

4th International Conference on Sustainable Chemical & Environmental Engineering



SUST
ENG
2025

1 - 4 September 2025

Thessaloniki, Greece

hybrid event

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organizers



Design of
Environmental
Processes Lab



Technical University of Crete
School of Chemical and
Environmental Engineering



Cyprus
University of
Technology

Dear friends and colleagues,

I am pleased to invite you to the **4th International Conference on Sustainable Chemical and Environmental Engineering (SUSTENG 2025)**, between **1st to 4th September 2025**.

The conference is organized by the "Design of Environmental Processes Laboratory", of the School of Chemical and Environmental Engineering, Technical University of Crete and the Cyprus University of Technology.

The **SUSTENG 2025** Conference will take place both with physical and virtual presence.

The conference aims to encourage the exchange of knowledge between academicians, scientists and engineers on hot issues and current developments in chemical and environmental engineering through sustainable perspective. The participants will have the opportunity to present their recent research findings on an extended spectrum of conference topics and be informed about new challenges, future trends, and technological innovations on sustainable processes following the principles of circular economy.

After the completion of the conference, all abstracts/full papers will be published in the **SUSTENG 2025** Conference Proceedings with ISBN number, while selected manuscripts will be published to at least one Special Issues at high IF scientific journals.

The conference Chair

Petros Gikas

Professor

Head of the "Design of Environmental Processes Lab"

School of Chemical and Environmental Engineering

Technical University of Crete, Greece



SESSIONS OF SUSTENG 2025

Session 1: Biochemical engineering and green chemistry

Session 2: Valorization of food waste

Session 3: Wastewater management

Session 4: Solid waste management and greenhouse gas monitoring

Session 5: Modeling and optimization in energy and environment

Session 6: Circular economy in waste management

Session 7: Sustainable agriculture and waste management

Session 8: Sustainable water management and reuse

Session 9: Biosolids management and valorization

Session 10: Life cycle assessment

Scientific committee:

Agboka, Komi, University of Lome, Togo

Akratos, Christos, Democritus University of Trace, Greece

Andreopoulos, Andreas, National Technical University of Athens, Greece

Angeli, Panagiota, University College, UK

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Chile, Chile

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Greece

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Juchelkova, Dagmar, Technical University of Ostrava, Czech Republic

Juizo, Dinis, University of Eduardo Mondlane, Mozambique Kakai,

Romain Lucas Glele, University of Abomey Calavi, Benin

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Lyberatos, Gerasimos, National Technical University of Athens, Greece

Mahdjoub, Noredine Loeid, University of Kwa Zulu-Natal, South Africa

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Moustakas, Konstantinos, National Technical University of Athens, Greece

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Ntougias, Spyridon, Democritus University of Thrace, Greece

Odirile, Phillimon, University of Botswana, Botswana

Panagiotopoulou, Paraskevi, Technical University of Crete, Greece

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Papadakis, Georgios, Agricultural University of Athens, Greece

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George, University of Thessaly, Greece

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Shaer, Mohamed El, Zagazig University, Egypt

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Stefanakis, Alexandros, Technical University of Crete, Greece

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Tchobanoglous, George, University of California Davis, USA

Tejeda, Lesly, University of Cartagena, Colombia

Tekerlekopoulou, Athanasia, University of Patras,

Greece Tiburue, Elvis, University of Ghana, Ghana

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Kolokotsa, Dionysia, Technical University of Crete, Greece

Komesli, Okan Tarık, Atatürk University, Turkey

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Kumar, Manish, University of Petroleum and Energy Studies, India

Kungolos, Athanasios, Aristotle University of Thessaloniki, Greece

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Kuroda, Keisuke, Toyama Prefectural University, Japan

Kyzas, George, International Hellenic University, Greece

Lin, Justin Chun-Te, Feng Chia University, Taiwan

Livingstone, Andrew, Queens Mary University, UK

Torosyan, Gagik, State Engineering University of Armenia, Armenia

Trois, Cristina, University of Kwa Zulu –Natal, South Africa

Tsanis, Ioannis, Technical University of Crete, Greece

Tsihrintzis, Vassilios, National Technical University of Athens, Greece

Tsoutsos, Theocharis, Technical University of Crete, Greece

Vakalis, Stergios, University of the Aegean, Greece

Vamvouka, Despoina, Technical University of Crete, Greece

Vayenas, Dimitris, University of Patras, Greece

Venieri, Danae, Technical University of Crete, Greece

Vlysidis, Anestis, National Technical University of Athens, Greece

Voulgarakis, Apostolos, Technical University of Crete, Greece

Vyrides, Ioannis, Cyprus University of Technology, Cyprus

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Zhuang, Wei-Qin, University of Auckland, New Zealand

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SUSTENG 2025- PROGRAMME AT A GLANCE

