ Alexander Reznichenko¹ and
Juha Lehtonen¹

¹VTT Technical Research Centre of Finland Ltd, Espoo, Finland

e-mail: sari.rautiainen@hotmail.com

Liquefaction of lignocellulosic biomass to bio-oils represents a promising pathway for the production of sustainable transportation fuels. However, in order to meet fuel-quality specifications, these bio-oils require subsequent upgrading and deoxygenation owing to their chemical instability, high acidity, elevated viscosity, and substantial oxygen content. Conventional fixed-bed hydrotreatment of such feedstocks is hindered by several operational challenges, most notably rapid catalyst deactivation and reactor plugging. In this context, catalytic slurry-phase hydrotreatment in a continuous stirred-tank reactor (CSTR) constitutes a robust alternative for the upgrading of problematic bio-oils, as it mitigates clogging and enables continuous catalyst replacement to control deactivation.^[1] In our previous study, unsupported cobalt–molybdenum sulfides were evaluated for the hydrodeoxygenation of isoeugenol as a model compound representative of bio-oils.^[2] In the present work, unsupported molybdenum sulfides are demonstrated to be effective catalysts for the slurry-phase hydrotreatment of real bio-oils. In addition, the synthesis conditions on catalyst structure—and consequently on deoxygenation performance—are systematically examined. Further deoxygenation of the slurry-stabilised intermediate fuel in fixed-bed reactor is shown to produce deoxygenated fuels. This work highlights the potential of slurry hydroprocessing with unsupported MoS-based catalysts and proposed two-step approach as a viable path for upgrading bio-oils to drop-in hydrocarbon fuels and chemicals such as steam cracker feedstocks.

REFERENCES

- [1] Dimitriadis A, Bergvall N, et al. [Article title]. *Fuel*. 2023;332:126153. doi:10.1016/j.fuel.2022.126153.
[2] Viertiö T, Vuorio N, Rautiainen S, et al. [Article title]. *Catal Sci Technol*. 2026;16:925-943.

O25: TUNABLE HYDROGEL ARCHITECTURES FOR ADVANCED ELECTROCHEMICAL ENERGY SYSTEMS: TOWARD ADAPTIVE MEMBRANES AND ELECTRODE INTERFACES FOR GREEN HYDROGEN TECHNOLOGIES

Vojtech Škopek,^{1,2,3} Vuk Martinović,^{1,2,3} Maja Čič,¹ Anja Verbič,²
 Blaž Stres,^{2,3} Blaž Likozar,² Filipa A. Vicente,^{2,3} Ilja Gasan Osojnik Črnivec^{1,3}

¹Laboratory for Demonstration of H₂ and CO₂ Technologies, National Institute of Chemistry, Slovenia

²Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Slovenia

³Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia

e-mail: gasan.osojnik@ki.si

Biopolymer-based hydrogels are emerging as highly promising multifunctional materials for next-generation electrochemical energy systems due to their tuneable physicochemical behaviour, sustainability, and compatibility with low-energy fabrication routes. Building upon interdisciplinary research in catalytic hydrogen production, mesoporous materials, encapsulation technologies, bio-derived functional materials, and hybrid polymer systems, this work explores the development of adaptive hydrogels prepared predominantly from bio-based ingredients and renewable biopolymers for applications in electrolyzers, electrochemical reactors, and hydrogen conversion technologies. The presented concepts focus on hydrogel systems with programmable physicochemical functionalities, including tuneable ionic conductivity, selective ion transport, adaptive swelling behaviour, controlled gas permeability, self-healing capability, redox responsiveness, thermal stability, and mechanically reinforced hierarchical porosity. Particular emphasis is placed on hydrogel-enabled electrode coatings and membrane materials capable of improving catalyst immobilization, mitigating gas bubble accumulation, stabilizing local electrochemical environments, and enhancing operational durability under alkaline and membrane-electrode assembly conditions, whereas metabolite, analyte and selective molecular permeability is also discussed and demonstrated. Building upon these concepts, hydrogel-enabled membranes and electrode-interface coatings for alkaline and membrane-based electrolyzers are further explored. In these systems, hydrogel layers may enhance catalyst dispersion, regulate local hydration environments, suppress bubble accumulation, reduce interfacial degradation, and improve operational durability. Beyond conventional membrane roles, the materials are envisioned as adaptive multifunctional interfaces capable of coupled catalytic, transport, and sensing behaviour. Potential advanced functionalities include pH-responsive conductivity modulation, electrochemically induced restructuring, selective molecular capture, antifouling behaviour, and dynamic regulation of mass transfer under operating conditions. Drawing from previous work on hydrogen generation systems, catalytic materials, encapsulation technologies, photocatalytic and thermochemical processes, as well as sustainable material synthesis, this contribution highlights how bio-based hydrogel architectures can bridge soft matter engineering with electrochemical energy technologies. The integration of renewable feedstocks, biodegradable polymers, and circular-material approaches positions tuneable biopolymer hydrogels as enabling materials for resilient and sustainable hydrogen production systems and future low-carbon energy infrastructures.

ACKNOWLEDGMENTS

This research was founded by the Scientists4Future Slovenia Doctoral Programme (HE MSCA SoE and Janko Jamnik Doctoral Scholarship, National Institute of Chemistry), the CarbNETS programme, co-funded by the Republic of Slovenia, Ministry of Higher Education, Science and Innovation, and by the European Union under the Cohesion Policy Fund as well as the Slovenian Research Agency under the research core funding No. P2-0152.

SAFE-AND-SUSTAINABLE-BY- DESIGN WORKSHOP

REGULATORY AND SUSTAINABILITY CONSIDERATIONS¹

¹Safe-and-Sustainable-by-Design Workshop is organised within the TOXBOX project, with the support of and in synergy with the SSbD4Chem project.

O26: SSbD IN PRACTICE WITHIN THREE INDUSTRIAL VALUE CHAINS – FIRST LESSONS FROM THE PLANETS APPROACH TO INNOVATION OF SAFER AND MORE SUSTAINABLE PLASTICIZERS, SURFACTANTS, AND FLAME RETARDANTS

Martin Himly,¹ Sabine Hofer,¹ Norbert Hofstätter,¹ Estela Perez Ventosa,¹ Laura Schmitz,¹ Mario Wenger,¹ Thomas Hennequin,² Neeraj Shandilya,² Loélia Fohet,³ Catherine Colin,³ Pierre-Emmanuel Dufils,⁴ Szymon Zdybel,⁵ Anita Sosnowska,⁵ Beatriz Alfaro-Serrano,⁶ Carl-Christoph Höhne,⁷ Josephine Munsch,⁸ Stéphanie Desrousseaux,⁹ Cécile Philippot,⁹ Joséphine Steck⁹

¹Department of Biosciences & Medical Biology, Division of Allergy & Immunology, Paris Lodron University of Salzburg, Austria, ²Netherlands Organisation for Applied Scientific Research, The Netherlands, ³Industrial Technical Center for Plastics and Composites, CT-IPC, Biopôle Clermont-Limagne, France, ⁴Syensqo, Performances & Care-RIC Lyon, France, ⁵QSAR Lab Ltd., Poland, ⁶BioNanoNet Forschungsgesellschaft mbH (BNN), Austria, ⁷Polymer Engineering, Fraunhofer-Institut für Chemische Technologie ICT, Pfinztal (Berghausen), ⁸Bluestar (former ELKEM) Silicones, France, ⁹CEA, Liten, DTNM, France

email: martin.himly@plus.ac.at

European policies, such as the European Clean Industrial Deal and the Chemicals Strategy for Sustainability, call for safer and more sustainable innovation. Hence, the Safe and Sustainable by Design (SSbD) approach has been elaborated to proactively embed safety and sustainability from the earliest stages of innovation. Within PLANETS we implement SSbD in three industrial value chains to innovate safer and more sustainable plasticizers, surfactants, and flame retardants. To do so, we developed a tiered and integrated SSbD workflow operationalizing the SSbD approach on three real-life innovation cases, starting with an extensive scoping analysis, clarifying the WHY (redesign definition), the WHAT (setting the system boundaries), the WHO (stakeholder / involved value chain actors definition) and the HOW (overviewing the essential / prioritized SSbD assessment requirements), followed by a simplified (Tier 1) assessment of the sets of alternatives highlighting potential red-flags or points of attention. Currently the respective innovation cases undergo the stage of intermediate (Tier 2) assessment, which is mainly based on computational tools and predictive modeling. Running through this SSbD workflow we encountered several key challenges and have entered the process of finding potential solutions. Those cover, for instance, integration of the scoping results or aspects from the functionality dimension into the tiered SSbD assessment approach, or how to deal with topics like collaborative and harmonized data collection, which is particularly complicated by confidentiality issues, or in respect to results at different Tier levels. For facilitating a smooth SSbD integration in the industrial value chains the PLANETS approach profits from the support through SSbD ambassadors. Moreover, we conduct extensive interdisciplinary knowledge building and undertake efforts for SSbD terminology harmonization running a DELPHI panel, jointly with PARC and IRIS, surveying relevance, clarity, and completeness of SSbD terms that are included in the PLANETS SSbD Wordbook. This presentation will provide critical insights for innovators aiming to embed SSbD in their industrial processes, highlighting the need for continued SSbD methodological development and domain-specific up-skilling in hazard identification for human health and the environment, exposure assessments or determination for the production and use phases, life cycle assessment, as well as an overall insight in the multi-criteria decision analysis across the diverse SSbD dimensions.

ACKNOWLEDGMENTS

This project received funding from the European Union's Horizon Europe research and innovation programme under grant agreement n° 101177608. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

O27: SAFE AND SUSTAINABLE BY DESIGN IMPLEMENTATION IN THE TOXBOX TOXICITY TESTING PLATFORM

Guillermo Ormazabal,¹ Filipa A. Vicente,² Andraž Teržan,² Adam Foley,³ Solène Moreau,⁴ Gemma Mendoza¹

¹Tekniker, Basque Research and Technology Alliance (BRTA), 20600 Eibar Spain

²National Institute of Chemistry, 1000, Ljubljana, Slovenia

³Dolmen design and innovation limited, Glasnevin D11KXN4, Ireland

⁴Univ. Grenoble Alpes, CEA-Leti, F-38000 Grenoble, France

e-mail: guillermo.ormazabal@tekniker.es

The Safe and Sustainable by Design (SSbD) concept has been introduced by the European Commission as a framework to guide the development of chemicals and materials that are safer and more sustainable across their life cycle.^[1-3] However, its practical implementation remains challenging, particularly due to the lack of integrated tools that can support decision-making at early stages of technology development. In this context, the European TOXBOX project contributes to SSbD implementation by developing an advanced ecotoxicity platform combining microfluidics and organ-on-chip technologies to enable high-throughput, mechanism-based toxicity assessment. This approach reduces the reliance on animal models, while supporting earlier and more informed decision-making in the development of chemicals and materials. During the design phase of TOXBOX equipment, SSbD principles are systematically applied alongside a stepwise sustainability assessment to guide early design choices. A preliminary life cycle assessment (LCA), following ISO 14040/44 and the Environmental Footprint (EF 3.1), together with a technoeconomic analysis (TEA) were conducted to support this design process. These tools enable the evaluation of alternative materials, structural optimisation to reduce resource use, recyclability and end-of-life options, and the identification of high-impact components in microfluidic and organ-on-chip systems. Overall, TOXBOX demonstrates how integrating SSbD, LCA and TEA tools during early design can steer technology development towards safer, more sustainable solutions, while maintaining performance and functionality. The project provides a practical example of how SSbD can be translated from policy ambition into concrete design and engineering decisions, with ongoing work extending towards social life cycle assessment.

ACKNOWLEDGMENTS

This research is funded by the European Union's Horizon Europe research and innovation programme under grant agreement No. 101138387 (TOXBOX). This article reflects only the authors' views. The European Commission is not responsible for any use that may be made for the information it contains.

REFERENCES

- [1] Commission Recommendation (EU) 2026/510 of 6 March 2026 on revising the European assessment framework for safe and sustainable by design chemicals and materials [Internet]. 2026 [cited 2026 Jul 21]. Available from: <http://data.europa.eu/eli/reco/2026/510/oj>
- [2] Garmendia Aguirre I, Abbate E, Bracalente G, Mancini L, Cappucci GM, et al. Safe and sustainable by design chemicals and materials: revised framework (2025) [Internet]. Bracalente G, Abbate E, Garmendia Aguirre I, editors. Luxembourg: Publications Office of the European Union; 2025. JRC143022. doi:10.2760/5103785.

[3] Abbate E, Garmendia Aguirre I, Bracalente G, Mancini L, Tosches D, Rasmussen K, et al. Safe and sustainable by design chemicals and materials: methodological guidance [Internet]. Luxembourg: Publications Office of the European Union; 2024. JRC138035. doi:10.2760/28450.

O28: IMPLEMENTING A SAFE-AND-SUSTAINABLE-BY-DESIGN (SSbD) FRAMEWORK FOR NANOCELLULOSE-ENHANCED COSMETIC FORMULATIONS

Ondrej Panák,¹ Uroš Novak,¹ Assaf Assis,² David Barak,² Dror Kohen,² Alexandra Blinderman,² Milica Velimirovic Fanfani,³ Barry Hardy,⁴ Panagiotis D. Kolokathis,⁵ Yvonne Kohl,⁶ Wouter A. Gebbink,³ Fruela Pérez Sánchez⁷

¹National Institute of Chemistry, Slovenia

²AHAVA - Dead Sea Laboratories Ltd., Israel

³Flemish Institute for Technological Research (VITO), Belgium

⁴Edelweiss Connect GmbH, Technology Park Basel, Switzerland

⁵NovaMechanics MIKE, Greece

⁶Fraunhofer Institute for Biomedical Engineering IBMT, Germany

⁷Instituto Tecnológico del Embalaje, Transporte y Logística (ITENE), Spain

e-mail: uros.novak@ki.si

The cosmetic industry is increasingly transitioning toward bio-based alternatives to replace synthetic polymers and microplastics. Within the SSbD4CheM project, this contribution presents an integrated Safe-and-Sustainable-by-Design (SSbD) approach to the development of skincare products that incorporate nanocellulose as a multifunctional additive to optimise rheological properties while enhancing texture, skin feel, smoothness, and stability. Cosmetic products are subject to strict safety regulations, with assessment of new ingredients relying on supplier safety data sheets, in-house testing, and external evaluation of the Margin of Safety. This case study aims to apply the multi-tiered SSbD framework to evaluate nanocellulose additives varying in type, size, and surface functionalisation in the development of four cosmetic products, and to extend the assessment to cover toxicological and hazard-related aspects, alongside worker safety, consumer safety, and environmental impact. At the first and second tiers, nanocellulose additives with different modifications were evaluated through data collection and curation, while AI-assisted knowledge mining tools are currently under development. Skin toxicity and sensitisation were assessed using the project's SbD4Skin *in silico* tool, indicating no toxicity and low or no sensitisation potential for the selected additives. Exposure assessment, covering workers, consumers, and the environment, is being performed through modelling based on identified exposure pathways. New Approach Methodologies (NAMs) under development include *ex vivo* skin models, *in vitro* gastrointestinal tract and lung models, and the zebrafish embryo model, which will further support hazard assessment and provide experimental benchmarks for *in silico* tools. Environmental and socioeconomic dimensions are addressed through ex-ante and Social Life Cycle Assessment. The integration of these tools into a holistic assessment framework continues to guide product development, supporting informed decisions that balance safety, sustainability, and product performance.

ACKNOWLEDGMENTS

The SSbD4CheM project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement number 101138475. UK participants in the SSbD4CheM project are supported by UKRI grant agreement number 10110559. CH participants in the SSbD4CheM project receive funding from the Swiss State Secretariat for Education, Research and Innovation (SERI). Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

O29: LINKING CHEMICAL STRUCTURE TO SPHEROID PHENOTYPES: AN INTERPRETABLE QSAR APPROACH FOR HEPATOTOXICITY IN THE CONTEXT OF SSBD

Milica Nikolic,^{1,2} Tijana Geroski,^{1,3} Nenad Filipovic^{1,3}

¹Steinbeis Advanced Risk Technologies Institute doo Kragujevac, 34000 Kragujevac, Serbia

²Faculty of Mechanical and Civil Engineering in Kraljevo, University of Kragujevac, 36000 Kraljevo, Serbia

³Faculty of Engineering, University of Kragujevac, 34000 Kragujevac, Serbia

e-mail: mnikolic@risk-technologies.com

In this research, we created a QSAR framework to analyse drug safety with the purpose of generating interpretable and relevant SSbD insights. We used public database containing approximately 20 000 brightfield microscopy images of human liver spheroids exposed to 108 drugs over a period of up to 7 days.^[1] CellProfiler open-source software was used to perform image segmentation and extract features related to spheroid size, shape, intensity and texture. These features were extracted from each image and exported into a CSV file, which served as input to the QSAR framework. The CSV file was further enriched with molecular fingerprints and physicochemical descriptors generated by RDKit library.^[2] We utilised scikit-learn library to train a Random Forest model, perform cross-validation and select the most relevant features. A simpler linear model was developed to compare interpretability against Random Forest model. The QSAR model investigated how the drug structure links to developed spheroid phenotype, that links to toxicity prediction. We interpreted the QSAR results using SHAP values, to identify key structural features which were further analysed in an open-source OECD QSAR Toolbox to provide mechanistic context and support grouping of compounds in line with the SSbD principles. The final output from this research is to derive conclusions of drug safety to the liver human spheroids based on correlation between chemical and structural composition of applied drugs and observed changes in spheroids upon exposure, leading to design-relevant insights, where specific aspects of drug chemical structure can be associated with toxicity level and the temporal progression of spheroid damage. These insights highlight structural motifs that should be avoided or reduced to decrease hepatotoxicity risk. Future research should include analysis of liver human spheroids toxicity response to drugs co-exposures, as combined exposures may lead to different toxicity profiles comparing to toxicity profile of single compound.

ACKNOWLEDGMENTS

This research is funded by the project that has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101138387 (TOXBOX). This article reflects only the authors' views. The European Commission is not responsible for any use that may be made for the information it contains. This research was also supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract numbers 451-03-34/2026-03/200108 (Faculty of Mechanical and Civil Engineering in Kraljevo, University of Kragujevac), 45103-33/2026-03/200378 (Institute for Information Technologies Kragujevac, University of Kragujevac) and 451-03-33/2026-03/200107 (Faculty of Engineering, University of Kragujevac). This research is in line with the strategic plan of the Republic of Serbia 2030, particularly in the section 2.2.2. Development of human resources, goal 3: Good health.

REFERENCES

[1] Dubinsky D, Harel S, Bein A, Nyska A, Ya'ari S, Koc B, et al. Non-invasive quantification of viability in spheroids using deep learning.

[2] RDKit. RDKit: Open-source cheminformatics [Internet]. Available from: <https://www.rdkit.org/> [cited 2026 Apr 27].

O30: LIFE CYCLE ASSESSMENT OF BLUE BIOREFINERIES: FROM CRUSTACEAN WASTE VALORISATION TO FUNCTIONAL CHITOSAN APPLICATIONS

Jan Puhar,¹ Rok Pučnik,¹ Filipa A. Vicente,² Lidija Čuček,¹ Blaž Likozar,² Annamaria Vujanović¹

¹University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor, Slovenia

²National Institute of Chemistry, Ljubljana, Slovenia

e-mail: jan.puhar@um.si

Blue biorefineries provide an opportunity to convert marine processing residues into high-value products while reducing waste-related environmental burdens. This work presents Life Cycle Assessment (LCA) as a process-design and decision-support tool for crustacean biorefineries, focusing on shrimp-shell valorisation and the production of chitin and chitosan from laboratory to industrial scale.

At laboratory scale, nine technological combinations were assessed, comprising three chitin extraction routes and three chitin-to-chitosan conversion routes. The resulting greenhouse-gas footprints ranged from approximately 149 to 1,532 g CO₂-eq per g of chitin converted to chitosan. Conventional eco-solvent extraction combined with conventional NaOH deacetylation showed the lowest impacts in most assessed categories. The biorefinery concept was subsequently evaluated through scale-up from laboratory conditions to a pilot plant processing 1 t of shrimp shells per day and an industrial scenario based on approximately 72,000 t of annually available shrimp-shell waste. The underlying process demonstrated recovery of up to 92% of proteins, approximately 79% of chitin and 34% of astaxanthin. Scale-up analysis further showed that direct linear extrapolation of laboratory energy consumption can substantially overestimate industrial impacts, highlighting the importance of equipment-specific modelling.

Future work will extend the sustainability assessment from chitosan production towards its modification and functionalisation, with particular emphasis on linking the environmental burdens with improvements in material performance. Chitosan-based coatings for food packaging represent one such pathway, where functional performance and biodegradability can be assessed against conventional LDPE packaging on an equivalent-function basis.

ACKNOWLEDGMENTS

Authors acknowledge the support from the European Climate, Infrastructure and Environment Executive Agency (CINEA, European Commission) for INSPIRE project (Grant Agreement Number: 101112879) and the Slovenian Research and Innovation Agency for project ARIS J2-70079.

O31: ORGANIZATIONAL PURCHASING BEHAVIOUR OF BIOPROCESSING TECHNOLOGIES IN A POST-PANDEMIC ENVIRONMENT: A QUALITATIVE RESEARCH APPROACH

Marcelo Fernandez Lahore,¹ Jessica Price²

¹Unit Biotechnologies, Luxembourg Institute of Science and Technology, Maison de l'innovation, 5 Avenue des Hauts-Fourneaux, L-4362 Esch-sur-Alzette, Luxembourg.

