# BOOK OF ABSTRACTS

Mini-symposium:

## Biocatalytic Process Intensification Using Deep Eutectic Solvents in Microflow Systems for Sustainable Waste Valorization

8 July 2025, Zagreb

## Welcome Message

The Organizing Committee of the mini-symposium *Biocatalytic Process Intensification Using Deep Eutectic Solvents in Microflow Systems for Sustainable Waste Valorization* warmly welcomes you to Zagreb and the University of Zagreb Faculty of Food Technology and Biotechnology.

This event brings together researchers to share knowledge and ideas on innovative strategies for enhancing the efficiency and sustainability of biocatalytic processes. The mini-symposium will focus on cutting-edge developments in reaction media, particularly deep eutectic solvents, and the use of microreactor systems to improve control, scalability, and process intensification. Our goal is to explore technologies that support the conversion of waste materials into valuable chemical products.

We hope the mini-symposium will inspire your ongoing work and future research. Thank you for your participation and valuable contribution.

*Marina Cvjetko Bubalo, Co-chair of the Mini-symposium*

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## Program

| TIME | ACTIVITY |
|---|---|
| **09:45** | **Registration** |
| **10:00** | **Opening Remarks**<br>*Polona Žnidaršič Plazl and Marina Cvjetko Bubalo*<br>Welcome and introduction to the project aims and outcomes, and an overview of the symposium topics |
| **SESSION I – SOLVENT DESIGN IN BIOCATALYSIS<br>CHAIR: MARINA CVJETKO BUBALO** | |
| **10:15** | *Rational design of deep eutectic solvents from natural metabolites: toward biocompatible media for enzyme-catalyzed reactions* (Anja Damjanović) |
| **10:35** | *Biocatalytic $CO_2$ conversion in deep eutectic solvents: toward sustainable solvent engineering* (Marijan Logarušić) |
| **10:55** | *Artificial neural networks in the rational design of DESs: promise and potential* (Ana Jurinjak Tušek) |
| **11:15** | *From lab to market: IP strategies for natural deep eutectic solvents innovation* (Manuela Panić) |
| **11:35** | **Brunch / Networking Break** |
| **SESSION II – MICROFLOW SYSTEMS FOR BIOCATALYTIC PROCESS INTENSIFICATION<br>CHAIR: POLONA ŽNIDARŠIČ PLAZL** | |
| **12:30** | *Self-assembled enzyme–nanoparticle structure for transamination* (Borut Šketa) |
| **12:50** | *DES-modulated hydrogels and nano-CLEAs in microreactors for advanced biocatalysis* (Tadej Menegatti) |
| **13:10** | *Sustainable synthesis of furfurylamine in a magnetic field-assisted microfluidic Reactor* (Marko Božinović) |
| **13:30** | *Microfluidic pervaporation unit and mathematical modeling for downstream intensification* (Igor Plazl) |
| **13:50** | **Discussion and Final Remarks**<br>Open floor for questions and discussion, closing comments by the organizers |

# ORAL PRESENTATIONS

# RATIONAL DESIGN OF DEEP EUTECTIC SOLVENTS FROM NATURAL METABOLITES: TOWARD BIOCOMPATIBLE MEDIA FOR ENZYME-CATALYZED REACTIONS

*Anja Damjanović[a]*, Marijan Logarušić[a], Lidija-Marija Tumir[b], Ana Jurinjak Tušek[a], Marina Cvjetko Bubalo[a], Ivana Radojčić Redovniković[a]*