DAY 1 – Monday 1 September Susteng 2025	
Aristotle University Research Dissemination Center (KEDEA)	
Registration	14:00 - 16:00
Session 1: Biochemical engineering and green chemistry	16:00 - 18:00
Welcome Speeches Stavros Kalafatis-Vice Minister of Development Konstantinos Gioutikas-Vice Governor of Thessaloniki Stelios Angeloudis-Mayor of Thessaloniki Dimitris Aslanidis, Mayor of the Municipality of Pavlos Melas Kyriakos Anastasiadis-Rector of the Aristotle University of Thessaloniki Michael Zervakis-Rector of the Technical University of Crete George Savaidis-Dean of the Faculty of Engineering, Aristotle University of Thessaloniki Vasileios Zaspalis-School of Chemical Engineering, Aristotle University of Thessaloniki Aris Gorogias-President at Hellenic Association of Chemical Engineers Michael Geranis-President of the Regional Association of Solid Waste Management Agencies of Central Macedonia Yiannis Tsouloftas-President of the Local Government Organization of Limassol District, Cyprus	18:00 - 18:30
PLENARY LECTURE: Professor Petros Gikas	18:30 - 19:15
Welcome reception	19:30 - 22:00
DAY 2 – Tuesday 2 September Susteng 2025	
Aristotle University Research Dissemination Center (KEDEA)	
Session 2: Valorization of food waste	09:30 - 10:45
Coffee break - Posters viewing	10:45 - 11:00
Session 3: Wastewater management	11:00 - 12:30
PLENARY LECTURE: Professor Güçlü Insel	12:30 - 13:15
Lunch break	13:15 - 14:45
Session 4: Solid waste management and greenhouse gas monitoring	14:45 - 16:30
PLENARY LECTURE: Professor Paraschos Melidis	16:30 - 17:15
Session 5: Modeling and optimization in energy and environment	17:15 - 19:00

Gala dinner	20:30 - 23:00
DAY 3 – Wednesday 3 September Susteng 2025	
Aristotle University Research Dissemination Center (KEDEA)	
Session 6: Circular economy in waste management	09:30 - 10:45
<i>Coffee break - Posters viewing</i>	10:45 - 11:00
PLENARY LECTURE: Professor Jacek Dach	11:00 - 11:45
Session 7: Sustainable agriculture and waste management	11:45 - 13:15
<i>Lunch break</i>	13:15 - 14:45
PLENARY LECTURE: Professor Konstantinos Kakosimos	14:45 - 15:30
Session 8: Sustainable water management and reuse	15:30 - 16:15
Session 9: Biosolids management and valorization	16:15 - 18:30
Session 10: Life cycle assessment	18:30 - 19:15
Closing ceremony	19:15 - 19:45
DAY 4 - Thursday 4 September Susteng 2025	
Conference excursion at the capital of ancient Macedonia Aegae, in Vergina, and the grave of Philip II, father of Alexander the Great, and the Byzantine town of Veria	09:00 - 17:00



SUSTENG 2025 PROGRAMME

DAY 1 – Monday 1 September Susteng 2025	
Aristotle University Research Dissemination Center (KEDEA)	
Registration	14:00 - 16:00
Session 1: Biochemical engineering and green chemistry Chair: Emine Çokgör, Manal Haj Zaroubi	16:00 - 18:00
Anaerobic production of biosurfactants from contaminated soils: a waste valorization approach to soil remediation M. Puddu, G. Beretta, S. Saponaro and E. Sezenna	16:00 - 16:15
Carbon dioxide capture from actual industrial flue gas using microalgae G. Makaroglou, P. Gikas	16:15 - 16:30
From lab to pilot scale Microalgae-based Photobioreactors: statistical optimization of 3 rd generation biofuel production A. Pavlou, G. Penloglou and C. Kiparissides	16:30 - 16:45
Willow (<i>salix acmophylla boiss.</i>) Leaf and Branch Extracts Inhibit in <i>vitro</i> Sporulation of <i>Coccidia</i> (<i>Eimeria</i> spp.) from Goats M. Haj-Zaroubi, N. Mattar, S. Awabdeh, R. Sweidan, A. Markovics, J. D. Klein and H. Azaizeh	16:45 - 17:00
Optimization of high-added value terpenes recovery from genetically engineered <i>Saccharomyces cerevisiae</i> and <i>Yarrowia</i> <i>lipolytica</i> strains A. Moschona, C. Gerasi, M. Moumin, F. Tsakiroglou, A. Assimopoulou and S.I. Patsios	17:00 - 17:15
Valorization of Invasive Macroalgae (<i>Asparagopsis armata</i> and <i>Sargassum</i> spp.) as Sources of Immune-Enhancing Aquafeed Ingredients: Recovery and In-Vitro Fish Digestibility Assessment S. Papadaki, C. Stergiopoulos, N. Tricha and M. Krokida	17:15 - 17:30
Life cycle assessment of systems exploiting microalgae for the wastewater treatment G. Dolci, M.J.F.A. Nichilo, M. Grosso, and L. Rigamonti	17:30 - 17:45