²LUNEX, Corporate Health and Wellbeing, 50, avenue du Parc des Sports L-4671 Differdange, Luxembourg

e-mail: marcelo.fernandez-lahore@list.lu

Organizational Purchasing Behavior (OBB) in the biomanufacturing industries can be understood as a process resulting in the adoption of new technologies following a series of complex steps and multiphases. Context-based junctures—such as the COVID-19 pandemic, societal trends, sustainability imperatives, and geopolitical risks—shape how these decisions are made. This perspective article revisits insights from a 2017 exploratory qualitative study of purchasing behaviors by experienced practitioners in downstream processing [DSP] and integrates them with findings from the most recent post-pandemic literature. By presenting these findings side by side, the analysis highlights areas of continuity, disruption, and reversal in purchasing behaviors.^[1] A case is made for the use of qualitative research methods when exploring the ‘human aspects’ (e.g., as gathered *via* qualitative research methods) of OBB, an approach rarely considered by engineering practitioners. Key lessons for those interested in the interpersonal and organizational dynamics of decision making emerge from this line of inquiry.

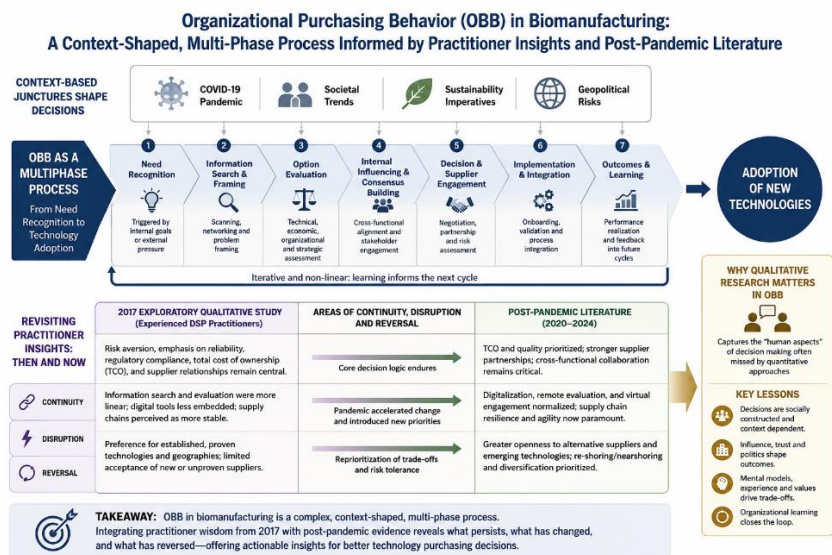


Figure 1: Lessons learned on organizational purchase behaviors decisions in the context of the biomanufacturing industry.

REFERENCES

[1] Price JAM, Fernandez Lahore HM. Organizational purchasing behaviour of bioprocessing technologies in a post-pandemic environment: a qualitative research approach. J Chem Technol Biotechnol. 2026;101(7):1291-9. doi:10.1002/jctb.70089.

BIOPRODUCTS ENGINEERING FOR BIOPROCESSING ENHANCEMENT

O32: SIMULTANEOUS EXTRACTION AND SEPARATION OF ANTHOCYANINS AND CARBOHYDRATES FROM BERRY POMACE IN AQUEOUS TWO-PHASE SYSTEMS

Marija Lemić,¹ Milica Perović,² Marija Kostić,¹ Mirjana Antov²

¹Institute Biosense, University of Novi Sad, Novi Sad, Serbia

²Faculty of Technology Novi Sad, University of Novi Sad, Novi Sad, Serbia

e-mail: marija.lemic@biosense.rs

Utilization of agricultural waste has been increasingly recognized as a sustainable and efficient approach for by-product valorization. Berry pomace is a fruit juice processing waste rich in bioactive compounds such as anthocyanins, phenolic acids, and pectin. However, despite having high antioxidant potential, anti-cancer, and anti-inflammatory properties, it often remains underutilized. Recovering flavonoids and pectin present in berry pomace can considerably increase the value of this residue.

In this study, aqueous two-phase system (ATPS) was used for simultaneous extraction and separation of anthocyanins and carbohydrates (expressed as galacturonic acid reducing equivalents) from chokeberry, cherry, and raspberry pomaces by partitioning them into the opposite phases. Conventional and ultrasound-assisted extractions were performed in ATPS consisting of ethanol and ammonium sulfate. Anthocyanin and carbohydrate contents, as well as ABTS radical scavenging activities, were determined in top and bottom phases of the systems. High partition coefficient demonstrated predominant anthocyanins' distribution in the top phase, while partition coefficient for carbohydrates indicated their predominant partition in the bottom phase in all systems. Generally, the use of ultrasound improved extraction yields of anthocyanins in top and carbohydrates in bottom phases, with maximal values of 82% and 98% in a system with cherry pomace, respectively. Both the bottom and top phases exhibited considerable antioxidant activity, consistent with the presence of pectin and anthocyanins. Moreover, antioxidant potential was increased in the top phases of ultrasound-treated systems, compared with conventional ATPS. The highest antioxidant potential was determined in a top phase of an ultrasound-assisted ATPS with raspberry pomace, equivalent to 1.91 mg Trolox/mL.

Overall, applied ethanol/salt ATPS allowed efficient extraction and *in situ* separation of anthocyanins and carbohydrates by their partitioning into opposite phases, i.e., in a single step, indicating its potential for simultaneous production of high-valuable compounds from berry waste.

ACKNOWLEDGEMENTS

The financial support by the Ministry of Education, Science and Technological Development, Republic of Serbia (Project no: 451-03-33/2026-03/200358, 451-03-34/2026-03/200134 and 451-03-33/2026-03/200134) is greatly acknowledged.

O33: EXTRACTION OF PROTEINS FROM *ACHETA DOMESTICUS* USING SUSTAINABLE STRATEGIES FOR THE DEVELOPMENT OF NEW FOOD PRODUCTS

Daniela B. Silva,¹ Pedro Moleiro,¹ Nair Cunha,² Emanuel V. Capela,¹ Ana P.M. Tavares,¹ Mara G. Freire¹

¹CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

²The Cricket Farming Co, Santarém

e-mail: danielabs@ua.pt

The world population is currently growing and is expected to continue growing in the next years; this fact has raised serious concerns about food scarcity and systems integrity, especially in underdeveloped countries, where food security may be impaired.^[1] Therefore, novel and sustainable protein sources, such as insects, are needed to override this problem. The conventional strategies that have been commonly used for insect protein extraction usually recurs to alkaline, acid and salt solubilization, and often coupled with several pre-treatments.^[2] However, such traditional methods present several drawbacks, since they considerably rely on organic solvents or highly acidic/basic conditions, and on experimental conditions that turn the processes unsustainable for the food industry. More efficient, milder and sustainable processes are, therefore, required.

In this work, the use of more conventional and alternative/sustainable solvents, such as ionic liquids and deep eutectic solvents, was explored for the extraction of the protein fraction from cricket (*Acheta domesticus*) flour. A wide plethora of solvents were screened, and the extraction conditions were studied to obtain a cost-effective and greener process. The optimisation of the process was performed, aiming to maximize the performance, to obtain protein fractions that are suitable for food formulations. The obtained protein fraction functionality and characteristics were also studied, and the impact of the alternative solvents on these parameters was assessed. Thus, a high-performance and more sustainable process was herein developed, that improves the protein extraction procedures often applied to these insects, and that can deeply impact the food industry.

ACKNOWLEDGMENTS

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). This study was also funded by the PRR - Recovery and Resilience Plan and by the NextGenerationEU funds at Universidade de Aveiro, through the scope of the Agenda for Business Innovation “InsectERA” (Project no. 20 with the application C644917393-00000032). Emanuel V. Capela acknowledge FCT for the research contract 2024.08677.CEECIND.

REFERENCES

- [1] OECD, Food and Agriculture Organization of the United Nations. OECD-FAO agricultural outlook 2022-2031 [Internet]. Paris: OECD Publishing; 2022 [cited 2026 Mar 24]. (OECD-FAO Agricultural Outlook). Available from: https://www.oecd.org/en/publications/oecd-fao-agricultural-outlook-2022-2031_f1b0b29c-en.html. doi:10.1787/f1b0b29c-en.
- [2] Rusindo-Rodríguez IM, Gutiérrez-Méndez N, Delgado E, Mateo J, Peralta-Pérez MDR, García-Triana A, et al. Extraction techniques for protein recovery from edible insects: a review. J Food Compos Anal. 2025;147:108070. doi:10.1016/j.jfca.2025.108070.

O34: MARINE-DERIVED HYDROGELS AND THE CIRCULAR BIOECONOMY

Katja Klun,¹ Eylem Atak,^{1,2} Arita Dubņika,³ Maksims Melniņuks,³ Karina Egle,³ Luen Zidar,¹ Ana Rotter¹

¹Marine Biology Station Piran, National Institute of Biology, Fornače 41, 6330 Piran, Slovenia

²Department of Environmental Technologies, Jožef Stefan Institute, Ljubljana, 1001, Slovenia

³Institute of Biomaterials and Bioengineering, Faculty of Natural Sciences and Technology, Riga Technical University, Pulka 3, Riga, LV-1007 Latvia

e-mail: katja.klun@nib.si

Marine-derived hydrogels are emerging as high-value biomaterials with strong potential to support the transition toward a sustainable circular bioeconomy. Their renewable origin, biocompatibility, and biodegradability provide opportunities for their utilization in biomedical, environmental, and various industrial applications. This research was structured in order to answer the following research questions:

1. Is there a growing research and innovation interest for marine-derived hydrogels?
2. What polymers are currently of highest strategic value to invest into?
3. What are the optimal crosslinking approaches?

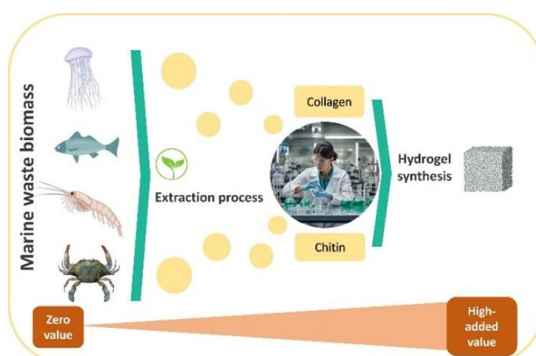


Figure 1: Marine waste valorization concept.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Slovenian Research and Innovation Agency (ARIS) (research core funding “Marine and microbial biotechnology”— grant number P4-0432 and bilateral project with Latvia “Bio-inspired material preparation from marine organisms” (No. BI-LV/25-27-002).

O35: AQUEOUS SOLUBILITY ENHANCEMENT OF PORPHYRINS THROUGH HYDROTROPY AND COMPUTATIONAL CHEMISTRY

Ricardo G. Rocha,^{1,2} Carlos. J. P. Monteiro,² Dinis O. Abranches,¹ M. Amparo F. Faustino ²

¹CICECO - Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Aveiro, Portugal

²LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal

e-mail: ricardogaspar.r23@ua.pt

Antimicrobial resistance is one of the top public health threats, caused mainly by the overuse of antimicrobial drugs. Antimicrobial photodynamic therapy (aPDT) emerges as an alternative solution, where a photosensitizer (PS), irradiated with visible, generates reactive oxygen species (ROS) through electron or energy transfer, causing microbial cell death. Porphyrins PS are extensively explored as multifunctional PS for cancer photodynamic therapy (PDT) and theranostic applications. However, their intrinsic hydrophobicity promotes aggregation and poor aqueous solubility, limiting biodistribution, target interaction and overall performance in physiological environments.^[1]

This work employs a series of computational techniques, namely predictive thermodynamic modelling (i.e., COSMO-RS) and machine learning (i.e., Gaussian processes) to predict and rationalize the aqueous solubility of porphyrins. Two avenues are explored: structural modifications in known porphyrins, such as 5,10,15,20-tetraphenylporphyrin (TPP) and 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (TPPS4), aiming at increasing their inherent solubility in pure water, as well as the addition of hydrotropes. The first strategy involves encoding molecular information using sigma profiles, enabling the transformation of complex molecular structures into numerical descriptors suitable for regression modelling.^[2] The posterior use of COSMO-RS allows a qualitative assessment of their solubility, which can later be tested experimentally. Hydrotropy offers a potential strategy to overcome the solubility barrier through the addition of amphiphilic small molecules that interact with the solute while remaining compatible with water. There are many possible combinations of solute-hydrotrope pairs, providing different results depending on the combination chosen. Despite its practical relevance, the molecular determinants governing solubility enhancement by different hydrotropes remain poorly understood.^[3] This dual approach suggested novel porphyrin structures, which were synthesized and explored in this work. Solubility curves of known porphyrin derivatives were also measured in the presence of selected hydrotropes.

ACKNOWLEDGEMENTS

This work is being developed within the scope of the Gulbenkian “Novos Talentos” program. R.G. Rocha thanks Calouste Gulbenkian Foundation for the “Novos Talentos 2025” grant. This work was developed within the scope of the project UID/50006/2025 (DOI 10.54499/UID/50006/2025) Laboratório Associado para a Química Verde-Tecnologias e Processos Limpos (LAQV-REQUIMTE), CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MECI (PIDDAC).

REFERENCES

- [1] Wainwright M, Maisch T, Nonell S, Plaetzer K, Almeida A, Teges GP, et al. Photoantimicrobials—are we afraid of the light? *Lancet Infect Dis.* 2017;17:e49-e55.
- [2] Abranches DO, Maginn EJ, Colón YJ. Stochastic machine learning via sigma profiles to build a digital chemical space. *Proc Natl Acad Sci U S A.* 2024;121:e2404676121.
- [3] Abranches DO, Benfica J, Soares BP, Leal-Duaso A, Sintra TE, Pires E, et al. Unveiling the mechanism of hydrotropy: evidence for water-mediated aggregation of hydrotropes around the solute. *Chem Commun (Camb).* 2020;56:7143-7146.

O36: UNABILITY OF SWELLING, AEROPHOBIC AND VISCOELASTIC PROPERTIES OF BIO-BASED CHITOSAN/DIALDEHYDE STARCH HYDROGELS FOR ELECTROCHEMICAL APPLICATIONS

Vojtěch Škopek,^{1,2,3} Filipa A. Vicente,^{1,3} Blaž Likozar,¹ Ilja Gasan Osojnik Črnivec^{2,3}

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, 1000 Ljubljana, Slovenia

²Laboratory for Demonstration of H₂ and CO₂ Technologies, National Institute of Chemistry, 1412 Kisovec, Slovenia

³Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, Ljubljana SI-1000, Slovenia

e-mail: vojtech.skopek@ki.si

Recent studies have shown many potential applications of hydrogels in numerous fields for energy systems. Hydrogel-based materials are promising alternatives to alkaline liquid electrolytes,^[1] selective gas-permeation membranes,^[2] and superaerophobic coatings for increasing bubble removal in gas production systems.^[3] This study focused on the preparation and characterization of bio-based hydrogel combining chitosan and modified dialdehyde starch (DAS) as a crosslinking agent. We demonstrate the tuning of structure-property relationship of hydrogels by varying the concentration and molecular weight of chitosan. Initial swelling stability test performed in a strongly alkaline medium (from neutral up to pH 12) showed that the hydrogels maintained their swelling properties during 7 days. Additionally, the aerophobicity, gelation kinetics, and porosity of the hydrogels could be systematically controlled, with several formulations reaching superaerophobicity. Gelation dynamics, including gelation time and storage modulus (mechanical stiffness), were measured by rheometry. Hydrogel coating performance on electrode was evaluated by contact angle measurement and electrochemical analysis. With their tunable structure, porosity, and bio-based origin, these hydrogels are a promising platform for functional coatings.

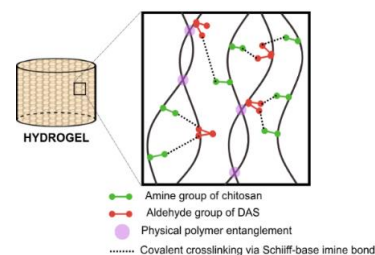


Figure 1: Graphical visualization of hydrogel formation via Schiff-base covalent bonding.

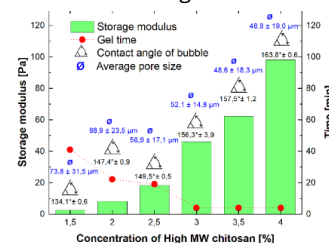


Figure 2: Influence of polymer concentration on gelation time, 30 min storage modulus, porosity and air bubble contact angle.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Slovenian Research Agency under the research core funding No. P2-0152, and the Scientists4Future Slovenia Doctoral Programme (HE MSCA SoE and Janko Jamnik Doctoral Scholarship, National Institute of Chemistry).

REFERENCES

- [1] Ding J, Yang Y, Poisson J, et al. Recent advances in biopolymer-based hydrogel electrolytes for flexible supercapacitors. *ACS Energy Lett.* 2024;9(4):1803-1825.
- [2] Lee S, Lee J, Lee S, et al. Superaerophobic hydrogels for diaphragm modification to suppress gas crossover in alkaline water electrolyzers. *J Mater Chem A*. Published online 2026.
- [3] Bae M, Kang Y, Lee DW, Jeon D, Ryu J. Superaerophobic polyethyleneimine hydrogels for improving electrochemical hydrogen production by promoting bubble detachment. *Adv Energy Mater.* 2022;12(29):2201452.

O37: ATALYTIC CONVERSION OF AMINO ACIDS TO HIGH-VALUE NITROGEN CHEMICALS

Rok Pogorevc,¹ Brigita Hočevár,¹ Miha Grilc,¹ Blaž Likozar¹

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia

e-mail: rok.pogorevc@ki.si

In recent years, the transition away from fossil fuels, which cause many environmental problems, has begun to take shape.^[1] The transition is moving toward the use of biomass such as lignocellulosic biomass (LB) to produce fuels, chemicals and materials.^[1] However, the lack of nitrogen in LB where only carbon, oxygen, and hydrogen elements are present, producing lactams, and nitriles requires additional steps, such as amination.^[2,3] While these processes are efficient, they lack sustainability; therefore, new, more direct, and safer processes need to be developed to obtain these chemicals.^[3] Potentially, one-step conversion using heterogeneous catalysis could be implemented with compounds that already contain fixed nitrogen in their structure (e.g., amino acids) sourced from nitrogen-rich biomass (e.g., algae) to obtain those chemicals.^[3] Among nitrogen-rich biomasses, microalgae have the highest potential, as they contain high percentages of two amino acids, glutamic acid and lysine, which are mainly produced in large quantities via fermentation (about 3 million tons per year).^[3,4] Therefore, microalgae, specifically their amino acids, offer the greatest potential for conversion to high-value nitrogen-containing chemicals.^[3,4] In this study, amino acids such as lysine, glutamic acid, or their derivatives were converted into valuable nitrogen-containing chemicals, including diamines and lactams. The conversion reactions were conducted at low temperatures, typically below 200 °C, under low pressure with a reductive (e.g., H₂) or inert (N₂) gas phase, using an appropriate solvent and various solid catalysts. Final analysis by liquid and gas chromatography showed promising results and trends, warranting further in-depth evaluation. In conclusion, the results on the catalytic conversion of amino acids such as lysine, glutamic acid, or their derivatives from biomass sources such as algae are highly insightful and offer a greener route to producing high-value nitrogen chemicals, which calls for further exploration.

ACKNOWLEDGMENTS

The research was funded by core funding from the Slovenian Research Agency (P2–0152) and the Young Researcher program from the Slovenian Research Agency.

REFERENCES

- [1] Kumar MBA, Gao Y, Shen W, He L. Valorisation of protein waste: an enzymatic approach to make commodity chemicals. *Front Chem Sci Eng.* 2015;9(3):295-307.
- [2] Chen X, Liu Y, Wang J. Lignocellulosic biomass upgrading into valuable nitrogen-containing compounds by heterogeneous catalysts. *Ind Eng Chem Res.* 2020;59(39):17008-17025.
- [3] Chen X, Song S, Li H, Gözaydın G, Yan N. Expanding the boundary of biorefinery: organonitrogen chemicals from biomass. *Acc Chem Res.* 2021;54(7):1711-1722.
- [4] Lammens TM, Franssen MCR, Scott EL, Sanders JPM. Availability of protein-derived amino acids as feedstock for the production of bio-based chemicals. *Biomass Bioenergy.* 2012;44:168-181.

POSTER PRESENTATIONS

DOWNSTREAM PROCESSING

P01: THE ELUTION DILEMMA IN PROTEIN A CHROMATOGRAPHY

Anna Wehlin,¹ [Mikael Andersson Schönn](#)¹

¹Bio-Works Technologies, Sweden

e-mail: Mikael.andersson@bio-works.com

Monoclonal antibodies (mAbs) require highly efficient purification processes to meet stringent regulatory standards for therapeutic applications. Protein A chromatography is the dominant capture step but represents a major contributor to cost of goods and a critical control point for product quality, particularly due to aggregation risks associated with acidic elution. Increased upstream titers and higher-capacity resins further elevate aggregation propensity by generating high local antibody concentrations during elution. In this study, aggregation formation during protein A purification of an aggregation-prone monoclonal antibody (Antibody X) was systematically evaluated with respect to elution pH, buffer system, and resin selection. Elution pH values between 2.7 and 4.0 were investigated, demonstrating that lower pH increased high molecular weight species (HMWS), while higher pH reduced aggregation but could compromise peak shape and process efficiency. Comparative evaluation of citrate and glycine-HCl buffers revealed distinct pH transition behaviours within the column, affecting peak broadening and aggregation levels.^[1] Citrate buffer at pH 3.5 provided the optimal balance between narrow peak shape, high yield, and moderate HMWS formation. Under these optimized conditions, the next-generation protein A resin WorkBeads™ affmAb Edge outperformed two widely used commercial resins, MabSelect PrismA and MabSelect SuRe 70, delivering higher recovery, lower aggregation levels, and improved overall purity. These findings demonstrate that careful optimization of elution conditions combined with high-performance resin selection can significantly enhance productivity, robustness, and cost efficiency in monoclonal antibody purification workflows.