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Deep eutectic solvents (DES), emerging as biocompatible and sustainable alternatives to conventional solvents, offer unique advantages for stabilizing proteins and enabling enzyme-catalyzed transformations. Comprising mixtures of hydrogen bond donors and acceptors, DES can maintain a liquid state at relatively low temperatures and be tailored from natural, non-toxic components. Their tunable properties and compatibility with biological systems make them promising media for biocatalysis and protein stabilization. In this study, we focused on the rational development of DES using naturally derived metabolites, with a particular emphasis on their interaction with the model protein lysozyme. The research addressed key objectives: preparation of DES systems, characterization of their physicochemical and toxicological properties, and assessment of their suitability for enzyme-related applications. Special attention was given to evaluating lysozyme stability in DES under stress conditions, including thermal shock and freeze–thaw cycles, to understand their potential as protective media. Bioinspired DES formulations were developed based on metabolites commonly associated with cellular stress protection. These DES showed enhanced stabilization of lysozyme during thermal shock (80 °C) and freeze–thaw cycles. Spectroscopic analyses revealed that DES with moderate water content (20–40% w/w) promoted conformational shifts toward more stable structures. Upon dilution in buffer, lysozyme regained its native structure and activity, confirming reversible stabilization. The study supports the concept that natural-metabolite-based DES can function similarly to osmolytes, protecting proteins under denaturing conditions. Detailed statistical analysis of the results enabled prediction of enzyme–solvent compatibility, streamlining solvent design. Overall, the findings highlight the potential of these DES as green, functional media for biocatalysis and enzyme formulation in biotechnology.

*Keywords: biocatalysis, deep eutectic solvents, enzyme stabilization, lysozyme, solvent design*

# BIOCATALYTIC $CO_2$ CONVERSION IN DEEP EUTECTIC SOLVENTS: TOWARD SUSTAINABLE SOLVENT ENGINEERING

*Marijan Logarušić[a*], Ana Jurinjak Tušek[a], Anja Damjanović[a], Mia Radović[a], Ivana Radojčić Redovniković[a], Polona Žnidaršič-Plazl[b], Marina Cvjetko Bubalo[a]*

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Biocatalytic conversion of $CO_2$ into value-added chemicals such as methanol offers a sustainable route toward circular economy and climate change mitigation. A well-studied cascade enzymatic process comprising formate dehydrogenase (FDH), formaldehyde dehydrogenase (FaldDH), and alcohol dehydrogenase (ADH) has demonstrated this potential, though challenges such as low $CO_2$ solubility, NADH instability, and enzyme deactivation limit its industrial feasibility. The presnted work explores deep eutectic solvents (DESs) as tunable, biocompatible media to overcome these hurdles. Using the reduction of $CO_2$ to formate by FDH as a case study, an integrated computational and experimental strategy identified a choline chloride:glycerol DES formulation that enhances NADH and enzyme stability, improves $CO_2$ solubility, and boosts volumetric productivity. COSMO-RS modeling and QSAR-based predictions guided solvent design, while experimental screening validated enzyme activity, stability, and NADH retention. These findings highlight the promise of DESs in enabling efficient and continuous $CO_2$ biotransformations by stabilizing key reaction components and facilitating cofactor recycling.

*Keywords: deep eutectic solvents (DESs), green biocatalysis, $CO_2$ reduction, enzyme stability*

# ARTIFICIAL NEURAL NETWORKS IN THE RATIONAL DESIGN OF DESs: PROMISE AND POTENTIAL

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Deep Eutectic Solvents (DES) have gained significant attention as innovative and tunable liquid media with diverse applications, particularly in biocatalysis, where they are crucial for enhancing substrate solubility, modulating enzyme stereoselectivity, and notably, improving enzyme activity and stability. However, the traditionally employed trial-and-error methods for identifying optimal DES for specific applications are time-consuming and inefficient due to the vast and potentially unlimited chemical space of possible DES structural combinations. This hinders the strategic and rational design of these solvents.

To overcome these challenges, the development of mathematical tools for rational DES design is imperative. The Conductor-like Screening Model for Real Solvents (COSMO-RS) provides a computational approach to generate molecular σ-profiles, which serve as crucial molecular descriptors. These *σ*-profiles contain essential information about electrostatic, hydrogen bonding, and dispersion interactions, allowing for the quantification of structural changes. When combined with quantitative structure-property relationship (QSPR) and machine learning methods, such as Artificial Neural Networks (ANN), these descriptors prove effective, reliable, and cost-efficient for predicting DES properties.