Efficient mechanochemical synthesis of conjugated porous (macro)molecules via multiple Sonogashira couplings and their energy storage application R. S. Rao, A. Shunnar, B. Pirzada, E. Oueis, A. B. Lowe and J. Shaya	17:45 - 18:00
Welcome Speeches Stavros Kalafatis-Vice Minister of Development Konstantinos Gioutikas-Vice Governor of Thessaloniki Stelios Angeloudis-Mayor of Thessaloniki Dimitris Aslanidis, Mayor of the Municipality of Pavlos Melas Kyriakos Anastasiadis-Rector of the Aristotle University of Thessaloniki Michael Zervakis-Rector of the Technical University of Crete George Savaidis-Dean of the Faculty of Engineering, Aristotle University of Thessaloniki Vasileios Zaspalis-School of Chemical Engineering, Aristotle University of Thessaloniki Aris Gorogias-President at Hellenic Association of Chemical Engineers Michael Geranis-President of the Regional Association of Solid Waste Management Agencies of Central Macedonia Yiannis Tsoulouftas-President of the Local Government Organization of Limassol District, Cyprus	18:00 - 18:30
PLENARY LECTURE: Professor Petros Gikas Energy Self-Sustainable Wastewater Treatment Plants Chair: Güçlü Insel, Jacek Dach	18:30 - 19:15
Welcome reception Aristotle University Research Dissemination Center (KEDEA)	19:30 - 22:00
DAY 2 – Tuesday 2 September Susteng 2025	
Aristotle University Research Dissemination Center (KEDEA)	
Session 2: Valorization of food waste Chair: Caro Dario, Emine Çokgör	09:30 - 10:45
Comparing the carbon footprint of traditional beef burger with burgers fortified with by-products D. Caro	09:30 - 09:45
Sustainable valorization of seafood waste: enzyme-based chitin extraction as an alternative to chemical processes I. Kan, A. Morales, L. Sillero and X. Erdocia	09:45 - 10:00
Pomegranate Peel Bioplastic: Transforming Food Waste into Eco-Friendly Materials for Product Design L. Mailat, Delia A. Prisecaru	10:00 - 10:15

Valorisation of watermelon peel into an adsorbent for removal of hexavalent chromium Mangwandi Chirangano, GartlanCiaran, Khong Justin	10:15 - 10:30
Integrated Approach for Sustainable Protein Production: Utilizing Agricultural Waste for Black Soldier Fly Rearing and Feed Applications A. Jonas-Levi, I. Opatovsky, A. Hurvitz, A. Salomon, R. Gutman, R. Sivan	10:30 - 10:45
<i>Coffee break - Posters viewing</i>	10:45 - 11:00
Session 3: Wastewater management Chair: Paraschos Melidis, Güven Didem	11:00 - 12:30
Microalgal treatment of AnMBR Effluent: a low-energy approach to nutrient reduction E. Politou, A. Makri, D. Matziri, N. Remmas, S. Ntougias and P. Melidis	11:00 - 11:15
Circular economy strategy in wastewater treatment: the example of Limassol district local government organisation sewage treatment plant in Moni, Cyprus Y. Tsouloftas, S. Metaxas, M. Vrionides, P. Marti, V. Konstantinides and G. Panayiotou	11:15 - 11:30
Integrating greywater treatment and green façade creation using vertical flow constructed wetlands E. Stefanatou and M.S. Fountoulakis	11:30 - 11:45
An Appraisal for a Sustainable Process Design and Operation for Leachate Treatment D. Guven, E. Çokgör, G.E. Zengin Balci, B. Kaya, G. Ozyildiz, D. Soylu, I. Takacs, G. Insel, S. Sözen, and D. Orhon	11:45 - 12:00
Removal of non-steroidal anti-inflammatory micropollutants and nitrification in hybrid systems operating at low sludge age D. Soylu, B. Ciftcioglu Gozuacik, Ş. Yilmaz, G.E.Zengin, İ. Pala-Ozkok, E. Topuz, G. Ozyildiz, D. Guven, G. Insel and E. Çokgör	12:00 - 12:15
Quantitative Evaluation of Nitrification Inhibition in Petrochemical Wastewater AT. Cetinkaya, P. Eryavuz, G. Ozyildiz, D.Güven, N. Artan, E. Çokgör, G. Insel	12:15 - 12:30
PLENARY LECTURE: Professor Güçlü Insel Novel hybrid nutrient removal process for advanced nutrient removal: experimental based process development Chair: Paraschos Melidis, Petros Gikas	12:30 - 13:15
<i>Lunch break</i>	13:15 - 14:45