REFERENCES

[1] Hahn R, Berger L, Beck J, Carta G. pH and conductivity transients during elution of IgG from protein A columns. *Biotechnol Prog.* 2025 May-Jun;41(3):e3534. doi:10.1002/btpr.3534.

P02: SUSTAINABLE EXTRACTION OF CURCUMINOIDS USING MICELLAR TWO-PHASE SYSTEMS

Assya Bellaadem,^{1,2} Gregor Lavrič,³ Gasan Osojnik,¹ Kamal K. Mohaideen,¹ Blaž Likozar,¹ Filipa A. Vicente¹

¹Department of Catalysis and Chemical Engineering, National Institute of Chemistry, Ljubljana Slovenia

²Faculty of Chemistry and Chemical Technology, University of Maribor, Maribor Slovenia

³Pulp and Paper Institute, Ljubljana Slovenia

e-mail: assya.bellaadem@ki.si

Downstream processing plays a vital role in production across numerous industries. Nevertheless, such processes are frequently complex, energy-demanding, and require substantial material inputs, which can lead to costs accounting for as much as 80% of total production expenses. As the focus shifts toward more sustainable and economically viable solutions, there is a growing need for innovative downstream strategies that enhance efficiency while preserving productivity. In this work, a greener method for the extraction of curcuminoids from *Curcuma longa* is presented, based on the use of micellar systems. The approach integrates solid–liquid extraction (SLE) and liquid–liquid extraction (LLE) into a unified process, thereby improving overall performance. The SLE medium, consisting of a buffer and a biodegradable surfactant, facilitates the formation of an aqueous micellar two-phase system (AMTPS), which functions as a specific type of LLE. By optimizing both SLE and LLE parameters, curcuminoid recovery above 95% was achieved in the surfactant-rich phase. In addition to extraction efficiency, the results indicate potential applications in smart packaging. The surfactant-rich phase exhibits notable stability, retaining its structure for over 50 hours under UV irradiation, with approximately 20% degradation. Furthermore, these systems were successfully utilized in paper dyeing, where AMTPS mixtures produced stronger coloration with increasing ionic liquid content. This behavior contrasts with conventional systems lacking ionic liquids, where higher concentrations did not lead to enhanced color intensity. Overall, the results highlight the potential of this approach for the development of sustainable materials with practical applications and the potential to replace synthetic pigments.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Slovenian Research Agency under the research core funding No. P2-0152 and scholarship grant No.139012.

P03: SALT EFFECTS ON HYDROGEN BONDING AND VISCOSITY IN AQUEOUS TWO-PHASE SYSTEMS

Lyndsey E. Corrigan,¹ Amber R. Titus,¹ Luisa A Ferreira,¹ Erez Podoly¹

¹Cleveland Diagnostics, 3615 Superior Ave., Cleveland, OH 44114

e-mail: lyndsey.corrigan@clevelanddx.com

Aqueous Two-Phase Systems (ATPS) are used in a variety of industries and applications and are crucial for biomolecule extraction and purification. ATPS are traditionally formed when two nonionic polymers, or a single polymer and salt, are mixed in water above a specific concentration, resulting in the emergence of phase separation and the formation of two immiscible aqueous phases.

The solvent properties and viscoelastic behavior of these systems are intricately linked to the hydrogen bond network within each phase. This research explores the impact of sodium, potassium, and ammonium salt additives on polyethylene glycol (PEG)-8000/Dextran-70, PEG-8000/Dextran-500, and Ficoll-70/Dextran-70 ATPSs. Utilizing Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectroscopy, we analyze changes in hydrogen bond arrangement, while rotational rheometry is used to quantify phase viscosities.

Salt addition significantly alters system composition, which affects phase separation and the distribution of polymers in each phase. Our results reveal that the addition of salts affect both the hydrogen bond network and the resulting phase viscosities, demonstrating a clear relationship between composition, molecular interactions, and macroscopic flow properties. This work contributes to a fundamental understanding of ATPS behavior, enabling better control over phase properties for optimized separation applications.

P04: RECOVERY AND STABILIZATION OF RECOMBINANT NUCLEIC-ACID BIOPHARMACEUTICALS WITH IONIC LIQUIDS

João L. Fernandes,¹ Miguel F. Galrinho,¹ Ana C. S. Marques,¹ Joana L. Costa,¹ Ana P. M. Tavares,¹ Augusto Q. Pedro,¹ Mara G. Freire¹

¹CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro, Aveiro, Portugal

e-mail: jluis.fernandes@ua.pt

Due to fast development times and efficient immune responses, messenger RNA (mRNA) vaccines were vital to combat the COVID-19 pandemic. Despite this, the widespread use of mRNA therapeutics is still challenged by high production costs and low stability of the finished product. The current gold standard for mRNA production is in vitro transcription (IVT) followed by several purification steps, ending in strict cold-chain storage at very low temperatures. This process is complex, expensive and dependent on reagents with limited availability.^[1] Building on these limitations, this work focuses on developing new, integrated, and cost-effective downstream processes for bioproduced mRNA, particularly for mRNA extraction from yeast intracellular compartments. To achieve this, a set of bio-based ionic liquids (IL) was synthesized and characterized, and mRNA produced in a modified yeast strain according to standard protocols. Initial results revealed that ILs comprising carboxylic acids as anions were effective at yeast cell disruption and the consequent release of the target biopharmaceutical. Furthermore, through electrophoresis and RT-qPCR analysis it was possible to conclude that IL-mediated lysis yielded a similar amount of target mRNA to the control method used (Glass beads), but with superior scalability. It was additionally shown that aqueous solutions of the selected ILs are able to maintain the stability of nucleic acids, acting thus as preservation media of these biopharmaceuticals at room temperature. Overall, this work shows the beneficial role of ILs in developing integrated extraction-preservation methods for RNA.

ACKNOWLEDGMENTS

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). This work was additionally funded by the EIC-Pathfinder YSCRIPT project with reference 101047214, supported by the budgets of the Horizon Europe Program. João L. Fernandes, Ana C. S. Marques, Miguel F. Galrinho and Joana L. Costa acknowledge FCT, respectively, for the PhD grants 2024.05338.BDANA, 2023.04854.BD, 2024.18530.PRT and 2025.04562.BDANA. Augusto Q. Pedro and Ana P. M. Tavares acknowledge FCT for the research contracts under the Scientific Stimulus – Individual Call, respectively, CEECIND/2020/02599 (DOI 10.54499/2020.02599.CEECIND/CP1589/CT0023) and CEECIND/2020/01867 (DOI 10.54499/2020.01867.CEECIND/CP1589/CT0013).

REFERENCES

[1] Ali N, Rampazzo RdeCP, Costa ADT, Krieger MA. Current nucleic acid extraction methods and their implications to point-of-care diagnostics. Biomed Res Int. 2017;2017:9306564.

P05: SELECTIVE LEACHING OF LITHIUM IRON PHOSPHATE BATTERY WASTE USING *DEEP* EUTECTIC SOLVENTS FOR SUSTAINABLE BATTERY RECYCLING

Marko Gabrovšek,^{1,2} Filipa A. Vicente,¹ Martin Šala,³ Blaž Likozar^{1,2}

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

²University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor

³Department of Analytical Chemistry, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

e-mail: marko.gabrovsek@ki.si

The demand for critical raw materials required for lithium-ion battery production is continuously increasing due to the rapid expansion of battery manufacturing. Currently, lithium iron phosphate (LFP) batteries have the largest market share, particularly in electric vehicles and stationary energy storage sectors, owing to their safety, long cycle life, and relatively low cost. Consequently, the generation of LFP battery waste is also rapidly increasing. The valorization of such waste as a secondary raw material requires efficient, scalable, and sustainable recycling technologies. Recycling LFP remains challenging because of its chemical stability and the low value of its raw components. Conventional hydrometallurgical processes rely on strong inorganic acids and reducing agents for leaching metals out of battery waste, resulting in high consumption of chemicals, limited selectivity, and significant secondary waste generation. Alternatively, *deep* eutectic solvents (DES) have emerged as a promising solution due to their greener character, tuneable physicochemical properties, such as adjustable acidity, complexation behaviour, and selective metal dissolution. Therefore, this work investigates the use of *DES* as an alternative leaching medium for hydrometallurgical treatment of LFP black mass. Experimental results demonstrate that selective lithium extraction can be achieved under mild conditions and that oxidative treatment significantly improves the dissolution of iron and phosphate, enabling complete leaching of the LFP active material. Thus, a cascade leaching strategy is proposed to allow sequential separation and recovery of valuable components from the LFP black mass. The behaviour of graphite was also evaluated, with the objective of preserving its structure for subsequent reuse. Overall, the proposed approach contributes to the development of more sustainable recycling pathways for LFP batteries by reducing dependence on aggressive mineral acids, hence lowering chemical consumption through solvent reuse, and improving selectivity during leaching. These findings support ongoing efforts toward circular battery value chains and advanced recycling strategies for next-generation battery technologies.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Slovenian Research Agency under the research core funding No. P2-0152, P1-0034 and the European Union's Horizon Europe research and innovation programme under Grant Agreement No. 101192383 (CIRCUBATT).

P06: NMR INVESTIGATION OF WATER-INDUCED STRUCTURAL TRANSITIONS IN DEEP EUTECTIC SOLVENT FOR STARCH DISSOLUTION

Marko Gabrovšek,^{1,2} Monika Vidmar,¹ Mónica A. R. Martins,³ Robin D. Rogers,⁴ Blaž Likozar,^{1,2} Filipa A. Vicente¹

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

²University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor

³Centro de Investigação de Montanha (CIMO) and Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal

⁴525 Solutions, Inc., P.O. Box 2206, Tuscaloosa, AL 35403, USA

e-mail: marko.gabrovsek@ki.si

Starch is a renewable biopolymer widely used in the food, pharmaceutical and packaging industries. However, its semi crystalline structure and extensive intra- and intermolecular hydrogen bonding hinder its dissolution in conventional solvents, limiting processing efficiency. In this context, the development of sustainable solvent systems is essential to advance biomass valorisation and reduce dependence on fossil-derived processing technologies. *Deep* eutectic solvents (*DES*) have emerged as a promising alternative for the biomass processing due to their tunable physicochemical properties. By selecting combinations of hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs), *DES* can be designed to enhance interactions with starch and improve its dissolution. Despite increasing interest, the role of water in *DES* systems remains insufficiently understood. Water significantly modifies the hydrogen bond network characteristic of *DES*, altering the solvent–solute interactions. In particular, the transition between water-in-*DES* and *DES*-in-water structures is expected to influence solvent functionality and starch dissolution behaviour. Therefore, this work investigates the relationship between *DES* structure, water content, and starch solubilisation. A range of *DES* composed of generally recognised as safe (GRAS) components were prepared and evaluated. Nuclear magnetic resonance spectroscopy (NMR) was used to monitor the ¹H water chemical shift as a function of water content, enabling identification of the transition from a *DES*-dominated structure to an aqueous solution regime. The structural transition was correlated with starch dissolution performance to identify the water composition range at which solvent properties change from *DES*-like to aqueous-like. To support solvent selection, Hansen solubility parameters were used to assess solvent–polymer compatibility and guide the design of suitable HBA:HBD combinations. Overall, these findings contribute to a deeper understanding of *DES*–water systems, correlating the ¹H NMR water chemical shift with the structural breakdown of the hydrogen-bond network in *DES* upon dilution, and provide a basis for tailoring solvent composition for efficient starch processing and broader biomass applications.

ACKNOWLEDGMENTS

This research was funded by the Slovenian Research Agency under research core funding P2-0152 and research projects NC-0024 and NC-24002. This work was also supported by the Foundation for Science and Technology through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020).

P07: METAL–SUPPORT EFFECTS IN RE CATALYSTS FOR BIO-BASED ADIPIC ACID DERIVATIVES

Brigita Hočevár,¹ Maja Gabrič,¹ Miha Grilc,¹ Blaž Likozar¹

¹National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

e-mail: brigita.hocevar@ki.si

The transition toward sustainable polymer production requires the development of efficient catalytic routes for converting lignocellulosic biomass into value-added monomers. In this work, rhenium-based catalysts supported on various materials were investigated for the selective deoxydehydration and hydrogenation of biomass-derived mucic acid to renewable adipic acid derivatives. The influence of catalyst support and reductive pre-treatment conditions on catalytic performance was systematically evaluated.

Among the tested materials, carbon-supported rhenium catalysts exhibited the highest activity, achieving up to 88% yield of dehydroxylated products under mild reaction conditions (120 °C, methanol as hydrogen donor). In contrast, oxide-supported catalysts showed significantly lower activity, highlighting the crucial role of metal–support interactions. Characterization results revealed that the superior performance of Re/C originates from the coexistence of metallic and high-valent rhenium species, enabling a bifunctional catalytic mechanism involving deoxydehydration and subsequent hydrogenation steps.^[1,2]

These findings provide new insights into catalyst design for biomass valorization and demonstrate a promising pathway toward sustainable production of bio-based adipic acid derivatives and polymer building blocks.

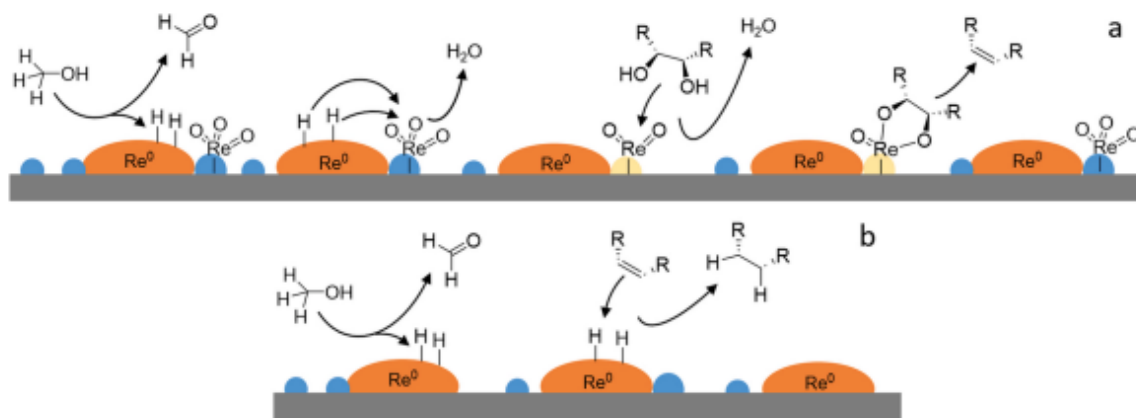


Figure 1: Bifunctional mechanism over Re/C catalyst showing interplay of metallic and high-valent Re sites for deoxydehydration, followed by hydrogenation on metallic Re sites.^[1]

REFERENCES

- [1] Harth FM, Gabrič M, Teržan J, Hočevár B, Gyergyek S, Likozar B, et al. Tailoring selective de-hydroxylation/hydrogenation reactions of bio-based aldaric acids towards adipic acid derivatives by Re catalyst metal-support interactions. *Catal Today*. 2024;441:114879. doi:10.1016/j.cattod.2024.114879.
- [2] Hočevár B, Prašnikar A, Huš M, Grilc M, Likozar B. H₂-free Re-based catalytic dehydroxylation of aldaric acid to muconic and adipic acid esters. *Angew Chem Int Ed*. 2021;60(3):1244-1253. doi:10.1002/anie.202010035.

P08: PRECIPITATION AND ATPS OF VIRUS LIKE PARTICLE

Giulia Polazzo,¹ Malte Schwarzer,¹ Jürgen Hubbuch¹

¹Institute of Engineering in Life Sciences, Section IV: Biomolecular Separation Engineering, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

e-mail: juergen.hubbuch@kit.edu

Virus-like particles (VLPs), including those derived from adeno-associated virus (AAV), are increasingly used in biotechnology and gene therapy; however, their purification remains challenging due to the co-precipitation of host-cell impurities during standard capture steps such as polyethylene glycol (PEG) precipitation. The lack of rapid, high-throughput analytical methods capable of monitoring VLP behaviour in complex mixtures like cell lysate also limits the systematic optimization of precipitation and phase partitioning conditions. In this work, AAV5-VLPs produced recombinantly in *Escherichia coli* via VP3-only expression were used as a model system. UV-derived light scattering in the 310–340 nm region was employed as a simple proxy to track particulate content and VLP behaviour during precipitation and aqueous two-phase system (ATPS) screening, and was validated against ELISA measurements, showing strong qualitative agreement. Scattering-corrected UV values at 280 nm and 260 nm were used for host-cell impurity evaluation (proteins and DNA). Using this approach, low-molecular-weight PEG (400) showed improved selectivity compared to higher molecular weights (600–1000), with higher protein retention and lower scattering: +7% impurity removal, -40% particle loss in the best ATPS with PEG400, compared with the same with PEG1000. Moreover, ATPS outperforms precipitation by +8% impurity removal, with same particle loss of ~10%. Overall, UV scattering provides a fast and accessible method to monitor VLP behaviour, enabling high-throughput screening of separation conditions and highlighting the potential of low-molecular-weight PEG for selective VLP capture.

P09: VALORIZATION OF ONION PEEL WASTE: EUTECTIC SOLVENT EXTRACTION OF QUERCETIN-ENRICHED EXTRACTS FOR COTTON DYEING

Helena F. Ribeiro,¹ Alexandre M. S. Jorge,¹ Ana M. A. Dias,¹ Jorge F. B. Pereira¹

¹ University of Coimbra, CERES, FCTUC, Department of Chemical Engineering, Rua Sílvia Lima, Pólo II - Pinhal de Marrocos, 3030-760 Coimbra, Portugal

e-mail: alexandrej@eq.uc.pt

The textile sector consumes significant resources and contributes substantially to environmental pollution, driving the need for sustainable processes based on renewable feedstocks and green solvents.^[1] Phenolic compounds obtained from agro-industrial residues represent valuable bioactive molecules; however, their efficient and selective extraction remains a key challenge in biopartitioning and downstream processing.

In this study, eutectic solvents (ES) based on choline chloride (ChCl) combined with urea (U), glycerol (Gly), and acetic acid (AA) were investigated as alternative media for the extraction and partitioning of phenolic compounds, particularly quercetin derivatives, from onion peel waste[2]. The performance of ES was benchmarked against conventional solvents, such as water, ethanol, and ethanol–water mixtures, with emphasis on extraction efficiency and selectivity.

ES systems enabled up to 4.8-fold increase in phenolic recovery, highlighting their strong solubilization capacity. However, differences in solvent composition significantly affected the availability and subsequent interactions of extracted compounds with cellulose substrates. Aqueous systems, despite lower extraction yields, promoted enhanced dye uptake (up to 15-fold higher), suggesting that water plays a critical role in modulating solute-fiber partitioning and intermolecular interactions. To further investigate these effects, the ChCl/U (1:2) system containing 30% (w/w) water was employed as a model system to evaluate the influence of water content on phenolic partitioning behavior. Additionally, the use of mordants (chitosan, tannic acid, and alum) was explored to assess their role in modifying solute affinity and fixation.

Overall, the results demonstrate that ES composition and water content govern not only extraction efficiency but also solute partitioning and interaction mechanisms, emphasizing the importance of solvent design in integrated extraction-application processes. These findings highlight the potential of tailored eutectic systems as versatile platforms for sustainable biopartitioning and valorization of agro-industrial residues.

ACKNOWLEDGMENTS

CERES is supported by the Fundação para a Ciência e a Tecnologia (FCT) through the projects UID/00102/2025 and PRR – Recovery and Resilience Program of the Portuguese Republic (UID/PRR/00102/2025). The authors acknowledge the FCT for funding the project HIPERBIOTEX (COMPETE2030-FEDER-00708800) and Fundação Calouste Gulbenkian for funding the project DyeLoop – Circular Technologies for Textile Dyeing.

REFERENCES

- [1] Jorge MS, Athira KK, Alves MB, Gardas RL, Pereira JFB. Textile dyes effluents: a current scenario and the use of aqueous biphasic systems for the recovery of dyes. *J Water Process Eng.* 2023;55:104125.
- [2] Kumar M, et al. Onion (*Allium cepa* L.) peels: a review on bioactive compounds and biomedical activities. *Biomed Pharmacother.* 2022;146:112498.

P10: NATURAL DEEP EUTECTIC SOLVENTS FOR STEROID HORMONE EXTRACTION FROM WATER: CHARACTERIZATION AND BIO-UPCYCLING POTENTIAL

Nazmus Samah,¹ Robert Karcz,¹ Ewelina Cichoń,¹ Joanna Kryściak-Czerwenka,¹ Maciej Guzik¹

¹Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek 8,
30-239 Krakow, Poland

e-mail: robert.karcz@ikifp.edu.pl

Steroid hormones present in aquatic ecosystems have become a significant environmental and public health concern. The direct cause is their endocrine-disrupting effect, even at concentrations as low as nanograms per liter of water. Conventional wastewater treatment methods are unable to completely remove these persistent micropollutants, making it necessary to develop new, efficient, selective and environmentally friendly solutions. To this end, we investigated the suitability of natural deep eutectic solvents (NADES). Mixtures of this type are biodegradable, non-volatile and considered to be green solvents.^[1] We tested the resulting NADES mixtures as media for the extraction of steroid hormones from water. We prepared NADES compositions using various hydrogen bond acceptors (menthol, thymol, carvone, vanillin, coumarin) and hydrogen bond donors (C5–C9 carboxylic acids), using constant 1:1 HBA to HBD ratio. Stable compositions were characterized in terms of polarity, density and interactions between functional groups of components. Then prepared NADES were then used to extract epiandrosterone from model aqueous solution. The analyses validated the proposed method for model contaminant recovery. The results obtained indicate that NADES represent a promising and environmentally friendly alternative to traditional organic solvents for waste water treatment.