Recent research underscores the significant potential of ANN modeling for predicting DES properties. ANN models have been successfully developed to predict enzyme inactivation constants for dehydrogenases like ADH-A, GDH, and Lk-ADH. For ADH-A and GDH, these models demonstrated high efficiency and reliability ($R^2$ > 0.75), with metrics indicating they are "excellent" (RPD>2) and suitable for screening (4<RER<11). Furthermore, ANNs have been effectively applied to predict the pH values of DES, achieving very high accuracy ($R^2$ independent validation > 0.8600) across a wide range of acidic and basic DES. QSAR models utilizing ANN also successfully predicted formate dehydrogenase (FDH) activity and stability, as well as NADH stability in DES, with models being considered "substantial" or "reliable".

The most important advantages of ANN modeling for predicting DES properties include: (i) high predictive accuracy and reliability, (ii) in silico screening, (iii) exploration of vast chemical space, (iv) increased efficiency and accelerated development and (v) reliable and efficient means for the rational design of DES, thereby significantly advancing the application of biocatalysis and other industrial processes where these versatile solvents are crucial.

*Keywords: artificial neural networks, biocatalysis, COSMO-RS, deep eutectic solvents, rational design*

# FROM LAB TO MARKET: IP STRATEGIES FOR NATURAL DEEP EUTECTIC SOLVENTS INNOVATION

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In light of increasing environmental awareness and technological progress, the demand for sustainable chemical processes and materials is intensifying. Natural Deep Eutectic Solvents (NADES) have emerged as promising components of green chemistry due to their biodegradability, non-toxicity, simple preparation, and customizable properties. These features make them especially suitable for use in sectors such as pharmaceuticals and the extraction of bioactive compounds.

Although NADES technologies have already contributed to significant scientific and industrial advancements, their broader market adoption depends heavily on effective intellectual property (IP) management. IP rights are essential for protecting innovations, facilitating technology transfer, and supporting sustainable economic development. This lecture aims to explore IP management as a critical mechanism in the development and commercialization of NADES-based technologies, highlighting the strategic intersection of scientific innovation, legal protection, and business application.

A case study on technology transfer from the University of Zagreb to the start-up company NADES Design is presented, offering practical insights into the commercialization process. Additionally, the lecture examines international examples of successful IP-based strategies involving patents and strategic partnerships, including companies such as Korres and Gattefossé.

The findings emphasize that robust IP management plays a key role in transforming scientific excellence into marketable solutions. Through strategic protection and knowledge transfer, NADES technology can significantly contribute to green innovation and sustainable industrial growth.

*Keywords: biotechnology, green chemistry, intellectual property, NADES, technology transfer*

# SELF-ASSEMBLED ENZYME-NANOPARTICLE STRUCTURE FOR TRANSAMINATION

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The immobilization of biocatalysts using nanomaterials has become increasingly popular in recent years as it offers several advantages. Nanosized carriers have high mechanical strength and a high surface-to-volume ratio, which enables higher enzyme loading and faster mass transport. At the same time, enzyme immobilization enables recovery and reuse of the enzyme and often stabilizes the enzyme itself. [1]

In this work, $SiO_2$ nanoparticles [2] were used as carriers for amine transaminase, which was immobilized via a $His_6$-tag. The aim was to optimize the self-assembling structures for later use in a microreactor. To this end, nanoparticles of 100, 250, and 500 nm were used in combination with three linkers of varying lengths. Cobalt, nickel, copper, iron, gadolinium and lanthanum ions were compared as chelating agents in the $His_6$-tag immobilization.

The results show a correlation between the particle size (and thus the surface-to-volume ratio) and the immobilization yield, with the smaller particles being preferred. Among the ions tested, cobalt showed the highest relative recovery activity of over 36 %. [3, 4] The immobilization of enzyme nanoparticle structures on a glass plate was performed and characterized using XPS analysis, which revealed the elemental composition after each step of the functionalization and immobilization process.