The bio-upcycling potential of the selected NADES mixtures was further investigated by examining their performance as a carbon source in bacterial fermentation screening experiment. The growth of the *Pseudomonas putida* CA3 strain and polyhydroxyalkanoate (PHA) production were assessed. The starter culture was maintained for 15 h at 30 °C in mineral salt medium (MSM) supplemented with sodium octanoate. Subsequently, the inoculum was transferred to a nitrogen-limited MSM medium containing selected NADES compositions at a concentration corresponding to 1.96 g C L⁻¹. The cultures were carried out in triplicate in 250 mL flasks (working volume 50 mL) for 48 h at 30 °C. After the cultivation period, the biomass was centrifuged, frozen and freeze-dried to determine the cell dry weight (CDW) and intracellular PHA content.

ACKNOWLEDGMENTS

Work was funded by National Science Centre (NCN) within the project Opus Lap no. 2024/55/I/ST8/02722.

REFERENCES

[1] Dai Y, van Spronsen J, Witkamp GJ, Verpoorte R, Choi YH. Natural deep eutectic solvents as new potential media for green technology. Anal Chim Acta. 2013;766:61-68. doi:10.1016/j.aca.2012.12.019.

P11: TUNABLE BIO-BASED SOLVENT MIXTURES FOR ENHANCED ASTAXANTHIN SEPARATION FROM MICROBIAL BIOMASS

Cassamo U. Mussagy,^{1,3} Cristóbal N. Alvarado-Holtheuer,¹ Fabiane O. Farias,² Adalberto Pessoa Jr³

¹Laboratorio de Desarrollo de Bioprocesos Sostenibles (Labisost), Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, Quillota, Chile

²Graduate Program of Food Engineering, Dept. Chem. Engineering, Federal University of Paraná, Curitiba, Brazil

³Department of Pharmaceutical-Biochemical Technology, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

e-mail: pessoajr@usp.br

The separation of astaxanthin from microbial biomass is a key step in its valorization as a high-value bioactive compound, yet conventional processes often rely on volatile organic solvents with unfavorable safety and environmental profiles.^[1] This study explores tunable bio-based solvent mixtures as alternative systems for astaxanthin separation from *Paracoccus carotinifaciens*, focusing on separation performance and process sustainability. Bio-derived and low-hazard solvents were evaluated individually and in binary mixtures. Solvent-solute interactions were assessed using molecular affinity predictions and supported by experimental assays under mild operating conditions. Bio-based solvents with predominantly nonpolar character exhibited superior astaxanthin separation compared with more polar alternatives. Binary mixtures further enhanced performance, indicating synergistic effects arising from controlled polarity adjustment.^[2] Several mixtures outperformed their pure components, demonstrating that solvent tunability can optimize extraction behavior. Process feasibility was assessed using a sustainability-oriented metric integrating separation efficiency, safety, cost, and operational simplicity. The most effective mixtures also showed favorable sustainability profiles, supporting their potential application in scalable downstream processing. In addition, microbial astaxanthin stability in a representative bio-solvent system was evaluated under thermal stress to guide extract handling and processing. Overall, rational tuning of bio-based solvent mixtures provides an effective strategy for improving astaxanthin separation while advancing greener and safer downstream processing.

ACKNOWLEDGMENTS

This research was supported by grants from the National Agency for Research and Development (ANID, Chile) under projects ANID/FONDECYT/1250170 and ANID/FOVI/250065, and by the São Paulo Research Foundation (FAPESP, Brazil) through the Programa FAPESP para o Atlântico Sul e Antártica (PROASA), Projects 25/18237-4, 25/24314-1, and 2023/18416-0.

REFERENCES

- [1] Mussagy CU, Gini ALR, Scarim CB, Duarte JL, Chorilli M, Militão GFG, et al. Comparative analysis of bacterial and microalgal natural astaxanthin: Part II—biocompatibility, bioaccessibility, encapsulation, mutagenicity, and aquatic toxicity. *Algal Res.* 2025;84:104343.
- [2] Mussagy CU, Alvarado-Holtheuer CN, Pessoa Jr A, Farias FO. Emerging biosolvents for microbial astaxanthin recovery: molecular insights into green solutions for the 21st century. *Sep Purif Technol.* 2026;383:136174.

P12: COMPUTATIONAL AND EXPERIMENTAL SCREENING OF SOLVENTS FOR HYDROXYTYROSOL EXTRACTION FROM OLIVE-DERIVED EXTRACTS

Érica Mendes,¹ [Joaquim F. Pinto](#),¹ Alexandre M. S. Jorge,¹ Paulo Vilaça,² Jorge F. B. Pereira¹

¹University of Coimbra, CERES, Department of Chemical Engineering, 3030-790 Coimbra, Portugal

²Silicolife, S.A. Rua do Parque Poente, Lote 37, N° 39, Sequeira 4705-002 Braga, Portugal

e-mail: joaquimfp99@gmail.com

Hydroxytyrosol (HT) is a phenolic alcohol predominantly found in olive-derived extracts and is well known for its antioxidant, anti-inflammatory, and neuroprotective properties. These bioactivities have been associated with the prevention of diseases such as diabetes, neurodegenerative disorders, and cancer, thereby increasing its commercial value and industrial demand. However, the low natural abundance of HT and the complexity of olive-derived matrices hinder its large-scale production and recovery. In this study, computational pre-screening was combined with experimental validation to evaluate the efficiency of liquid–liquid extraction (LLE) for the recovery of HT from olive extract. Initially, the COSMO-RS thermodynamic model was employed due to its ability to predict solvent–solute interactions without requiring experimental input, enabling rapid and cost-effective solvent screening prior to laboratory testing. Experimental screening was subsequently carried out using 32 solvents with an aqueous/organic phase ratio of 1:1 (v/v). Ultra-high-performance liquid chromatography (UHPLC) was used to evaluate extraction performance through the determination of the partition coefficient (K) and extraction efficiency (EE). Solvent–solute affinity was predicted by COSMO-RS through interaction energy values ($\ln \gamma$), where more negative values indicate higher affinity for HT, ranging from -1.019 to 12.311 . The best-performing solvents showed K values between 1.3 to 3.75 and extraction efficiencies ranging from 55% and 86%, in agreement with computational predictions. In contrast, several solvents showed very low K values and negligible extraction efficiencies, indicating poor extraction performance. Overall, the strong agreement between computational predictions and experimental results demonstrates that COSMO-RS is an effective tool for solvent screening and selection in LLE processes. The integration of thermodynamic modelling with experimental validation reduces time and resource consumption and shows significant potential to accelerate the development of sustainable extraction and purification processes for high-value phenolic compounds at an industrial scale.

ACKNOWLEDGEMENTS

The authors acknowledge Silicolife, S.A, for providing the extracts and funding this project. CERES acknowledges financial support from FCT through the projects UID/00102/2025 (<https://doi.org/10.54499/UID/00102/2025>), UID/PRR/00102/2025 under the Recovery and Resilience Programme (PRR) of the Portuguese Republic (<https://doi.org/10.54499/UID/PRR/00102/2025>) and Equipar+2 (<https://doi.org/10.54499/UID/PRR2/00102/2025>).

P13: LIGNIN-BASED MEMBRANES FOR SUSTAINABLE SEPARATION PROCESSES

Filipe H. B. Sosa,¹ Noemie Dijoux,² João A. P. Coutinho,¹ Pedro Carvalho¹

¹CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal

²ENSIACET – Toulouse Graduate School of Chemical, Materials and Industrial Engineering, Materials Department, Toulouse INP, 31030 Toulouse, France

e-mail: filipesosa@ua.pt

The increasing demand for sustainable separation technologies has intensified the search for bio-based materials capable of reducing the environmental impact of conventional membrane processes. In this context, the development of renewable and low-footprint alternatives to petroleum-derived polymers is of particular relevance. In this work, lignin, an abundant and underutilized byproduct of the pulp and paper industry, is explored as a functional building block for membrane fabrication.^[1] Owing to its rich chemical functionality and film-forming capability, lignin-based formulations were prepared by tuning crosslinker type, lignin-to-solvent ratio, and processing conditions.^[2] Given the limited mechanical stability of standalone lignin films, the formulations were implemented as thin selective layers deposited onto polypropylene supports, yielding thin-film composite membranes. The resulting materials were characterized by FTIR, SEM, TGA, and contact angle measurements to assess their chemical structure, morphology, thermal stability, and surface properties. Separation performance was evaluated through filtration experiments using water, salts, and polyethylene glycol (PEG) to estimate molecular weight cut-off. Additionally, dye filtration experiments with Tartrazine (Acid Yellow 23) were conducted to investigate solute retention and enrichment capabilities. Starting from an initial concentration of 50 mg·L⁻¹, four consecutive filtration cycles using a lignin–erythritol selective layer led to an increase up to 68.3 mg·L⁻¹, corresponding to a 36.6% enrichment, while the bare support showed negligible rejection. Projections suggest that higher concentrations could be achieved with additional cycles. These findings demonstrate the potential of lignin-based thin-film composite membranes as sustainable alternatives for selective separations and solute recovery processes.

ACKNOWLEDGEMENTS

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020, UIDP/ 50011/2020 & LA/P/0006/2020, financed by national funds through the FCT/MEC (PIDDAC). Filipe H. B. Sosa acknowledges FCT for the researcher contract CEECIND/07209/2022 under the Scientific Employment Stimulus - Individual Call 2022. ACKNOWLEDGMENTS.

REFERENCES

- [1] Boarino A, Charmillot J, Bernardes Figueirêdo M, Le TTH, Carrara N, Klok HA. Ductile, high-lignin-content thermoset films and coatings. *ACS Sustain Chem Eng.* 2023;11(46):16442-16452. doi:10.1021/acssuschemeng.3c03030.
- [2] Shamaei L. Studying the application of lignin for the fabrication of high-performance membranes [PhD thesis]. Edmonton (AB): University of Alberta; 2021.

P14: REMOVAL OF dsRNA FROM *IN VITRO* TRANSCRIBED mRNA USING MULTI-STAGE AQUEOUS BIPHASIC SYSTEMS

Filipa Domingues,¹ [Maria I. Sousa](#),¹ Augusto Q. Pedro,¹ Mara G. Freire¹

¹CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, Portugal

e-mail: mineissousa@ua.pt

Messenger RNA (mRNA) vaccines have demonstrated promising clinical outcomes in the prophylaxis of infections, capturing the interest of the scientific community and the healthcare industry.^[1,2] However, the conventional mRNA production process, which involves *in vitro* transcription (IVT), results in generation of the byproduct double stranded RNA (dsRNA).^[3] This contaminant reduces mRNA translation efficiency making its efficient removal imperative. Currently, mRNA purification, including dsRNA removal, relies on multi-step procedures that involve chromatographic operations, enzymatic digestion, and filtration. Therefore, reducing process complexity and the number of unit operations requires developing an effective clarification step that removes dsRNA prior to chromatography.^[3] In this context, aqueous biphasic systems (ABS), typically formed by mixing two water-soluble components at concentrations above specific thresholds,^[4] represent a promising alternative.

To enhance the separation performance, this work employs a multi-stage ABS strategy using polymer/polysaccharide compositions towards more economical and scalable processes. In this method, fresh top phases are sequentially introduced at each stage, promoting stepwise partitioning and progressive enrichment of the target molecule and dsRNA removal, thereby improving selectivity and purity.

Initially, mRNA production by IVT was performed. Next, phase diagrams were determined for selected ABS. The separation performance was evaluated using agarose gel electrophoresis and dot-blot analysis. The results demonstrated that dsRNA preferentially partitions to the bottom phase of the system, whilst the target mRNA is enriched in the top phase, enabling its effective separation.

In conclusion, this work demonstrates the potential of a multi-stage ABS-based approach as an efficient, scalable, and cost-effective strategy for dsRNA removal, contributing to the current mRNA purification process.

ACKNOWLEDGMENTS

This work was developed within the scope of the project CICECO – Aveiro Institute of Materials, UID/50011/2025 & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC). Augusto Q. Pedro acknowledges FCT for the research contract CEEC-IND/02599/2020 (DOI 10.54499/2020.02599.CEECIND/CP1589/CT0023) under the Scientific Stimulus–Individual Call. Maria I. Sousa acknowledges FCT for the PhD grant 2022.11476.BD.

REFERENCES

- [1] Gote V, Bolla PK, Kommineni N, Butreddy A, Nukala PK, Palakurthi SS, et al. A comprehensive review of mRNA vaccines. *Int J Mol Sci.* 2023 Jan 31;24(3):2700. doi:10.3390/ijms24032700.
- [2] Lee K, Kim M, Seo Y, Lee H. Development of mRNA vaccines and their prophylactic and therapeutic applications. *Nano Res.* 2018 Oct;11(10):5173-5192. doi:10.1007/s12274-018-2095-8.
- [3] Nowak CJA, Liu S, Falconer RJ, Gerstweiler L. Process and analytical strategies for the safe production of mRNA vaccines and therapeutics. *Mol Biol Rep.* 2026 Dec;53(1):306. doi:10.1007/s11033-026-11455-0.
- [4] Iqbal M, Tao Y, Xie S, Zhu Y, Chen D, Wang X, et al. Aqueous two-phase system (ATPS): an overview and advances in its applications. *Biol Proced Online.* 2016;18(1):1-18. doi:10.1186/s12575-016-0048-8.

P15: SALT EFFECTS ON BOUND AND APO ALPHA-1-ACID GLYCOPROTEIN PARTITIONING IN AQUEOUS TWO-PHASE SYSTEMS

Amber R. Titus,¹ Luisa A Ferreira,¹ Tharangini Ranjith Somi,¹ Matthew R Wolf,¹ Jennifer J Arnold,¹ Tejasvi Dudiki,¹ Erez Podoly¹

¹Cleveland Diagnostics, 3615 Superior Ave., Cleveland, OH 44114

e-mail: amber.titus@clevelanddx.com

Alpha-1-acid glycoprotein (AGP) is a predominant plasma protein which transports small hydrophobic molecules such as steroids, retinoids, and lipids. AGP exhibits high-affinity binding to ligands such as chlorpromazine (CPZ), influencing its drug distribution and efficacy. CPZ binds deep within the central beta-barrel pocket of AGP, inducing a minor but measurable structural change (RMSD ~ 0.81 Å) in the flexible loops and specific pocket residues. Here, we applied polymer-polymer Aqueous Two-Phase Systems (ATPS) to study the partitioning behavior of bound and apo AGP in the presence of nine salt additives, consisting of three cations (Na^+ , K^+ , NH_4^+) and three anions (H_2PO_4^- , Cl^- , SCN^-). Salt additives are known to differentially affect the electrostatic properties of the phases in polymer-polymer ATPS, depending on the polymers used. The partition coefficient of AGP was highly sensitive to the chemical nature of the phase-forming polymers (polyethylene glycol (PEG)-8000/Dextran-70, PEG-8000/Dextran-500, and Ficoll-70/Dextran-70). For PEG-Dextran-based ATPS, we observed clear anion trends that follow the classical Hofmeister series of salting-in and salting-out of proteins. For the Ficoll-Dextran based systems, we observed unique AGP partitioning trends that appear to be both cation- and anion-dependent. Overall, this study demonstrates that ATPS are a sensitive and reliable method for studying protein-drug interactions and subtle conformational changes, even in physiologically relevant complex matrices such as human serum. This approach offers a promising platform for screening and characterization of drug binding relevant to pharmacokinetics and disease-relevant plasma environments.

P16: ALKALINE AND AUTOCLAVE-ASSISTED PROTEIN EXTRACTION FROM OLIVE POMACE: RECOVERY EFFICIENCY

Gokcem Tonyali Karsli,¹ Zeynep Canan Baş,¹ Orçun Ataseven,¹ Frederick Lia,² Karen Attard,^{2,3} Mecit Halil Oztıp¹

¹Department of Food Engineering, Middle East Technical University, Ankara 06800, Turkey

²BKEA Ltd., Rabat, RBT 3140, Malta

³Institute of Applied Science, Malta College of Arts, Science and Technology, PLA 6032 Paola, Malta

email: gokcem@metu.edu.tr

Olive pomace is the major semi-solid waste generated during olive oil production and remains an underutilized source of protein for potential food and nutraceutical applications. In this study, twelve extraction routes were compared using deseeded and defatted olive pomace, including conventional alkaline extraction at pH 11 for different extraction times (1h, 2h, 3h, 4h and overnight) concentrated NaOH treatments, and autoclave-assisted alkaline extraction. The deseeded and defatted pomace fractions contained 7.22% and 7.17% crude protein, respectively.^[1] Protein recovery was evaluated using Kjeldahl analysis for crude protein and the Bradford assay for soluble protein.

Among the tested conditions, autoclave-assisted alkaline extraction at pH 11 with overnight treatment resulted in the highest recovery, yielding 6.97% crude protein and 27.4 ± 2.5 mg soluble protein per gram of extract. Addition of NaOH immediately before autoclaving gave comparable results, whereas autoclave pretreatment alone was less effective. In contrast, highly concentrated alkali treatment using 10 M NaOH at a 1:100 g/ml solid-to-solvent ratio produced the lowest recovery, with 0.87% crude protein and soluble protein below the limit of quantification. Bradford values corresponded to only 25–45% of Kjeldahl-derived protein, further indicating that a substantial fraction of extracted nitrogen was not present as soluble Bradford-reactive protein.

FTIR analysis was used to compare the overall chemical profiles of the extracts. Differences were observed particularly in the protein-associated amide regions. Amide A and B were mainly related to N–H stretching, Amide I ($1600\text{--}1700\text{ cm}^{-1}$) to C=O stretching of peptide bonds, Amide II ($1500\text{--}1600\text{ cm}^{-1}$) to N–H bending and C–N stretching, and Amide III ($1200\text{--}1400\text{ cm}^{-1}$) to C–N stretching and N–H bending. Bands in the fingerprint region, especially around $1000\text{--}1200\text{ cm}^{-1}$, may also reflect C–O and C–O–C vibrations from co-extracted carbohydrate-related components. Since the extracts were not purified protein fractions, contributions from non-protein constituents such as lignin should also be considered. Overall, autoclave-assisted alkaline extraction at pH 11 was the most promising condition for obtaining protein-containing extracts for subsequent enzymatic hydrolysis and peptide production.

ACKNOWLEDGMENTS

This research was funded by Scientific and Technological Research Council of Turkey (TUBITAK) under the Grant Number 124N564.

REFERENCES

[1] López-Huertas E, Rubí-Villegas J, Sánchez-Moreno L, Nieto R. Olive Pomace Extract Contains Low Molecular Weight Peptides and Possesses ACE Inhibitory Activity. *International Journal of Molecular Sciences*. 2024;25(7). <https://doi.org/10.3390/ijms25073962>.

P17: DEVELOPMENT OF A CURCUMIN-BASED DYEING PLATFORM USING AQUEOUS MICELLAR SYSTEMS

Assya Bellaadem,^{1,2} Alexandre M. S. Jorge,³ Blaž Likozar,¹ Jorge F. B. Pereira,³ Filipa A. Vicente¹

¹Department of catalysis and chemical engineering, National Institute of Chemistry, Ljubljana Slovenia

²Faculty of Chemistry and Chemical Technology, University of Maribor, Maribor Slovenia

³Department of Chemical Engineering, Faculty of Sciences and Technology, University of Coimbra, Coimbra Portugal

e-mail: filipa.andre.vicente@ki.si

Concerns associated with synthetic colors, particularly their environmental and health impacts, have increased user awareness, leading to a growing demand for natural colorants. Synthetic colors' high stability, wide color range, ease of application, compatibility with various fibers, reproducibility, consistency and intense coloration are key reasons for their widespread use. However, during industrial dyeing processes, approximately 10–15% of these dyes are released into the ecosystem. These are known to be toxic, mutagenic, carcinogenic, and bioaccumulative, while their stability makes them difficult to degrade using conventional wastewater treatment methods. The main challenges associated with their removal include high operational costs, complex handling, low efficiency, and the risk of secondary pollution. Therefore, the transition from synthetic to natural dyes has become an increasingly popular approach in the textile industry. Building on this paradigm shift, this study explores the use of extracts from *Curcuma longa* as a sustainable dye for textile applications employing aqueous two-phase systems with ionic liquids as co-surfactants to facilitate curcumin-based dyeing processes. The surfactant-rich phase was directly employed as a dye bath, providing effective coloration in both cases. The dyeing process was optimized for four different types of fibers, resulting in consistent color intensity across all materials. Furthermore, the same dye bath was successfully reused over multiple dyeing cycles without replenishment, maintaining comparable color intensity throughout successive uses. This performance was sustained until depletion of the dye bath volume. In addition, washing conditions were simulated, demonstrating that the achieved coloration remained stable without significant loss of intensity. UV stability was also evaluated, confirming that the system retains its performance under prolonged exposure. Overall, these findings highlight the robustness, reusability, and stability of these systems, supporting their potential as sustainable alternatives for dyeing applications.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Slovenian Research Agency under the research core funding No. P2-0152 and scholarship grant No.139012. CERES acknowledges financial support from FCT through the projects UID/00102/2025 (<https://doi.org/10.54499/UID/00102/2025>), UID/PRR/00102/2025 under the Recovery and Resilience Program (PRR) of the Portuguese Republic (<https://doi.org/10.54499/UID/PRR/00102/2025>) and Equipar+2 (<https://doi.org/10.54499/UID/PRR2/00102/2025>). The authors acknowledge FCT for funding HIPERBIOTEX (COMPETE2030-FEDER-00708800) and Calouste Gulbenkian Foundation for funding DyeLoop – Circular Technologies for Textile Dyeing. The authors also acknowledge Casa da Malha – C5M, Lda. for supplying the textile fibers.

P18: EVALUATION OF NADES-MODIFIED BREWERS' SPENT GRAIN FOR THE REMOVAL OF SYNTHETIC DYES FROM AQUEOUS SOLUTIONS

Maja Adamović,¹ Ivan Zdjelarević,² Natalija Velić,² Marija Stjepanović,² Assya Bellaadem,³ Maja Čič,⁴ Ilja Gasan Osojnik Črnivec,⁴ Blaž Likozar,³ Filipa A. Vicente^{3*}

¹EKOS d.o.o., Trg Lava Mirskog 3, Osijek, Croatia

²University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 18, Osijek, Croatia

³National Institute of Chemistry, Department of Catalysis and Chemical Reaction Engineering, Hajdrihova 19, Ljubljana, Slovenia

⁴National Institute of Chemistry, Centre for Development, Demonstration and Training for Carbon-Free Technologies, Loke pri Zagorju 14b, Kisovec, Slovenia

e-mail: filipa.andre.vicente@ki.si

Natural deep eutectic solvents (NADES) have emerged as promising green alternatives to conventional extraction solvents, offering the possibility of recovering valuable bioactive compounds from biomass, such as brewers' spent grain (BSG), a major by-product of the brewing industry, while reducing the environmental impact associated with traditional solvent-based processes. Their low toxicity, biodegradability, and flexible composition make them suitable for the development of sustainable, integrated biomass valorisation strategies. Seeking to extend valorisation beyond the recovery of bioactive compounds, this preliminary investigation explores the feasibility of coupling NADES-based extraction of BSG with subsequent removal of pollutants (synthetic dyes) from aqueous solutions, thereby proposing a sequential utilisation approach in which a single biomass residue can serve multiple purposes. Lyophilised BSG was subjected to protein extraction using six different. After extraction, the remaining solvents were removed and the solid material was subsequently washed with water, dried, and used as a biosorbent for the removal of synthetic dyes, namely the cationic dye Methylene Blue (MB) and the anionic dye Congo Red (CR). The batch adsorption technique was used to screen the biosorptive removal efficiency of NADES-modified BSG for CR and MB. The results showed that NADES-modified BSG holds promise for efficient removal of synthetic dyes, with the initial dye removal percentage ranging from 50% to over 90%.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the Ministry of Science, Education and Youth and the Slovenian Research and Innovation Agency under grants BI-HR/25-27-032, P2-0152, NC-26001, J2-70079, and N2-0433.

PROCESS INTEGRATION & OPTIMIZATION

P19: ANTHOCYANIN EXTRACTION FROM BILBERRY POMACE (*Vaccinium myrtillus* L.) USING DEEP EUTECTIC SOLVENTS: SOLVENT SCREENING AND PROCESS OPTIMISATION

Ana Todorović,¹ Maja Čič,² Ilja Gasan Osojnik Črnivec,² Anissa Ouabibi,³ Steva Lević,¹ Viktor Nedović,¹ Filipa A. Vicente²

¹University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia

²National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

³Université Clermont Auvergne, Sigma Clermont, Clermont-Ferrand, France

e-mail: maja.cic@ki.si

Anthocyanins are polyphenolic plant pigments with antioxidant and antimicrobial properties, giving them strong potential for several applications. In recent decades, *deep* eutectic solvents (DES) have become an increasingly attractive option for their extraction due to their biodegradability, tunable composition, and stabilising effects. In this study, twelve DES were screened for anthocyanin extraction from bilberry pomace, followed by a stability assessment and optimisation of key process parameters (solid–liquid ratio and temperature) using response surface methodology. The two most promising systems differed only in the type of hydrogen bond acceptor, yet showed clear differences in anthocyanin stability during storage, with one preserving the compounds under both room temperature and refrigerated conditions. Furthermore, this system was successfully optimised, with temperature identified as the most influential factor, while the second system showed limited capacity to capture the experimental variability. Overall, this approach supports green principles not only through the choice of solvent but also through the valorisation of fruit pomace as a food industry by-product. These results show that similar extraction performance at the screening stage does not guarantee consistent behaviour during storage and optimisation, reinforcing the need to include stability and process optimisation early in solvent selection.

ACKNOWLEDGMENTS

This study was financially supported by the Slovenian Research Agency under research core funding P2-0152 and by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-34/2026-03/200116).

P20: DESIGN OF WATER-DRIVEN FRACTIONATION OF ORGANOSOLV LIGNIN TOWARD TAILORED MOLECULAR WEIGHT AND FUNCTIONALITY

Edita Jasiukaitytė-Grojzdek,¹ Rok Pogorevc,¹ Tina Ročnik Kozmelj,¹ Giorgio Tofani,¹ Miha Grilc,¹ Blaž Likozar¹

¹National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia

e-mail: edita.jasiukaityte@ki.si

Lignin is the largest renewable source of aromatic compounds and holds strong potential for the sustainable production of chemicals and materials. However, its direct utilization is limited by pronounced heterogeneity in molecular weight and chemical structure, which strongly depends on extraction and downstream processing conditions. Organosolv lignin, a sulfur-free technical lignin with relatively preserved ether linkages, is a promising feedstock, yet controlled fractionation is required to unlock its full valorization potential.^[1]

In this work, a sustainable strategy for the direct fractional precipitation of organosolv lignin from black liquor using water as a green antisolvent is developed. By systematically varying the organosolv process temperature (140–180 °C), antisolvent addition protocol, and lignin source (hardwood beech and softwood spruce), lignin fractions with tunable molecular weight (1–11 kDa), dispersity, and functionality were obtained. Fractionation was governed by the solubility parameter of ethanol–water mixtures, enabling selective separation without additional chemicals.^[2]

Structural characterization by size-exclusion chromatography and multidimensional NMR spectroscopy revealed strong correlations between processing conditions and lignin properties. Increasing temperature promoted depolymerization, reduced β -O-4 content, and increased aromatic OH groups, while controlled antisolvent addition enabled tuning of the aliphatic-to-aromatic OH group ratio over a wide range with minimal change in total OH content. The degree of ethoxylation and interunit linkage distribution could be adjusted independently, providing fine control over lignin reactivity. Differences between hardwood and softwood lignins further highlighted the role of lignin source in determining fractionation behavior.^[3,4]

This water-assisted fractionation approach enables the production of well-defined lignin fractions tailored for targeted applications such as catalytic depolymerization, polymer synthesis, and functional materials, while supporting sustainable and scalable lignin valorization within a circular bioeconomy framework.

ACKNOWLEDGMENTS

This research was funded by grants from the Slovenian Research Agency for research program P2-0152 and research project L2-50050.

REFERENCES

- [1] Bertella S, Luterbacher JS. Lignin functionalization for the production of novel materials. *Trends Chem.* 2020;2(5):440–453.
- [2] Jasiukaitytė-Grojzdek E, Ročnik Kozmelj T, Tofani G, Segers B, Nimmegeers P, Billen P, et al. Design of organosolv lignin fractionation: influence of temperature, antisolvent, and source on molecular weight, structure, and functionality of lignin fragments. *ACS Sustain Chem Eng.* 2025;13(9):3452–3466.
- [3] Tofani G, Jasiukaitytė-Grojzdek E, Grilc M, Likozar B. Organosolv biorefinery: resource-based process optimisation, pilot technology scale-up and economics. *Green Chem.* 2024;26(1):186–201.
- [4] Gillet S, Aguedo M, Petitjean L, Morais ARC, da Costa Lopes AM, Łukasik RM, et al. Lignin transformations for high value applications: towards targeted modifications using green chemistry. *Green Chem.* 2017;19(18):4200–4233.

P21: TAILORED DISTRIBUTION, STRUCTURE, AND FUNCTIONAL PROPERTIES OF OLIGOMERIC FRAGMENTS VIA RUTHENIUM-CATALYZED DEPOLYMERIZATION OF TECHNICAL LIGNIN

Edita Jasiukaitytė-Grojzdek,¹ Rok Pogorevc,¹ Tina Ročnik Kozmelj,¹ Marcell Gyurkač,¹ Miha Grilc,¹ Blaž Likozar¹

¹National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia

e-mail: edita.jasiukaityte@ki.si

Technical lignin represents a major renewable source of aromatic compounds with considerable potential for the sustainable production of chemicals and materials; however, its efficient valorization is limited by pronounced structural heterogeneity. Catalytic depolymerization offers a promising route to overcome these limitations by converting lignin into value-added products.^[1]

In this work, ruthenium-catalyzed depolymerization of technical lignin was systematically investigated to elucidate the distribution, structural features, and functional properties of the resulting oligomeric fragments. The process was conducted under controlled conditions, followed by fractionation and comprehensive characterization using chromatographic and spectroscopic techniques. The obtained results showed the formation of a broad range of oligomeric species with tunable molecular weight distributions and diverse structural motifs. Significant transformation of lignin interunit linkages was observed, accompanied by an increase in aromatic hydroxyl group content and modification of aliphatic functionalities. The extent of depolymerization and functional group evolution strongly depended on reaction conditions, demonstrating the possibility of controlling product properties. Furthermore, the generated oligomeric fractions exhibited reactive functional groups suitable for further chemical modification, highlighting their potential for downstream applications in advanced materials, polymers, and specialty chemicals.^[2,3]

These findings confirm that ruthenium-catalyzed depolymerization represents an effective strategy for producing structurally diverse and functionally enriched lignin-derived intermediates and contributes to the development of sustainable lignin valorization pathways within a biorefinery framework.

ACKNOWLEDGMENTS

This research was funded by grants from the Slovenian Research Agency for research program P2-0152 and research project L2-50050.

REFERENCES

- [1] Bertella S, Luterbacher JS. Lignin functionalization for the production of novel materials. *Trends Chem.* 2020;2(5):440–453.
- [2] Ročnik Kozmelj T, Bartolomei E, Dufour A, Leclerc S, Arnoux P, Likozar B, et al. Oligomeric fragments distribution, structure and functionalities upon ruthenium-catalyzed technical lignin depolymerization. *Biomass Bioenergy.* 2024;181:107056.
- [3] Jasiukaitytė-Grojzdek E, Ročnik Kozmelj T, Tofani G, Segers B, Nimmegeers P, Billen P, et al. Design of organosolv lignin fractionation: influence of temperature, antisolvent, and source on molecular weight, structure, and functionality of lignin fragments. *ACS Sustain Chem Eng.* 2025;13(9):3452–3466.

P22: APPLICATION AND IMPACT OF NOVEL EXTRACTION TECHNOLOGIES FOR XYLOGLUCAN FROM NASTURTIUM SEEDS (*TROPAEOLUM MAJUS*)

Simon Körber,^{1,2} Özlem Özmutlu Karslioglu,¹ Ute Weisz²

¹University of Applied Sciences Weihenstephan-Triesdorf, Institute of Food Technology

²TUM School of Life Sciences, Plant Proteins and Nutrition

e-mail: simon.koerber@hswt.de

Introduction: Research at HSWT (University of Applied Sciences Weihenstephan-Triesdorf) and partnerships with industry stakeholders enable the RegioTexFood project, which aims to optimize food supply chains by developing cultivation concepts for raw materials like nasturtium used in hydrocolloids, proteins, and fibers. Various cultivation systems are considered, including open-field and indoor vertical farming systems. Underutilized plants are introduced as sustainable sources of texturizing ingredients. Green technologies like pulsed electric fields (PEF), ultrasound, and high-pressure homogenization are used to enhance the extraction efficiency of xyloglucan.

Aim: The enhancement of extraction processes, the potential synergistic outcomes of combining innovative, green extraction methods, and the consequent impact on the rheological and functional characteristics of the extracted texturizing ingredients were identified as pivotal objectives of the present study.

Methods: Hexane defatting was performed as described by Nguyen et al. (2019).^[1] The polysaccharide xyloglucan is extracted from defatted nasturtium seed flour using hot-water extraction. The hot-water extraction method was adapted from Limsangouan et al. (2020) with minor modifications.^[2] Ultrasound-assisted extraction is being investigated both in comparison with conventional extraction and in combination with it.

Results: Hexane defatting of the nasturtium seed powder (1:10 w/v, 5 min, two steps) reduced fat content from 10.43 ± 1.10 % to 0.31 ± 0.04 %. The protein content increased from 19.10 ± 0.11 % in the raw material powder to 21.20 ± 0.09 % in the two-step defatted powder. The extraction methods yielded a powder with thickening properties, indicating that nasturtium seeds contain the polysaccharide xyloglucan^[3]. The rheological properties, content, and purity of the extracted thickening agent are being confirmed through further analysis. The use of ultrasound in the extraction process increased the xyloglucan yield from tamarind seeds, as reported.^[4]

Conclusion: Recent research indicates that nasturtium seeds have potential for extracting thickening agents, a possibility attributable to the presence of xyloglucan in the seeds.^[3] New green technologies can improve the extraction process of xyloglucan.

REFERENCES

- [1] Nguyen TT, Jittanit W, Srichamnong W. Production of xyloglucan component extracted from tamarind (*Tamarindus indica*) seeds using microwave treatment for seed decortication. J Food Process Preserv 2019;43. <https://doi.org/10.1111/jfpp.14055>.
- [2] Limsangouan N, Charunuch C, Sastry SK, et al. High pressure processing of tamarind (*Tamarindus indica*) seed for xyloglucan extraction. LWT 2020;134:110112. <https://doi.org/10.1016/j.lwt.2020.110112>.
- [3] FRY SC. The Structure and Functions of Xyloglucan. Journal of Experimental Botany 1989;40:1–11.
- [4] Jiang X, Yang T, Li Y, et al. Ultrasound-assisted extraction of tamarind xyloglucan: an effective approach to reduce the viscosity and improve the α -AMYLASE inhibition of xyloglucan. J Sci Food Agric 2023;103:4047–57. <https://doi.org/10.1002/jsfa.12366>.

P23: HYDROGEN PEROXIDE GENERATION FROM PERSULFATE: EXPERIMENTAL INSIGHTS AND THEORETICAL MODELLING

Katja Makovšek,¹ Brigita Hočevar,¹ Emina Novak,¹ Jurij Golobič,¹ Tomislav Blagec,¹ Matej Huš,¹ Blaž Likozar¹

¹National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

e-mail: katja.makovsek@ki.si

Hydrogen peroxide is an important green oxidant widely used in chemical synthesis, environmental applications, and industrial processes. In this work, the acidic hydrolysis of sodium and ammonium persulfates was investigated as an alternative route for hydrogen peroxide production, combining experimental studies with the development of a radical-based kinetic model.

Experiments were performed at persulfate concentrations between 0.7–1.4 mol L⁻¹ and sulfuric acid concentrations of 1.5–3.5 mol L⁻¹, reaching reaction temperatures between 105 and 120 °C. Under optimized conditions, hydrogen peroxide yields close to 100% were achieved within 10–15 minutes. High sulfuric acid concentrations (>3 mol L⁻¹) and temperatures above 110 °C were identified as key factors enabling rapid and complete conversion. In contrast, lower acid concentrations or reduced temperatures resulted in incomplete conversion, with hydrogen peroxide yields ranging from 66 to 92%. Prolonged heating led to gradual hydrogen peroxide degradation, highlighting the importance of timely reaction termination to maximize yield.

A detailed radical-based kinetic model was developed to describe persulfate decomposition, hydrogen peroxide formation, and subsequent degradation. The model successfully reproduced experimental concentration–time profiles and captured the strong influence of acidity and temperature on reaction kinetics. The extracted kinetic parameters were consistent with literature values, confirming the validity of the proposed reaction network.

The combined experimental and modelling results provide important insights into persulfate hydrolysis chemistry and offer a basis for process optimization and scale-up, supporting the development of more sustainable and flexible hydrogen peroxide production routes.

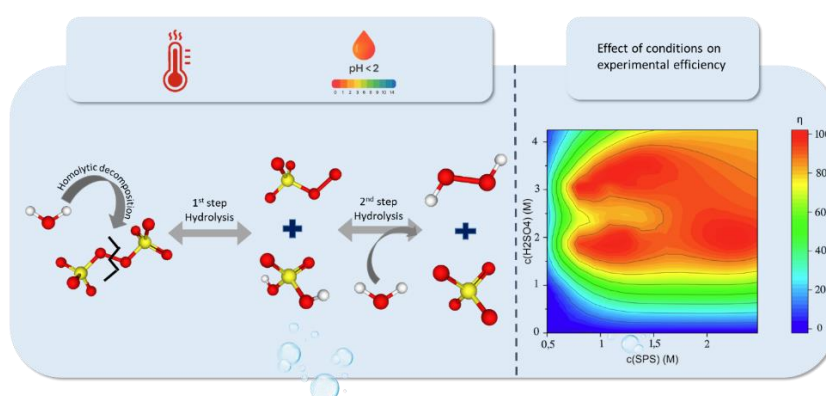


Figure 1: Persulfate decomposition under acidic conditions leading to hydrogen peroxide formation.

P24: ILIMITED PROJECT: UNDERSTANDING THE WATER LIQUID SORBENTS FOR HIGH TEMPERATURE APPLICATIONS

Anže Prašnikar,¹ Špela Gašperlin,² Jurij Golobič,¹ Jošt Knez,² Igor Shlyapnikov,³ Blaž Likozar¹

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia

²Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia

³Centre of Excellence for Low-Carbon Technologies (CO NOT), Hajdrihova 19, SI-1000 Ljubljana, Slovenia

e-mail: anze.prasnikar@ki.si

The ILIMITED project aims to intensify CO₂ hydrogenation to methanol through the in-situ removal of methanol and water, thereby shifting the reaction equilibrium and targeting yields above 80%. Since operating temperatures may reach 513 K, this concept requires liquid sorbents combining product affinity with negligible volatility and high thermal and chemical stability.

Temperature-programmed thermogravimetric analysis was used to screen phosphonium- and ammonium-based ionic liquids with different cations, anions, and functional groups. Molecular sieve 3A, TEGDME (tetraethylene glycole dimethyl ether), Santovac 5 (polyphenyl ether), and a UiO 66-containing composite were included as benchmarks.

The LiNTf₂-[P₁₄₄₄][NTf₂] reference showed pronounced low-temperature sorption and high stability, whereas neat [P₁₄₄₄][NTf₂] showed no detectable sorption. [P₆₆₆₁₄][Br] retained relatively high sorption but was slightly less stable. Other [NTf₂]⁻-based ionic liquids exhibited comparable stability, while dicyanamide and dimethyl phosphate substantially reduced it. Ether functionalization did increased sorption midly, while UiO-66 additive did not improved it. A solid sorbent molecular sieve 3A provided greater high-temperature uptake but was solid, TEGDME was highly volatile, and Santovac 5 was less stable than [P₁₄₄₄][NTf₂].

The results identify thermally stable [NTf₂]⁻ ionic liquids as a promising platform while highlighting the remaining challenge of achieving strong sorption at ILIMITED operating temperatures.

ACKNOWLEDGMENTS

This work was supported by the ILIMITED project, funded by the European Union's Horizon Europe research and innovation programme under Grant Agreement No. 101192964.

P25: DESIGNING SAFE AND SUSTAINABLE PROTEIN HYDROLYSIS FROM DAIRY WASTE USING DEEP EUTECTIC SOLVENTS FOR INTEGRATED PROCESSING AND SEPARATION

Pardis Saadatmand,¹ Gasan Osojnik,¹ Blaž Likozar,¹ Maciej Guzik,² Filipa A. Vicente¹

¹National Institute of Chemistry (NIC), Ljubljana, Slovenia

²Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Kraków, Poland

e-mail: pardis.saadatmand@ki.si

Dairy waste, particularly expired milk and whey, is both an environmental burden and an underutilized protein source. Through hydrolysis, these proteins are converted into peptides and amino acids with potential applications as functional ingredients in pharmaceutical and cosmetic formulations. Conventional acid hydrolysis (e.g., HCl) involves harsh conditions less sustainable than greener alternatives, while enzymatic approaches are costly. In this context, deep eutectic solvents (DES) are explored as greener, more sustainable, and tunable media for integrated protein isolation and hydrolysis from dairy waste. The approach exploits the dual functionality of DES as solvents and reaction media in a single system. Acidic DES systems are investigated for their ability to promote controlled protein breakdown into peptides and amino acids under milder conditions. Key process parameters, including pH, temperature, and reaction time, are systematically optimized. The performance of these systems is evaluated in terms of efficiency and selectivity using analytical techniques such as SDS-PAGE, Western blot, and HPLC. Particular attention is given to protein conversion and to the composition of hydrolysates and separated fractions. Overall, DES-based systems demonstrate strong potential for valorizing dairy waste, offering a viable route to recover functional ingredients of industrial interest. Their dual role as reaction and separation media further supports the development of scalable and more sustainable bioprocessing strategies.

ACKNOWLEDGMENTS

This work was supported through the WEAVE programme under project N2-0433, funded by the Slovenian Research and Innovation Agency (ARIS) and the National Science Centre (NCN), Poland. Additional support from ARIS through projects P2-0152, J2-70079, and NC-26001 is also gratefully acknowledged.

P26: OPTIMIZATION OF THE PURIFICATION OF C-PHYCOCYANIN FROM *LIMNOSPIRA PLATENSIS* BIOMASS USING A QUALITY BY DESIGN APPROACH

Allandelon Alencastro Barbosa,¹ Isabella dos Reis Aguera,¹ Valéria de Carvalho Santos-Ebinuma,² Felipe Rebello Lourenço,³ Nathalia Vieira Porphirio Veríssimo¹

¹School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, SP, Brazil.

²School of Pharmaceutical Sciences of Araraquara, São Paulo State University (UNESP), Araraquara, SP, Brazil.