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# DES-MODULATED HYDROGELS AND NANO-CLEAs IN MICROREACTORS FOR ADVANCED BIOCATALYSIS

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Hydrogels are promising carriers for enzyme immobilization in flow biocatalysis due to their tunable structure and biocompatibility [1]. When using deep eutectic solvents (DESs) as green reaction media, tailoring hydrogel properties becomes crucial. DESs can modify hydrogel porosity, mechanical stability, and polarity, thereby improving enzyme performance and mass transfer under continuous conditions. In this work, we investigated DES-enhanced poly(vinyl alcohol)-hydrogels, optimizing crosslinking and solvent compatibility for improved structural integrity and enzyme support[2]. Systematic tuning of DES content and hydrogel formulation led to better rheological properties and stability in DES-rich environments, establishing a platform for effective immobilization in non-aqueous biocatalytic systems.

In parallel, we developed a microfluidic method for producing nanoscale cross-linked enzyme aggregates (nano-CLEAs) of ω-transaminase (ATA), addressing limitations of conventional CLEA synthesis. Traditional batch processes yield heterogeneous, fragile particles unsuitable for continuous reactors. Our approach spatially separates acetone-induced precipitation and glutaraldehyde crosslinking, enabling precise control over particle formation. The resulting nano-CLEAs (~100 nm) retained up to 87.1% activity—2.4× higher than batch CLEAs—and demonstrated superior stability. Integration with a membrane microreactor enabled one-step purification and immobilization with 100% immobilization yield. The immobilized nano-CLEAs reached 68% immobilization efficiency and showed a 45% higher turnover number than the non-aggregated enzyme over five days of continuous operation [3].

We now aim to combine both advances by embedding ATA nano-CLEAs into DES-optimized hydrogels within a microreactor, creating a modular system for efficient and sustainable continuous biocatalysis.

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*Keywords: hydrogel, biocatalysis, microreactor, immobilization, CLEA*

# SUSTAINABLE SYNTHESIS OF FURFURYLAMINE IN A MAGNETIC FIELD-ASSISTED MICROFLUIDIC REACTOR

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Furfurylamine is a valuable chemical intermediate with wide-ranging industrial applications, particularly in pharmaceutical synthesis [1-2]. To promote more sustainable production, recent efforts have focused on biocatalytic approaches using amine transaminases [2-4]. In this study, we present an efficient biocatalytic transamination employing N-$His_6$-ATA-wt with (*S*)-(−)-*α*-methylbenzylamine as the amine donor. An impressive 97% furfurylamine yield was achieved within just 30 min of batch reaction using equimolar substrate concentrations, enhancing cost efficiency, reducing waste, and aligning with green chemistry principles [5].

To move to continuous production, the enzyme was immobilized on custom-made and functionalized magnetite nanoparticles, and the immobilization protocol was optimized, achieving a high recovered activity of 92.8%. The stability test of the immobilized enzyme revealed a total turnover number of $2.04 \cdot 10^7$, underlining its promising suitability for industrial-scale applications [5]. This work demonstrates a highly efficient, scalable, and environmentally sustainable strategy for the continuous synthesis of furfurylamine.

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*Keywords: furfural, furfurylamine, amine transaminase, biomass valorization, enzyme immobilization, green chemistry*

# MICROFLUIDIC PERVAPORATION UNIT AND MATHEMATICAL MODELING FOR DOWNSTREAM INTENSIFICATION

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Downstream separation remains a major bottleneck in the development of continuous bioprocesses, particularly in the removal of volatile organic by-products such as acetone and acetophenone. Here we present a novel microfluidic pervaporation device developed for efficient VOC extraction from aqueous media under steady-state conditions. The system integrates a 3D-printed channel architecture with a PDMS membrane and leverages a first-principles mathematical model that does not require customised parameters. Experimental validation with acetone–water and acetophenone buffer mixtures demonstrated excellent separation performance with a separation efficiency of over 97 %. A detailed time scale analysis confirmed that membrane diffusion is the dominant transport resistance, guiding optimization strategies toward geometric design and membrane material selection. The predictive power of the developed mathematical model enabled a rational tuning of geometry and flow conditions, while the modular fabrication approach ensures adaptability and scalability. This work highlights the potential of combining microfluidics, modeling and additive manufacturing to intensify separations and enable seamless integration into upstream biocatalytic or pharmaceutical production systems.

*Keywords: microfluidic pervaporation, volatile organic compounds (VOCs), process intensification, mathematical modeling, continuous separation*