³School of Pharmaceutical Sciences, University of São Paulo (USP), São Paulo, SP, Brazil.

e-mail: valeria.ebinuma@unesp.br

C-phycoerythrin (C-PC) is a blue phycobiliprotein derived from cyanobacteria with relevant applications as a natural colorant and bioactive ingredient for food, cosmetics, and biopharmaceutical products.^[1] However, the large-scale application of C-PC remains challenging due to its instability during processing and storage, together with the need for economically viable and flexible purification strategies. Thus, this study aimed to develop a simpler, adaptable, and low-cost purification strategy for C-PC from *Limnospira platensis* biomass based on Quality by Design (QbD) principles,^[2] using stabilization assays to support process design. Initially, preservation assays were performed using different classes of compounds, including polyethylene glycols (PEGs) and salts such as ammonium sulfate, to evaluate the maintenance of the C-PC chromophore through spectral profiles. PEG-300 and PEG-6000 showed promising effects on the preservation of optical properties, while assays with ammonium sulfate supported the selection of salt conditions compatible with both chromophore maintenance and subsequent purification steps. Initially, an aqueous extraction step was performed, resulting in above 70% C-PC recovery and purity values compatible with food-grade applications. Subsequently, aiming to obtain cosmetic-grade C-PC, the purification step was investigated by ammonium sulfate precipitation using an experimental design in which pH, salt saturation, and ultrasonic treatment time were evaluated as critical process parameters. Process performance was monitored by recovery yield and spectrophotometric purity ratios associated with the presence of contaminants naturally produced by *L. platensis*, including proteins, chlorophylls, and carotenoids. C-PC was preferentially recovered in the precipitated fractions, and the most favorable region of the design space provided purity values compatible with cosmetic-grade applications, with recovery yields above 70%. The optimal condition was observed around pH 6.5, 50% ammonium sulfate saturation, and approximately 67 s of ultrasonic treatment, providing a better balance between C-PC enrichment and recovery. Hence, QbD tools can support the development of more flexible and lower-cost purification strategies for obtaining high-value C-PC fractions from *L. platensis* biomass.

ACKNOWLEDGEMENTS

University of São Paulo, CAPES 001.

REFERENCES

- [1] Eriksen NT. Production of phycocyanin - a pigment with applications in biology, biotechnology, foods and medicine. Appl Microbiol Biotechnol. 2008;80:1-14.
- [2] International Council for Harmonisation. ICH Q8(R2): Pharmaceutical development. Geneva: International Council for Harmonisation; 2009.

P27: CIRCULAR APPROACH FOR THE DEVELOPMENT OF SUSTAINABLE FUNCTIONAL COATINGS FROM BIOMASS-DERIVED POLYMERS

Anja Verbič,¹ Vuk Martinović,¹ Miša Mojca Cajnko,¹ Blaž Likozar,¹ Blaž Stres¹

¹National Institute of Chemistry, Department of Catalysis and Chemical Reaction Engineering

e-mail: anja.verbic@ki.si

The growing demand for sustainable and environmentally responsible materials has intensified the development of advanced functional coatings. This study explored a circular approach for the development of hydrophobic coatings based on natural biopolymers. These materials can be derived from renewable biomass and biowaste streams, enabling the valorization of polysaccharide-rich feedstocks derived from biowaste to develop functional coatings, thereby contributing to resource efficiency and waste minimization. The proposed approach combines material development with systematic assessment of both functional and environmental behavior, integrating a Safe and Sustainable by Design (SSbD) principles together with mathematical modelling tools as an additional option to guide material development and reduce experimental workload. In this context, data-driven modelling can be applied as decision-support tool to identify key relationships between formulation parameters and resulting surface functionality, enabling efficient formulation design and reducing the need for extensive experimentation. In parallel, environmental compatibility was addressed through systematic evaluation of biodegradation, ensuring that developed coatings can undergo natural degradation after disposal, while retaining their hydrophobic surface functionality during service life. This dual requirement highlights the importance of balancing performance and environmental fate in functional material design. The combination of circular feedstock utilization, sustainable development and end-of-life (EoL) evaluation provides a comprehensive framework for the safe and sustainable design of functional biopolymer-based coatings, supporting the transition towards more circular and environmentally compatible material systems.

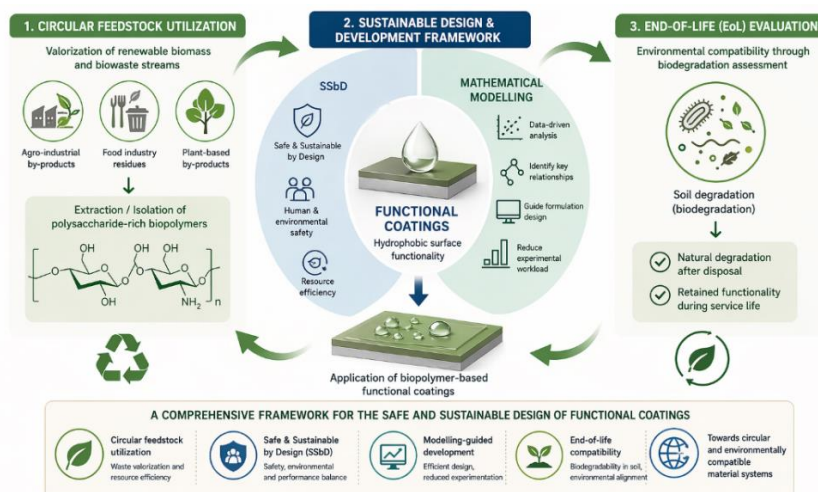


Figure 1: Schematic presentation of circular, sustainable design approach for the development of functional coatings.

ACKNOWLEDGEMENTS

This research was funded by the Slovenian Research Agency under the research core funding No. P2-0152 and project J4-50148 (MicroBeast).

BIOMASS PROCESSING

P28: INTEGRATING IN SILICO SCREENING AND GREEN EXTRACTION FOR LIMONENE RECOVERY FROM CITRUS WASTE

Monika Arnič,¹ Kristina Andrejč,¹ Mónia A. R. Martins,² Filipe H. B. Sosa,³ João A. P. Coutinho,³ Blaž Likozar,¹ Filipa A. Vicente¹

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Ljubljana, Slovenia

²CIMO, LA SusTEC, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

³CICECO – Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

e-mail: monika.arnic@ki.si

The food-processing industry generates millions of tonnes of orange peel waste annually, representing an underutilised source of valuable bioactive compounds such as limonene. Limonene, the main constituent of orange essential oil, is highly valued for its aroma and antimicrobial properties across various industries. Conventional extraction methods typically use toxic organic solvents like hexane, prompting a shift towards greener alternatives such as deep eutectic solvents (DES). DES, composed of hydrogen bond donors (HBDs) and acceptors (HBAs), are tunable, environmentally friendly systems that can improve both extraction efficiency and product applicability. This study aimed to design a DES capable of efficiently extracting limonene from orange peels while being directly suitable for cosmetic formulations, thereby eliminating the need for post-extraction purification. Initially, in silico screening using the COSMO-RS model was employed to evaluate limonene solubility in various solvents. Results indicated that limonene primarily engages in hydrophobic interactions. Interestingly, one identified HBD demonstrated strong extraction potential despite not being conventionally hydrophobic. Based on these findings, selected HBD–HBA combinations were experimentally tested. DES systems were prepared and optimised in terms of molar ratios and water content to achieve suitable viscosity. Extraction conditions were fine-tuned to maximise limonene extraction yield. A total of 15 DES and 5 conventional organic solvents were evaluated comparatively. Limonene content was quantified using gas chromatography–mass spectrometry, while flavonoids and the furanocoumarin bergapten were analysed by high-performance liquid chromatography. The combined in silico and experimental approach enabled efficient identification of high-performing DES, reducing time and resource consumption. Several DES outperformed traditional solvents in limonene extraction efficiency. Additionally, the resulting extracts contained beneficial flavonoids, enhancing their potential value in cosmetic applications. This work demonstrates a sustainable strategy for valorising orange peel waste through green extraction technologies, offering a viable alternative to conventional solvent-based processes.^[1]

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Slovenian Research Agency (P2-0152, BI-US/24-26-017, NC-0024, NC-24002) and Foundation for Science and Technology (UIDB/00690/2020, UIDP/00690/2020, LA/P/0007/2020, UIDB/50011/2020, UIDP/50011/2020, LA/P/0006/2020, CEECIND/07209/2022).

REFERENCES

[1] Arnič M, Andrejč K, Martins MAR, Sosa FHB, Coutinho JAP, Likozar B, et al. Repurposing orange peel waste for limonene extraction using deep eutectic solvents for cosmetic applications. *Sustain Chem Pharm.* 2026;50:102339.

P29: COLLAGEN TYPE I EXTRACTION FROM EGGSHELL MEMBRANES USING EUTECTIC SOLVENTS AT AMBIENT TEMPERATURE

Victoire Biard O'Meara,^{1,2} Abolfazl Keshmirshekan,² João A. P. Coutinho,² Sónia P.M. Ventura²

¹École Européenne de Chimie, Polymères et Matériaux (ECPM), Université de Strasbourg, France

²CICECO – Aveiro, Institute of Materials, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-163 Aveiro, Portugal

email: jcoutinho@ua.pt

Eggshell membranes (ESM) are a widely available yet underexploited biomaterial enriched with structural proteins, particularly collagen type I (CTI). However, the efficient isolation of CTI from ESM remains difficult due to its complex protein matrix and the coexistence of multiple collagen subtypes. Conventional extraction approaches generally rely on low-temperature conditions (4 °C) to maintain collagen integrity and functionality, but these protocols are largely limited to laboratory-scale applications and are not readily adaptable for industrial production. In this study, CTI extraction from ESM was performed at ambient temperature over 72 h using three engineered aqueous eutectic solvent (DES) systems. The DES formulations were designed to regulate hydrogen-bond donor/acceptor (HBD/HBA) interactions, solvent acidity, and ionic network characteristics in order to investigate their influence on collagen yield, purity, and structural preservation. DES systems with and without acetic acid were also compared to evaluate the contribution of acetic acid under extraction environments. All DES formulations successfully extracted collagen type I, as evidenced by distinct α - and β -chain profiles in SDS-PAGE that were comparable to commercial rat tail collagen and indicative of collagen type, and high purity. FTIR spectra confirmed the presence of characteristic amide bands, while XRD analysis demonstrated preserved molecular packing consistent with retention of the triple-helical structure. Among the investigated systems, DES2 exhibited the most balanced performance, achieving yields above 14% while maintaining favorable structural integrity. DES1 showed superior structural preservation but relatively low extraction efficiency (<7%), whereas DES3 produced yields exceeded 10% but with lower structural organization. In conclusion, with additional optimization, this ambient-temperature DES-based strategy presents strong potential as a scalable and sustainable approach for collagen recovery from extracellular matrix sources for different industrial applications.

ACKNOWLEDGMENTS

This work was developed within the scope of the project CICECO–Aveiro Institute of Materials, UID/50011 s LA/P/0006/2020 (DOI 10.54466/LA/P/0006/2020), financed by national funds through the FCT/MCTES (PIDDAC). A.K. acknowledges the financial support of Fundação para a Ciência e a Tecnologia by his PhD grant with the reference 2024.05467.BDANA.

P30: LIGNIN-BASED CANS WITH SELF-HEALING PROPERTIES AS SUSTAINABLE ALTERNATIVES TO CONVENTIONAL EPOXY THERMOSETS

Iva Edrovska,^{1,2} Giorgio Tofani,² Edita Jasiukaitytė-Grojzdek,² Filipa A. Vicente,² Miha Grilc,² Rok Pogorevc,² Blaž Likozar^{1,2}

¹Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

²Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Ljubljana, Slovenia

e-mail: iva.edrovska@gmail.com

In recent years, significant efforts have been directed towards the development of polymeric materials that combine the structural stability of thermosets with the reprocessability of thermoplastics. In this context, covalent adaptable networks (CANs), offer a promising pathway towards recyclable and self-healing thermosetting systems.^[1] In this study, lignin was employed as a renewable aromatic precursor to replace conventional Bisphenol A-based components in epoxy resins.^[1] CAN was introduced through the functionalization of lignin with cyclic anhydrides,^[2] forming ester linkages capable of reversible bond exchange.^[1] The functionalized lignins were subsequently cross-linked with diepoxides to yield CAN-based epoxy resins with a self-healing behavior. This enables for reversible bond exchange under thermal stimuli.^[1] By varying the lignin type (kraft and organosolv) and the epoxide structure, two distinct material classes were obtained: rigid resins with high tensile strength and low elongation, and elastic resins with lower strength but enhanced flexibility. The recycling potential of these materials was evaluated using solvent dissolution and thermally induced self-healing via hot-pressing. While dissolution in green solvents enabled solubilization of rigid kraft lignin-based resins, the reshaped materials exhibited a significant loss of mechanical integrity. In contrast, the self-healing approach proved effective for the elastic organosolv lignin-based resins. Successful mechanical reprocessing was achieved at higher temperatures, with improved healing efficiency observed when additional epoxide was applied as an adhesive. A multi-cycle self-healing study was conducted using an organosolv lignin-based resin. The system containing added epoxide retained self-healing capability for up to five cycles, exhibiting a gradual decrease in mechanical performance. In contrast, the resin without additional epoxide failed after three cycles. All tests were performed under identical conditions.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from the Slovenian Research Agency (grant P2-0152), the European Union's Horizon Europe research and innovation programme under grant agreements No. 101058371 (ESTELLA) and No. 101070302 (HyPELignum) and the program (Interreg SI-AT) Slovenia-Austria under the reCANet project.

REFERENCES

- [1] Tiz DB, Vicente FA, Kroflič A, Likozar B. Lignin-based covalent adaptable network polymers—when bio-based thermosets meet recyclable by design. *ACS Sustainable Chem Eng.* 2023;11(38):13836-13867.
- [2] Scarica C, Suriano R, Levi M, Turri S, Griffini G. Lignin functionalized with succinic anhydride as building block for biobased thermosetting polyester coatings. *ACS Sustainable Chem Eng.* 2018;6(3):3392-3401.

P31: THE THERMOMECHANICAL PROPERTIES OF LIGNIN-EPOXY

Meta Florjančič,^{1,2} Giorgio Tofani,¹ Edita Jasiukaitytė-Grojzdek,¹ Filipa A. Vicente,¹ Miha Grilc,¹ Rok Pogorevc,¹ Blaž Likozar^{1,2}

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Ljubljana, Slovenia

²Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

e-mail: meta.florjancic3@gmail.com

Lignin-based epoxy thermosets are emerging as sustainable alternatives to conventional bisphenol-A-derived systems, offering reduced environmental impact while maintaining competitive material performance.^[1] Lignins sourced from different extraction processes were subjected to fractionation, functionalisation with a cyclic anhydride using a single catalyst in tetrahydrofuran (THF), and afterwards cured with various aliphatic epoxides to form irreversible thermoset networks.

All resins demonstrated consistently high thermal stability, with degradation onset temperatures (T_{onset}) exceeding 350 °C across all formulations, regardless of the epoxide utilized. This indicates strong resistance to thermal decomposition and potential suitability for elevated-temperature applications.

Mechanical properties varied with resin composition, allowing modulation between rigid and flexible behavior. The rigid formulation achieved tensile strengths of up to approximately 30 MPa, demonstrating load-bearing capability. The flexible formulation reached elongation at break values of up to approximately 60 %, reflecting a more compliant and deformable network architecture. Notably, complete curing of the rigid systems required an additional curing step. These results indicate that such systems offer a promising combination of sustainability and tunable material properties. Overall, they show potential as viable alternatives to conventional thermoset materials.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from the Slovenian Research Agency (grant P2-0152), the European Union's Horizon Europe research and innovation programme under grant agreements No. 101058371 (ESTELLA) and No. 101070302 (HyPELignum), and the program (Interreg SI-AT) Slovenia-Austria under the reCANet project.

REFERENCES

[1] Lu X, Gu X. A review on lignin-based epoxy resins: lignin effects on their synthesis and properties. *Int J Biol Macromol.* 2023;229:778-790.

P32: TAILORING RHENIUM-CATALYSED DEOXYDEHYDRATION OF GLYCERIC ACID TOWARDS BIO-BASED ACRYLATES

Maja Gabrič,¹ Miha Grilc,¹ Blaž Likozar,¹ Brigita Hočevar¹

¹National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

e-mail: maja.gabric@ki.si

The sustainable production of acrylic acid and acrylic esters from renewable feedstocks represents an attractive alternative to conventional petrochemical processes. Glyceric acid, obtainable from biomass-derived glycerol, is a promising platform molecule for the synthesis of bio-based acrylates via catalytic deoxydehydration (DODH). In this work, the catalytic performance of several supported rhenium catalysts was evaluated for the conversion of glyceric acid in alcoholic media, while systematically investigating the effects of catalyst support, reaction temperature, gas atmosphere, and solvent on product distribution.

The reactions were carried out in a stirred high-pressure batch reactor using methanol and other alcohols as solvents under either nitrogen or hydrogen atmosphere. Product formation was quantified by GC-MS analysis. Among the investigated catalysts, commercial Re/C exhibited the highest activity and selectivity, achieving a combined yield of acrylic acid and methyl acrylate of 60.6%. The optimum reaction conditions were identified as 150 °C in methanol under nitrogen atmosphere for 72 h. Increasing the reaction temperature or replacing nitrogen with hydrogen promoted undesired hydrogenation pathways, leading to higher propionate formation. The results further indicate that methanol plays a multifunctional role as solvent, hydrogen donor through transfer hydrogenation, and esterification agent, thereby significantly influencing both reaction pathway and product selectivity.

The study demonstrates the critical influence of catalyst support and reaction conditions on the selective deoxydehydration of glyceric acid and provides valuable insights for the development of efficient catalytic routes towards renewable acrylic monomers. Ongoing work aims to elucidate the origin of the exceptional performance of carbon-supported rhenium catalysts and to establish structure–activity relationships that can guide the design of next-generation DODH catalysts.

P33: IONIC LIQUID-ISOLATED LIGNIN PELLETS AS ADVANCED CLEAN SOLID FUELS

Marcell Gyurkač,^{1,2} Edita Jasiukaitytė-Grojzdek,² Tina Ročnik Kozmelj², Blaž Likozar,² Miha Grilc²

¹Laboratory of Separation Processes and Product Design, Faculty of Chemistry and Chemical Engineering,
 University of Maribor, Smetanova ulica 17, SI-2000 Maribor, Slovenia

²Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19, SI-
 1000 Ljubljana, Slovenia

e-mail: marcell.gyurkac@ki.si

Lignocellulosic biomass, particularly agricultural residues, represents an untapped renewable energy resource. Wheat straw, which is highly abundant across the EU, contains up to one third lignin, an aromatic biopolymer with an energy density approximately 35% higher than that of commercial wood pellets. The successful extraction and isolation of this high-quality biofuel enables the development of an efficient biomass-to-energy conversion pathway from waste materials. This transition to renewable resources is crucial for creating sustainable heating and industrial energy applications as the EU pursues climate neutrality.

In this study we investigate a novel approach to valorise wheat straw waste by selectively extracting lignin using distillable and recyclable ionic liquids, yielding high-quality, sulphur and halide-free lignin powder suitable for thermal energy applications. Optimal extraction conditions (165°C, 90 min) yielded over 78 wt% lignin recovery with excellent physicochemical properties. The addition of small portions of glycerol (up to 2.5 wt%) further enabled processing of the lignin powder into durable lignin pellets ready for combustion. We thus demonstrated that the extracted pure lignin with low ash content (below 2.0 wt%) exhibits superior combustion properties compared to traditional biomass fuels, achieving high heating values of 22 MJ/kg and beyond, making it suitable for industrial heating applications and district heating systems. This novel and recyclable (>95 wt% distillable ionic liquid recycling rate achieved) process demonstrates industrial scalability for converting agricultural waste into clean-burning biofuel. The process thus addresses two key energy transition challenges: efficient biomass utilization and solvent sustainability, both supporting rural energy independence and industrial decarbonization.

P34: COUPLING SUPERCRITICAL FLUIDS WITH BIOBASED IONIC LIQUIDS FOR THE GREEN ISOLATION OF PIGMENTS FROM BROWN MACROALGAE

Marcell Gyurkač,^{1,2} Taja Žitek Makoter,¹ Miha Grilo,² Blaž Likozar,² Maša Knez Marevci^{1,3}

¹Laboratory of Separation Processes and Product Design, Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova ulica 17, SI-2000 Maribor, Slovenia

²Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

³Faculty of Medicine, University of Maribor, Taborska 8, SI-2000 Maribor, Slovenia

e-mail: marcell.gyurkac@ki.si

Due to its exceptional transport properties, supercritical carbon dioxide (scCO₂) serves as a tunable, environmentally friendly solvent. It enables efficient recovery of non-polar and mildly polar compounds from biomass while eliminating solvent waste. As it is highly selective, inert, and scalable, scCO₂ is well suited to isolating valuable bioactive molecules. One such target is the pigment fucoxanthin (Fx), recognised for its antioxidant, anti-inflammatory, and potential anti-obesity effects.

The delicious and highly nutritious Wakame (*Undaria pinnatifida*) has rapidly spread in culinary use worldwide but, unfortunately, has also become invasive in coastal ecosystems globally. Its unchecked growth threatens local biodiversity, as it outcompetes native algae and alters marine habitats. However, Wakame's high Fx content offers both ecological and economic valorisation. Harvesting this invasive species restores ecosystems while yielding valuable extracts.

In this study, we optimised scCO₂ extraction to recover Fx from Wakame harvested in Europe. We also performed extractions using two biobased ionic liquids (BILs) for cell wall disruption and subsequent solvent extraction. Notably, our comparative analysis showed that ethyl acetate performed slightly better than scCO₂ in terms of Fx extraction efficiency, and the BIL1 pre-treated sample gave significantly higher Fx yields than both the BIL2-treated and non-pre-treated samples.

Despite lower extraction efficiency, scCO₂ provided excellent selectivity for Fx. By systematically optimising temperature and pressure profiles, we achieved a concentration of 80.9 mg/g in the final extract, representing an eightfold improvement in purity compared to conventional extraction methods (below 10 mg/g extract).

Targeting invasive European Wakame aligns with circular economy principles, transforming an ecological threat into a sustainable source for cosmetics and nutraceuticals. Future work will focus on optimising extraction pressures and yields, developing encapsulation techniques for Fx-rich extracts, and evaluating green co-solvents to enhance efficiency.

P35: CONVERSION OF WOOD CELLULOSE PULP RESIDUE INTO 5-HMF AND ENERGY-DENSE HYDROCHAR VIA WET TORREFACTION UNDER NITROGEN ATMOSPHERE

Andrii Kostyniuk,¹ Blaž Likozar¹

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19,
1001 Ljubljana, Slovenia

e-mail: andrii.kostyniuk@ki.si

Wet torrefaction (WT) has emerged as a promising thermochemical strategy for the sustainable upgrading of lignocellulosic residues into carbon-rich solid fuels and valuable bio-based chemicals. In the present work, wood cellulose pulp residue (WCPR) was subjected to WT treatment in a nitrogen atmosphere to investigate the influence of operating conditions on hydrochar properties and liquid product distribution. The process was performed at temperatures between 180 and 260 °C, reaction times of 15–60 min, and H₂O/WCPR mass ratios ranging from 10 to 25. The obtained results demonstrated that WT conditions strongly affected both the physicochemical characteristics of the hydrochar and the formation of liquid intermediates. Increasing temperature and residence time promoted dehydration and decarboxylation reactions, leading to progressive carbon enrichment and oxygen removal from the solid phase. The highest carbon content (68.3 wt%) and higher heating value (27.3 MJ kg⁻¹) were achieved at 260 °C after 30 min, corresponding to a hydrochar enhancement factor of 1.43. In parallel, selective formation of 5-hydroxymethylfurfural (5-HMF) was observed in the liquid fraction without the use of any catalyst. The optimal conditions for 5-HMF generation were identified at 220 °C with a reaction time of 30 min and an H₂O/WCPR ratio of 10, resulting in a selectivity of 73.3%. Comprehensive characterization of the produced hydrochars included elemental composition, energy yield, deoxygenation degree, surface area, and pore structure analysis. Furthermore, a mechanistic reaction pathway describing the conversion of WCPR during WT was proposed, providing insights into the simultaneous formation of energy-dense hydrochar and oxygenated platform molecules. These findings highlight the potential of WT as an efficient route for integrated biomass waste valorization into renewable fuels and high-value chemicals.^[1–4]

ACKNOWLEDGMENTS

The authors acknowledge financial support from the CARBIOW project (Carbon Negative Biofuels from Organic Waste), funded by the European Commission under the Horizon Europe Programme (Grant Agreement No. 101084443). The authors also acknowledge the BioTrainValue project (Grant Agreement No. 101086411, Horizon Europe Marie Skłodowska-Curie Staff Exchanges).

REFERENCES

- [1] Kostyniuk A, Likozar B. Wet torrefaction of biomass waste into value-added liquid product (5-HMF) and high quality solid fuel (hydrochar) in a nitrogen atmosphere. *Renew Energy*. 2024;226:120450.
- [2] Kostyniuk A, Likozar B. Catalytic wet torrefaction of biomass waste into bio-ethanol, levulinic acid, and high quality solid fuel. *Chem Eng J*. 2024;485:149779.
- [3] Kostyniuk A, Likozar B. Wet torrefaction of biomass waste into high quality hydrochar and value-added liquid products using different zeolite catalysts. *Renew Energy*. 2024;227:120509.
- [4] Kostyniuk A, Likozar B. Wet torrefaction of biomass waste into levulinic acid and high-quality hydrochar using H-beta zeolite catalyst. *J Clean Prod*. 2024;449:141735.

P36: CHEMOCATALYTIC WET TORREFACTION OF BIOMASS WASTE OVER ZEOLITE CATALYSTS FOR THE PRODUCTION OF BIO-ETHANOL, LEVULINIC ACID, AND HIGH-QUALITY HYDROCHAR

Andrii Kostyniuk,¹ Blaž Likozar¹

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19,
1001 Ljubljana, Slovenia

e-mail: andrii.kostyniuk@ki.si

Lignocellulosic residues from the pulp and paper industry represent an underutilized renewable feedstock for producing carbon-rich fuels and bio-based platform chemicals. Herein, a zeolite-assisted wet torrefaction (WT) approach was developed for the integrated valorization of wood cellulose pulp residue (WCPR) under a nitrogen atmosphere. Reactions were carried out in stainless-steel batch reactors at 220–260 °C using an H₂O/WCPR ratio of 10 and reaction times of 15–60 min. A series of acidic zeolites, including H-ZSM-5, H-Beta, H-Y, H-USY and H-Mordenite, was evaluated to tune the distribution of liquid products while improving the fuel properties of the resulting hydrochar. The catalytic WT route enabled the one-pot formation of levulinic acid (LA), bio-ethanol and upgraded hydrochar. Product selectivity was strongly governed by zeolite topology, acidity and reaction severity. H-Y favored rapid ethanol formation, reaching 59.0% selectivity at 220 °C after 15 min, whereas H-USY provided the highest ethanol yield of 75.6% at 260 °C after 60 min. In contrast, H-ZSM-5 promoted the formation of LA, with a maximum selectivity of 62.0% at 220 °C after 60 min. H-Beta also showed strong potential for LA production, achieving 62.8% selectivity at 260 °C after 30 min. Simultaneously, zeolite-assisted WT substantially enhanced the solid fraction. Hydrochar obtained over H-Mordenite at 220 °C for 60 min reached a carbon content of 71.5%, a higher heating value of 27.3 MJ kg⁻¹, an energy densification ratio of 1.36 and a carbon enrichment factor of 1.48. With H-Beta, even stronger carbon concentration was observed, with hydrochar containing up to 78.9% carbon and an HHV of 30.3 MJ kg⁻¹. The proposed reaction network involves cellulose hydrolysis to glucose, dehydration to 5-HMF and subsequent rehydration to LA, while ethanol formation is associated with C-C bond cleavage of oxygenated intermediates such as hydroxyacetone. These results demonstrate that acidic zeolites can direct WT toward simultaneous production of renewable fuels, platform molecules and high-quality hydrochar from biomass waste. ^[1–4]

ACKNOWLEDGMENTS

The authors acknowledge financial support from the CARBIOW project (Carbon Negative Biofuels from Organic Waste), funded by the European Commission under the Horizon Europe Programme (Grant Agreement No. 101084443). The authors also acknowledge the BioTrainValue project (Grant Agreement No. 101086411, Horizon Europe Marie Skłodowska-Curie Staff Exchanges).

REFERENCES

- [1] Kostyniuk A, Likozar B. Wet torrefaction of biomass waste into levulinic acid and high-quality hydrochar using H-beta zeolite catalyst. *J Clean Prod.* 2024;449:141735.
- [2] Kostyniuk A, Likozar B. Wet torrefaction of biomass waste into value-added liquid product (5-HMF) and high quality solid fuel (hydrochar) in a nitrogen atmosphere. *Renew Energy.* 2024;226:120450.
- [3] Kostyniuk A, Likozar B. Catalytic wet torrefaction of biomass waste into bio-ethanol, levulinic acid, and high quality solid fuel. *Chem Eng J.* 2024;485:149779.
- [4] Kostyniuk A, Likozar B. Wet torrefaction of biomass waste into high quality hydrochar and value-added liquid products using different zeolite catalysts. *Renew Energy.* 2024;227:120509.

P37: VALORISATION OF RESIDUAL MICROALGAL BIOMASS BY LIQUEFACTION AND CATALYTIC UPGRADING

Dana Marinič,^{1,2} Rok Pogorevc,^{1,2} Florian Delrue,³ Blaž Likozar,¹ Filipa A. Vicente¹

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

²University of Maribor, Faculty of Chemistry and Chemical Engineering, Maribor, Slovenia

³MicroAlgae Processes Platform, CEA, CEA Tech Région Sud - Provence-Alpes Côte d'Azur, F-13108 Saint Paul lez Durance, France

e-mail: dana.marinic@ki.si

Microalgae are considered a promising renewable feedstock for sustainable biofuel production due to their rapid growth, high carbon capture potential, and ability to accumulate energy-rich compounds. The valorisation of residual microalgal biomass into hydrocarbon fuels offers a potential pathway toward reducing waste generation and dependence on fossil resources.

In this study, residual biomass of *Chlorella vulgaris* remaining after pigment extraction with *deep* eutectic solvents (DES) and biosolvents was converted into hydrocarbon-rich products through solvent-assisted liquefaction, catalytic hydroprocessing and upgrading. Different DES systems, including menthol:octanoic acid and thymol:octanoic acid at various ratios, as well as octanoic acid as a biosolvent, were investigated.

Liquefaction and upgrading experiments were performed in a three-phase slurry reactor at 290 °C under 50 bar H₂ using dodecane as solvent. A sulfided NiMo/ γ -Al₂O₃ catalyst was primarily employed due to its industrial relevance, while Rh and Ru catalysts were additionally evaluated for the thymol:octanoic acid system. Product characterization was carried out using GC–MS analysis.

The obtained product mixtures consisted predominantly of gasoline-range hydrocarbons (C₆–C₈), with octane as the major product and the highest yields observed for the menthol:octanoic acid system. The predominance of gasoline-range hydrocarbons indicates efficient deoxygenation during catalytic upgrading, although significant cracking of heavier intermediates was also observed. Residual solvent-derived compounds, including thymol and menthol, were detected in the product phase, indicating partial solvent carryover. Additional catalytic tests showed a strong influence of catalyst selection on hydrocarbon distribution. Rh catalysts promoted the formation of longer-chain hydrocarbons (>C₁₀), corresponding to diesel-range fractions, whereas the sulfided NiMo/ γ -Al₂O₃ catalyst favored lighter gasoline-range products. In contrast, Ru catalysts exhibited substantially lower activity.

The results demonstrate the feasibility of integrating green extraction processes with catalytic liquefaction for the valorisation of residual microalgal biomass into hydrocarbon fuels and highlight the importance of catalyst selection in controlling product distribution.

ACKNOWLEDGMENTS

This research was funded by the Slovenian Research and Innovation Agency (ARIS) through research projects NC-24002.

P38: TERPENE-BASED SOLVENTS FOR ARTEMISININ EXTRACTION

Luan B. Corso,¹ Isabella W. Cordova,² Inês Moraes,³ Fátima Nogueira,³ Adriana C. S. Pais,² Sónia A. O. Santos,²
João A. P. Coutinho,² André Zuber,⁴ Olga Ferreira,¹ Simão P. Pinho,¹ Mónia A. R. Martins¹

¹CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal

²CICECO, Aveiro Institute of Materials, University of Aveiro, Campus Universitário de Santiago, Aveiro, Portugal

³Global Health and Tropical Medicine, Associate Laboratory in Translation and Innovation Towards Global Health,
Institute of Hygiene and Tropical Medicine, Universidade Nova de Lisboa, 1349-008 Lisbon, Portugal

⁴Academic Department of Engineering, Federal University of Technology – Paraná, Francisco Beltrão-PR 85601-
970, Brazil

e-mail: moniamartins@ipb.pt

Malaria caused 282 million cases in 2024, maintaining high demand for artemisinin-based therapies. However, conventional extraction of artemisinin from *Artemisia annua* uses petroleum ether, hexane, or halogenated solvents that pose flammability, toxicity, and waste-management risks. This study aimed to identify lower-hazard bio-derived solvents that maintain or improve extraction performance while reducing environmental and occupational risks.

The Conductor-like Screening Model for Realistic Solvents (COSMO-RS) was used to predict solute-solvent affinities for artemisinin and six comparator antimalarial compounds. Promising candidates were experimentally validated through solid-liquid extraction using terpene solvents and compared with dichloromethane benchmarks. The best terpene system increased artemisinin recovery by about 15% while eliminating halogenated solvent use, and the extracts showed IC₅₀ values comparable to pure artemisinin, supporting these natural solvents as sustainable alternatives for green extraction and potential antimalarial formulations.

These results demonstrate that computationally guided selection of bio-derived solvents can enhance extraction efficiency while reducing hazardous inputs, enabling safer and scalable antimalarial manufacturing aligned with green chemistry and sustainable industrial practices.

ACKNOWLEDGMENTS

This work is also part of the "Artemisinin bioavailability enhancement through eutectic formation with natural excipients" project financed by PhosAgro/UNESCO/IUPAC Partnership in Green Chemistry for Life (contract number 4500513457). This work was also supported by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020); CICECO – Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020).

P39: FROM SOLID LIGNIN TO LIQUID FEEDSTOCK: SUSTAINABLE SOLUBILIZATION WITH GREENER SOLVENTS

Irina Modrušan,^{1,2} Sabina Berne,² Filipa A. Vicente,¹ Blaž Likozar,¹ Edita Jasiukaitytė-Grojzdek¹

¹National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia

²University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

e-mail: irina.modrusan@ki.si

Lignin, the largest renewable source of aromatic compounds, has the potential to replace fossil resources, yet it is often treated as waste. Each year, around 100 million tonnes of lignin are produced worldwide, but only 2% is recovered and used for material applications, while the rest is burned for energy recovery.^[1] Annual lignin production is expected to surpass 225 million tonnes by 2030, further widening the gap between availability and effective utilization.^[2] The main limitation is the processability of technical lignins, as they are structurally complex, heterogeneous, and largely insoluble in neutral aqueous environments. Lignin can be solubilized with conventional organic solvents, but these often compromise its structure and raise environmental and safety concerns, limiting both sustainability and scalability.^[3] In this context, *deep* eutectic solvents (DES) offer a greener alternative for sustainable lignin processing. Additionally, due to their relatively mild, non-reactive nature and gentle operating conditions, they may also help preserve the native lignin structure during solubilization, reducing unwanted chemical changes.^[4]

In this work, we show that carefully designed DES can solubilize both Kraft and organosolv hardwood lignins at high loadings under mild conditions, including near-neutral pH, ambient temperature (30 °C), and controlled water content, without the need for additives or external energy input. The DES components are biodegradable, biocompatible, and low-volatile, making the overall process significantly greener than conventional solvents.

Notably, these conditions are compatible with the functional range of enzymes such as laccases, allowing the same DES to serve as a reaction medium for subsequent enzymatic depolymerization of dissolved lignin. Mild solubilization thus becomes the first step toward sustainable lignin valorization within a circular bioeconomy framework.

ACKNOWLEDGMENTS

This research was funded by grants from the Slovenian Research Agency for research program P2-0152 and research project L2-50050.

REFERENCES

- [1] Yuan JS, Pavlovich MJ, Ragauskas A, Han B. Biotechnology for a sustainable future: biomass and beyond. *Trends Biotechnol.* 2022;40(12):1490-1503.
- [2] Mateo S, Fabbri G, Moya A. Lignin from plant-based agro-industrial biowastes: from extraction to sustainable applications. *Polymers (Basel).* 2025;17(7):952.
- [3] Raikwar D, Van Aelst K, Vangeel T, Corderi S, Van Aelst J, Van den Bosch S, et al. Elucidating the effect of the physicochemical properties of organosolv lignins on their solubility and reductive catalytic depolymerization. *Chem Eng J.* 2023;472:141999.
- [4] rovost V, Dumarcay S, Ziegler-Devin I, Boltoeva M, Trebouet D, Villain-Gambier M. Deep eutectic solvent pretreatment of biomass: influence of hydrogen bond donor and temperature on lignin extraction with high β -O-4 content. *Bioresour Technol.* 2022;362:126837.

P40: HETEROGENEOUS CATALYSIS FOR BIOMASS CONVERSION

Matej Rukše,¹ Žan Lavrič,¹ Blaž Likozar,¹ Miha Grilc¹

¹Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Ljubljana, Slovenia

e-mail: matej.rukse11@gmail.com

Biomass transformation to value added chemicals is improving its competitiveness in the last decades. In parallel with the development of new catalysts for biomass valorisation, process intensification is crucial to raise technology readiness level (TRL) and compare it to the current industrial benchmark.

Selective oxidation of sugars to functionalized aldehydes or carboxylic acids with solid catalysts is one such alternative, compared to conventional nitric acid oxidation. The latter is operated at lower yields, to prevent a runaway of this highly exothermic reaction. The alternative process uses a solid catalyst with aqueous solution of glucose under relatively low temperature and pressure. The main challenge is to develop a stable catalyst, capable to withstand long time on stream (TOS) and is actively giving a reasonable yield and selectivity of the desired product.

Our study focuses on the development of a trickle-bed reactor (TBR) that will enable the highest-yield possible and selective oxidation of glucose to glucaric acid.

Batch reactor experiments were initially conducted to identify the optimal reaction conditions for the oxidation of glucose to glucaric acid. The highest glucaric acid yield in a batch reactor was 51 %. These optimized conditions were used as a basis for reactions in the trickle-bed reactor.

Reactions in the trickle-bed reactor are still underway, with the highest glucaric acid yield so far being similar to batch experiments, but the residence time in TBR is shorter than the reaction time in batch experiments.

ACKNOWLEDGMENTS

This research was funded by the Slovenian Research and Innovation Agency (ARIS) (research projects N2-0242, GC-0007 and J1-3020 and research core funding P2-0152).

P41: CASCADE RECOVERY OF ASTAXANTHIN AND CHITIN FROM SHRIMP SHELLS USING *DEEP* EUTECTIC SOLVENTS

Sunčica Srzentić,^{1,2} Ilja Gasan Osojnik Črnivec,¹ Blaž Likozar,¹ Filipa A. Vicente¹

¹National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

²University of Ljubljana, Faculty of Chemistry and Chemical Technology, Večna pot 113, 1000 Ljubljana, Slovenia

e-mail: suncica.srzentic@ki.si

The crustacean industry is a rapidly growing sector driven by high demand, generating nearly 10 million tons of shell waste annually. The shells are rich in chitin, proteins, minerals, and lower amounts of astaxanthin, yet remain largely unutilized and typically end up in landfills creating a range of environmental problems. This work develops a cascade process that sequentially extracts the aforementioned compounds with the use of *deep* eutectic solvents (*DES*). Shrimp shells were first characterized for their composition and establish baseline values, which were used as reference to guide solvent selection. Given the differences in polarity, sensitivity and stability among the compounds, the use of *DES* allows for tunable properties of solvents which enables selective extraction. Two solvent matrices were screened: hydrophilic *DES* for the removal of proteins and minerals, and hydrophobic *DES* for the selective extraction of astaxanthin, with the remaining solid fraction consisting of chitin. Over 50 combinations for each matrix were evaluated for their ability to form stable liquids at room temperature. Component selection was guided by polarity, viscosity, hydrogen-bonding capacity and Generally Recognized as Safe (GRAS)/Cosmetic Ingredient Review (CIR) status. Of these, 15 hydrophilic and 15 hydrophobic *DES* were identified as viable candidates for extraction. The cascade process follows a three-stage sequence: (1) the use of hydrophilic *DES* to solubilize proteins and minerals, (2) the use of hydrophobic *DES* to extract astaxanthin, and (3) recovery of the chitin fraction. Extraction efficiency was monitored by UV-Vis spectrophotometry to quantify astaxanthin and protein concentrations in the extracts. This approach aims to maximize the recovery of valuable compounds from shrimp shells through a greener and more sustainable cascade process.

ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Slovenian Research Agency under the research core funding No. P2-0152, research projects J2-70079, NC-26001 and N2-0433, as well as the scholarship grant No.139205.

P42: GREEN EXTRACTION OF PROTEINS FROM MICROALGAE USING DEEP EUTECTIC SOLVENTS AS AN ALTERNATIVE TO CONVENTIONAL EXTRACTION METHODS

Anja Verbič,¹ Petja Logar,¹ Assya Bellaadem,¹ Florian Delrue,² Ilja Gasan Osojnik Črnivec,¹ Blaž Likozar,¹ Filipa A. Vicente¹

¹National Institute of Chemistry, Department of Catalysis and Chemical Reaction Engineering

²French Alternative Energies and Atomic Energy Commission (CEA)

e-mail: anja.verbic@ki.si

The increasing demand for sustainable and efficient biorefinery processes has driven the exploitation of green extraction media for the recovery of high-value compounds from renewable biomass. Microalgae, such as *Chlorella vulgaris*, represent promising sources of proteins. However, conventional extraction methods often rely on harsh chemicals or energy-intensive processes that may compromise protein integrity and environmental sustainability. In this study, deep eutectic solvents (DES) were investigated as environmentally benign alternative for the extraction of proteins from microalgal biomass. A systematic screening of different DES formulations was proposed, focusing on combinations of different hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD). In addition, the influence of different HBA:HBD molar ratios and water content was evaluated to optimize solvent properties, such as viscosity and extraction efficiency. The extraction performance was assessed to evaluate how DES composition influences protein recovery within a single microalgal system. Protein recovery efficiency was quantified and compared across different DES systems to identify optimal solvent formulations. The successful extraction was confirmed and quantified using UV/Vis spectroscopy. The results highlighted the relationship between DES composition and extraction efficiency, supporting the identification of optimal, tailor-made solvent systems. This work contributes to the development of sustainable microalgae biorefinery strategies, by demonstrating the potential DES as green, tunable solvents for efficient protein recovery, while reducing reliance on conventional extraction methods and supporting the transition towards more environmentally compatible processing routes.

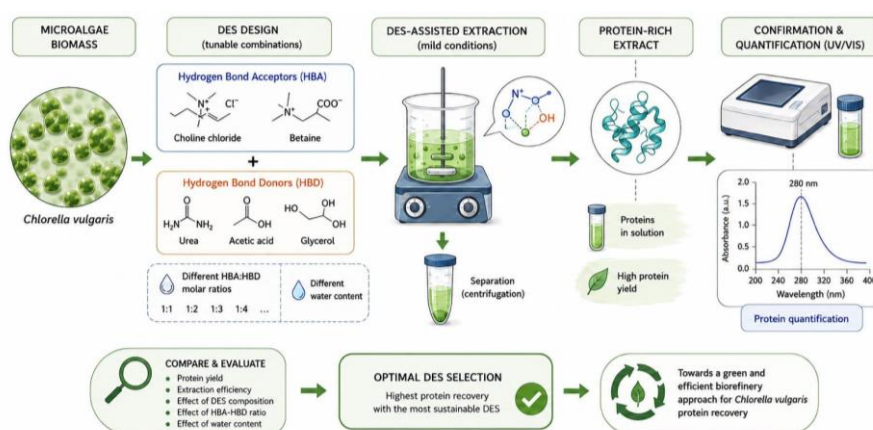


Figure 1: Schematic overview of DES-based protein extraction from microalgae biomass.

ACKNOWLEDGEMENTS

This research was funded by the Slovenian Research Agency under the research projects No. NC-26001, J2-70079 and N2-0433 and research core funding No. P2-0152.

REGULATORY AND SUSTAINABILITY CONSIDERATIONS

P43: CARBNETS PILLARS III AND IV: ADVANCED CARBON-SMART MATERIALS, WASTE HEAT RECOVERY, AND INDUSTRIAL WATER CIRCULARITY

Leon Kaluža,¹ Blaž Likozar,² Ilja Gasan Osojnik Črnivec¹

¹Center for Development, Demonstration and Training for Carbon-Free Technologies, National Institute of Chemistry, Kisovec, Slovenia

²Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Ljubljana, Slovenia

e-mail: leon.kaluza@ki.si

In the framework of the national flagship CarbNETs programme, the two of five interconnected pillars, Pillars III and IV, deal with secondary raw materials and the industrial waste heat recovery and wastewater circularity, respectively.^[1] Pillar III tackles the challenge of converting complex secondary raw materials into functional, high-value carbon-smart applications. Every year, substantial volumes of organic residues - including paper production sludges, lignocellulosic digestate, and mineral-rich industrial by-products - are discarded due to processing complexities. By employing green pre-treatment strategies with deep eutectic solvents and ionic liquids, Pillar III aims to achieve $\geq 80\%$ mineral impurity reduction. Advanced thermochemical processes, including Flash Joule Heating (FJH) and optimized pyrolysis, yield turbostratic graphene and biochar with tailored porosity and polarity for catalysts, electrodes, and sorbents. Simultaneously, mineral carbonation of industrial $\text{Ca}(\text{OH})_2$ waste with captured CO_2 produces high-purity ($>95\%$) nano-precipitated CaCO_3 . These fillers are incorporated into bio-based and recycled thermoplastic composites (bioPA, rPA, rPP, rABS), achieving up to 90% renewable/recycled content, a 40% improvement in mechanical properties, a 20% higher thermal resistance, and a 30% carbon footprint reduction. Complementing material synthesis, Pillar IV delivers a first-of-its-kind modular platform for industrial waste heat recovery and wastewater circularity.^[1] Thermal losses account for 20–50% of industrial energy inputs, while industrial freshwater withdrawal remains unsustainable. Pillar IV addresses these inefficiencies by recovering low-grade heat from compressors, furnaces, and CO_2 processes via thermochemical recuperators. This thermal energy is reused for sorbent regeneration, solvent preheating, and biological sludge drying, cutting sorbent regeneration energy demand by $\geq 25\%$, improving overall process efficiency by $\geq 15\%$, and reducing CO_2 emissions by $\geq 10\%$. In parallel, an integrated wastewater treatment system combining biosorbents (biochar, alginates), CO_2 -based pH regulation, and electrochemical purification achieves $>30\%$ removal of COD, TOC, NH_4^+ , and phosphorus. This enables closed-loop process water reuse across paper, chemical, and glass manufacturing sectors. Together, Pillars III and IV establish robust TRL 3 to TRL 6 technologies driving industrial decarbonization. in line with the Net-Zero Industry Act^[2] and the European Green Deal^[3].

ACKNOWLEDGMENTS

The CarbNETs programme is co-funded by the Republic of Slovenia, Ministry of Education, Science and Youth, and by the European Union under the Cohesion Policy Fund.

REFERENCES

- [1] CarbNETs Consortium. Presentation of the R&D Programme CarbNETs (Form 2). Public Call JR R&D PROGRAMMES (TRL 3-6); 2025.
- [2] European Commission. Net-Zero Industry Act: Accelerating the transition to climate neutrality. Brussels: EU Publishing; 2024.
- [3] European Commission, Fit for 55: Delivering the EU's 2030 Climate Target on the Way to Climate Neutrality, Brussels: EU Publishing; 2021.

P44: INTEGRATED LIFE CYCLE AND COST–BENEFIT ASSESSMENT OF POWDER AND CONVENTIONAL SHAMPOO SYSTEMS

Jan Puhar,¹ Tanja Tajnik, ¹ Martin Leban, Annamaria Vujanović¹

¹University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor, Slovenia

²123zero d.o.o., Ulica Petra Podleska 12, 2000 Maribor, Slovenia

e-mail: jan.puhar@um.si

The cosmetics sector is associated with significant environmental burdens related to packaging waste, water use, and transport intensity. Water-free formulations have recently emerged as an alternative to conventional liquid cosmetic products due to their reduced material and packaging requirements. In this study, a comparative life cycle assessment (LCA) of a powder shampoo system and a conventional liquid shampoo system was conducted in accordance with ISO 14040/44 standards and the Product Environmental Footprint (PEF) methodology.

The assessment was performed using a cradle-to-grave approach, including raw material acquisition, manufacturing, distribution, use, and end-of-life stages. The functional unit was defined as one hair wash under average European conditions. Environmental impacts were calculated using the EF 3.1 and ReCiPe methods with inventory data derived from industrial production data and Ecoinvent 3.9 datasets. In addition to product-level LCA, a scaling methodology was applied to evaluate large-scale deployment scenarios. Techno-economic parameters, including capital expenditure (CAPEX), operational expenditure (OPEX), and monetised environmental costs, were integrated into the assessment framework.

Results showed that the use phase dominated environmental impacts, accounting for approximately 78% of the total weighted score. Raw material acquisition contributed 28%, while manufacturing impacts remained below 1%. The powder shampoo system demonstrated lower impacts across most environmental categories, primarily due to reduced packaging requirements and lower transport intensity. The total environmental costs of the scaled system amounted to €11,616.12, with approximately 85% originating from the use phase.

The results demonstrate the environmental potential of water-free cosmetic systems and highlight the importance of integrating life cycle assessment with techno-environmental scaling approaches for sustainable product development.

ACKNOWLEDGMENTS

The authors acknowledge the support from the European Climate, Infrastructure and Environment Executive Agency (CINEA) for INSPIRE project (Grant Agreement Number: 101112879).

P45: PRELIMINARY TECHNO-ECONOMICAL ANALYSIS AND SOCIAL LIFE CYCLE ASSESSMENT FRAMEWORK FOR THE TOXBOX CHIP SYSTEM

Anja Verbič,¹ Andraž Teržan,¹ Blaž Likozar,¹ Filipa A. Vicente¹

¹National Institute of Chemistry, Department of Catalysis and Chemical Reaction Engineering

e-mail: anja.verbic@ki.si

TOXBOX is an advanced toxicological testing platform designed to provide a more reliable, human-relevant assessment of chemical and material effects. By combining innovative biological models, sensor technologies, and data-driven analysis, it supports faster, more reproducible, and more ethical toxicity screening while helping improve safety evaluation and product development. This work presents the preliminary TEA and S-LCA activities carried out for the TOXBOX chip system as an early-stage decision-support framework for future development and scale-up. The TEA focused on organizing available cost-related information for the consumable chip and its functional components. The chip was assessed as a recurring-use element within the wider platform, with attention to its role in long-term operating costs, commercial feasibility, market adoption, and future pricing or scale-up strategies. In parallel, the S-LCA was initiated as a preliminary hotspot and risk-screening exercise. It focused on mapping the value chain, identifying relevant stakeholder groups, and highlighting key social themes such as labor conditions, occupational health and safety, responsible sourcing, supplier practices, access to innovation, and potential societal benefits. Together, the TEA and S-LCA provide an early economic and social sustainability perspective. Future work should refine the value-chain model, improve data quality, validate assumptions, and collect supplier- and region-specific information to support responsible scale-up and market readiness.

ACKNOWLEDGEMENTS

This research was funded by the HORIZON-CL4-2023-RESILIENCE-01 Research and Innovation programme under Grant Agreement No. 101138387 (TOXBOX) and Slovenian Research Agency under the research core funding No. P2-0152.

BIOPRODUCTS ENGINEERING FOR BIOPROCESSING ENHANCEMENT

P46: EXPLORING THE ENZYMATIC DEGRADATION POTENTIAL OF BENZOXAZINE-BASED VITRIMERS

Miša Mojca Cajnko,¹ Antoine Adjaoud,² Filipa A. Vicente,¹ Pierre Verge²

¹National Institute of Chemistry, Slovenia

²Luxembourg Institute of Science and Technology, Luxembourg

e-mail: misa.cajnko@ki.si

Global plastics production and its accumulation in the environment has been steadily increasing, driven by its versatile applications, high production volumes and inadequate waste management.^[1,2] Although recycling is a potential solution, most of the plastic wastes are not reused due to technological constraints. This is particularly true for high-performance polymers, such as thermosets. Vitrimers have recently emerged as alternatives to thermosets,^[3] with similar 3D structures resulting from the crosslinking process, allowing for high thermal and mechanical properties, but composed of reversible covalent bonds that can be triggered under a specific stimulus.^[4]

Benzoxazines are another family of thermosets that has recently shown promising capabilities as vitrimers.^[5] In our study, we aimed to determine if benzoxazine-based vitrimers have the potential for enzymatic degradation, a more environmentally friendly method for polymer recycling. Three vitrimeric materials, differing in chain polarity, crosslinking density, and shape were synthesized and subjected to treatment with 2 ester bond-active enzymes, lipase and esterase. Additionally, choline chloride-based deep eutectic solvents (DES) were used as pre-treatments (choline chloride:oxalic acid; ChCl:U) and/or reaction medium (choline chloride:urea; ChCl:OxA) with the aim of increasing material susceptibility to enzymatic degradation or increasing enzyme activity. The enzymes showed no significant activity towards vitrimer 1 and two, which were more rigid and hard with high crosslinking density and low polarity, however, ChCl:OxA used as pre-treatment did cause notable structural changes such as increased flexibility or brittleness. On the other hand, with vitrimer 3, a soft polymeric film with low crosslinking density and high polarity, notable degradation with esterase was observed. This was confirmed by the FTIR analysis which showed cleavage of ester and C-N bonds within the polymer chain. The focus of future work will be to further investigate the DES-assisted enzymatic degradation of vitrimers, and the reuse of their degradation by-products, with aim of identifying chemical structures of vitrimers that are most suitable for enzymatic degradation.

ACKNOWLEDGMENTS

The work is the result of a preliminary study for the Weave FNR/2024 project VITRENZA (ARIS N2-0438).

REFERENCES

- [1] Isangedighi IA, David GS, Obot OI. Plastic waste in the aquatic environment: impacts and management. In: Analytical methods for nanoplastics and microplastics in food. Boca Raton (FL): CRC Press; 2020. p. 15-43.
- [2] Stapleton PA. Toxicological considerations of nano-sized plastics. AIMS Environ Sci. 2019;6(5):367-378.
- [3] Denissen W, Winne JM, Du Prez FE. Vitrimers: permanent organic networks with glass-like fluidity. Chem Sci. 2016;7(1):30-38.
- [4] Montarnal D, Capelot M, Tournilhac F, Leibler L. Silica-like malleable materials from permanent organic networks. Science. 2011;334(6058):965-968.
- [5] Adjaoud A, Puchot L, Verge P. Polybenzoxazine-based covalent adaptable networks: a mini-review. Polymer. 2023;287:126426.

P47: ECO-FRIENDLY METHODS FOR CAPTURING CO₂ WITH DEEP EUTECTIC SOLVENTS AND UTILIZING MICROALGAE FOR BIO FIXATION

Muhammad Irshad,¹ Ilja Gasan Osojnik Črnivec,¹ Dana Marinič,² Florian Delrue,³ Filipa A. Vicente²

¹Center for Development, Demonstration and Training for Carbon-Free Technologies, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia

²Department of Catalysis and Chemical Reaction Engineering, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

³MicroAlgae Processes Platform, CEA, CEA Tech Région Sud - Provence-Alpes Côte d'Azur, F-13108 Saint Paul lez Durance, France

e-mail: muhammad.irshad@ki.si

Increasing atmospheric CO₂ levels significantly contribute to climate change, highlighting the urgent need for effective and sustainable carbon capture technologies. This research investigates a unified method that merges green deep eutectic solvents (DES) for CO₂ capture with the following biofixation through microalgae.^[1] DES presents a viable alternative to traditional amine-based solvents, characterized by low toxicity, flexible properties, cost efficiency, and a lesser environmental footprint. This work evaluates selected DES formulations (choline chloride + urea (Reline), choline chloride + glycerol, and choline chloride + amino acids) for their CO₂ absorption capacity, stability, and recyclability under different operational conditions.^[2] Up to 40 DES will be screened for CO₂ adsorption to achieve over 70% CO₂ capture efficiency using microalgae.

CO₂ capture serves as a carbon source for photosynthesis in microalgal cultures. Microalgae effectively capture carbon and generate biomass suitable for biofuels, pharmaceuticals, and nutraceuticals. DES-based capture and algae biofixation form a closed-loop system that optimizes carbon utilization and minimizes emissions.

The research indicates that physicochemical and biological carbon management strategies are integrated. Solvent regeneration, mass transfer limitations, and changes to algal growth conditions are analyzed to enhance the efficiency and effectiveness of the integrated system. A hybrid strategy integrates effective CO₂ absorption with renewable biomass production to achieve a sustainable path to carbon neutrality.

REFERENCES

- [1] Ruan J, Chen L, Qi Z. Deep eutectic solvents as a versatile platform toward CO₂ capture and utilization. *Green Chem.* 2023;25(21):8328-8348.
- [2] Long S, Li J, Zhou W, Xin Y, Dong Z, Ning H, et al. Engineering low-viscosity and low-cost porous liquids using deep eutectic solvents for CO₂ selective separation. *Chem Eng Sci.* 2026;307:123708.

P48: ATR-FTIR CHEMOMETRICS REVEALS BATCH AND SOURCE VARIABILITY IN BIOMASS-DERIVED POLYSACCHARIDES FOR HYDROGEL MEMBRANE DEVELOPMENT

Vuk Martinović,¹ Ilja Gasan Osojnik Črnivec,¹ Anja Verbič,¹ Robert Dominko,¹ Blaž Stres,² Robert Dominko¹

¹National Institute of Chemistry, Center for Development, Demonstration and Training for Carbon-Free Technologies

²National Institute of Chemistry, Department of Catalysis and Chemical Reaction Engineering

e-mail: vuk.martinovic@ki.si

Biomass-derived polysaccharides are promising feedstocks for the development of sustainable hydrogel membranes because they are renewable, biodegradable, and chemically modifiable into functional materials with tunable swelling, hydration, and transport behavior.^[1,2] Among the most relevant candidates are chitosan, cellulose, starch, and alginate, which can be processed through chemical modification, blending, and crosslinking to obtain membrane-forming hydrogel systems.^[1,2] A major challenge in their development, however, is not only achieving the desired functionality, but also ensuring reproducibility between independently prepared batches.

This poster presents a proposed development and monitoring workflow in which batch-variability assessment is integrated directly into the process of polysaccharide-based hydrogel membrane design. In the proposed approach, biomass-derived polysaccharides are first selected and chemically tailored toward membrane-relevant properties, after which the resulting materials are formulated into hydrogel systems. Fourier-transform infrared (FTIR) spectroscopy is then used as a rapid analytical tool to monitor structural changes introduced during modification and to compare independently prepared batches.^[3] To evaluate reproducibility, the FTIR datasets are coupled with multivariate analysis, where principal component analysis enables visualization of replicate clustering and outlier detection, and PERMANOVA is used to distinguish within-batch variability from true between-batch differences.^[3]

The aim of the proposed workflow is to connect biomass valorization, functional material development, and analytical process control within a single framework. Such an approach could support more systematic optimization of polysaccharide modification procedures and provide a transferable strategy for monitoring quality and reproducibility in the development of sustainable hydrogel membrane materials.

REFERENCES

- [1] Li Z, Lin Z. Recent advances in polysaccharide-based hydrogels for synthesis and applications. *Aggregate*. 2021;2(2):e21.
- [2] Hameed MU, Ali L, Ahmad A, Zhang P, Raza S, Khan S, et al. Recent progress in biomass polymer based hydrogel membranes for adsorption and photocatalytic pollutants removal: fabrication, challenges and future perspectives. *Desalination*. 2025;615:119216.
- [3] Derksen GCH, Blommaert L, Bastiaens L, Haşşerbetçi C, Fremouw R, van Groenigen J, et al. ATR-FTIR spectroscopy combined with multivariate analysis as a rapid tool to infer the biochemical composition of *Ulva laetevirens* (Chlorophyta). *Front Mar Sci*. 2023;10:1154461.

P49: BEYOND SEPARATION: ATPS-BASED PLATFORMS FOR NEXT-GENERATION HEALTH APPLICATIONS

Mirna González-González,¹ Marco Rito-Palomares¹

¹Tecnológico de Monterrey, Institute for Obesity Research, Mexico

e-mail: mrto@tec.mx

Aqueous two-phase systems (ATPS), traditionally applied in downstream bioprocessing for biomolecule separation, are emerging as versatile platforms for engineering physiologically relevant three-dimensional (3D) cell microenvironments. In this work, we present the development of 3D-ATPS cell culture platforms that integrate biocompatibility, reproducibility, and cost-effectiveness to enhance the performance of cell-based bioprocesses. A systematic roadmap was established to optimize phase-forming polymer compositions, droplet volumes, and construction strategies (added, immersed, or covered), enabling the controlled formation of stable and reproducible microenvironments. Digital image analysis tools were implemented to quantify droplet size, circularity, and stability; guiding the selection of optimal conditions across different cell types.

The platform was validated through multiple applications. First, MCF-7 breast cancer cells were successfully encapsulated within PEG–dextran droplets.^[1] Second, uniform MDA-MB-231 spheroids were generated, reproducing key *in vivo* tumor features such as nutrient diffusion gradients and sustained metabolic activity.^[2] Third, a 3T3-L1 adipocyte 3D model was developed to study metabolic processes associated with obesity. Across all systems, 3D-ATPS enabled reproducible spheroid formation using standard laboratory infrastructure, demonstrating strong potential for low-cost and scalable implementation.

These results expand the functional scope of ATPS beyond conventional separation processes toward integrated bioprocess design. The proposed platforms support applications in high-throughput screening, metabolic modeling, and bioproduct evaluation, positioning 3D-ATPS cultures as enabling technologies for next-generation bioprocessing and translational health research.

ACKNOWLEDGMENTS

The authors wish to acknowledge the financial support of Tecnológico de Monterrey through the Bioengineering in Health Unit, as well as SECIHTI through Ciencia Básica y de Frontera grant CBF2023–2024-3252.

REFERENCES

- [1] Chairez-Cantu K, González-González M, Rito-Palomares M. Generation of MCF-7 spheroids in polyethylene glycol-dextran droplets for cancer niche studies using aqueous two-phase system-3D platforms. *Biotechnol J.* 2025;20(4):e70014.
- [2] González-González M, Villarreal-Alejandro J, Rito-Palomares M. Development and characterization of a polymer-polymer aqueous two-phase system 3D cell culture platform for MDA-MB-231 cancer cell spheroid analysis. *J Chem Technol Biotechnol.* 2026.

P50: BIOACTIVE AND METABOLOMIC POTENTIAL OF THE ENDEMIC BROWN MACROALGA *FUCUS VIRSOIDES*

Drago Šubarić,¹ Aly Castillo,² Marta Lores,² Krunoslav Aladić,¹ Ivana Flanjak,¹ Stela Jokić¹

¹Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Franje Kuhača 18, 31000 Osijek, Croatia

²Laboratory of Research and Development of Analytical Solutions (LIDSA), Department of Analytical Chemistry, Nutrition and Food Science, University of Santiago de Compostela, E-15782 Santiago de Compostela, Spain

e-mail: drago.subaric@ptfos.hr

The endemic brown macroalga *Fucus virsoides* J. Agardh, exclusive to the Adriatic Sea, underwent comprehensive bioactive and metabolomic characterization. Matrix solid-phase dispersion (MSPD) with GRAS solvents (ethanol/water, 50:50 v/v) was applied as a sustainable, mild-condition extraction strategy.

Targeted LC–MS/MS analysis identified key phenolics, including rosmarinic acid — reported here for the first time in *F. virsoides* — along with chlorogenic acid, caffeic acid, and catechin. The presence of rosmarinic acid, linked to desiccation stress responses, aligns with the intertidal ecology of this species under high Adriatic solar irradiance. Untargeted UHPLC–QToF metabolomics annotated eighty metabolites across twelve species, revealing a distinctive fingerprint for *F. virsoides*, enriched in glutamic and aspartic acids, mannitol, tagatose, succinic acid derivatives, sulphated phenolics — including 2,4-dihydroxyacetophenone 5-sulphate, reported for the first time in macroalgae — and hydroxybenzoates. PCA and PLS-DA confirmed *F. virsoides* as the most metabolomically distinct of the twelve species studied. These findings establish *F. virsoides* as a priority target for marine bioprospecting, underscoring its chemical richness and promise as a source of functional natural compounds.

ACKNOWLEDGMENTS

This work was funded by the Croatian Government and the European Union, through the European Regional Development Fund—the Competitiveness and Cohesion Operational Program (KK.01.1.1.01), for funding this research through the project Sustainable Bioprospecting of Organisms from the Adriatic Sea for Innovative Natural Products (KK.1.1.10.0001)—BioProCro, granted to the Center of Research Excellence for Marine Bioprospecting.

GOLD SPONSORS



SILVER SPONSORS



SUPPORTERS



OTHER