Session 4: Solid waste management and greenhouse gas monitoring Chair: Francesca Villa, Güçlü Insel	14:45 - 16:30
Dewatering of landfill leachate by combined flocculation with geotextile filtration B. Kaya, S. Dogruel, V. Oztug and E. Çokgör	14:45 - 15:00
Air Quality Assessment in the Municipality of Pavlos Melas, Thessaloniki S. Cheristanidis, E. Pitsalidis and D. Melas	15:00 - 15:15
The Problem Tree: a strategic tool for priorities' identification, applied to the assessment of operational and design issues of a solid waste treatment facility in Lebanon F. Villa, A. De Robertis and M. Grosso	15:15 - 15:30
Management of household hazardous waste: survey and comparative analysis M. Markantoni, T. Daras and A. Giannis	15:30 - 15:45
Ternary Deep Eutectic Solvent-Mediated Leaching of Valuable Metals from Waste Printed Circuit Boards A. Alaydaroos, N. Chola, S. Stephen, G.E. Luckachan, G. Xavier, M. Elkadi, E. AlNashef, M. Abi Jaoude	15:45 - 16:00
Comparison of source and site level methane emissions measurements at gas installations N. Tsochatzidis	16:00 - 16:15
Preliminary investigation of removal of pharmaceuticals from aqueous solutions by potato peel based biochars produced from hydrothermal conversion and pyrolysis: a comparative study J.Khong, and C. Mangwandi	16:15 - 16:30
PLENARY LECTURE: Professor Paraschos Melidis Anaerobic Membrane Bioreactors for Sustainable Municipal Wastewater Treatment Chair: Petros Gikas, Jacek Dach	16:30 - 17:15
Session 5: Modeling and optimization in energy and environment Chair: Thomas Thomaidis, Vassilis Charitopoulos	17:15 - 19:00
An optimisation approach towards the integration of hydrogen in heat and power decarbonization M.E. Efthymiadou, V.M. Charitopoulos and L.G. Papageorgiou	17:15 - 17:30
Deciphering the impact of AI's energy demand on Europe's Green Deal M. Hemmati, V. M Charitopoulos	17:30 - 17:45

Assessment of water-energy-food-ecosystem nexus of sakarya river basin S. Yaykiran and A. Ekdal	17:45 - 18:00
Integrating sustainability into decision support for the transition to resilient business models I. Mavrogiannis	18:00 - 18:15
Development of a decision support system for shallow lakes under the impact of eutrophication and climate change and its application to a hypertrophic lake A. Ekdal, M. Gürel, C. Gurevin and A. Ertürk	18:15 - 18:30
Comparative Assessment of Alternative Waste Management Scenarios for Mixed Municipal Solid Waste: Case study of Cyprus S. Mai, E.M. Barampouti, D. Malamis, K. Moustakas and M. Loizidou	18:30 - 18:45
Policy Pathways for Marine Litter Prevention and Control: Insights from the SeaClear2.0 Project D. Orthodoxou, X. Loizidou	18:45 - 19:00
Gala dinner	20:30 - 23:00
DAY 3 – Wednesday 3 September Susteng 2025	
Aristotle University Research Dissemination Center (KEDEA)	
Session 6: Circular economy in waste management Chair: Fabio Hernández-Ramos, Abeer Albalawneh	09:30 - 10:45
Deep eutectic solvents and microwave heating for efficient oligosaccharide recovery from apple tree pruning waste L. Sillero, J. Labidi and A. Morales	09:30 - 09:45
From Waste to Value: Pilot-Scale EV Battery Recycling in Armenia H. Akarmazyan, G. Vardanyan, V. Marukhyan, A. Tadevosyan	09:45 - 10:00
Metal leaching from e-waste using phosphonium-based hydrophobic deep eutectic solvent N. M. Chola, S. Stephen, G. E. Luckachan, G. Xavier, M. Elkadi, I. Alnashef, M. Abi Jaoude	10:00 - 10:15
Sustainable Consumption Principles of the Circular Economy: Consumer Preferences and Willingness to Pay for Electric Vehicle (EV) Purchase Options: A Study in Klang Valley, Malaysia F. Shahudin, R. Ya'cob, L. Yiyang, G. Zihan	10:15 - 10:30
From urban pruning to sustainable films: unlocking the value of tree residues F. Hernández-Ramos, A. Morales, P. de Hoyos-Martínez, J. Pérez-Arce, X. Erdocia, L. Sillero	10:30 - 10:45
Coffee break - Posters viewing	10:45 - 11:00

PLENARY LECTURE: Professor Jacek Dach Biogas and biomethane as a key element of the circular economy and carbon footprint reduction in the products Chair: Konstantinos Kakosimos, Petros Gikas	11:00 - 11:45
Session 7: Sustainable agriculture and waste management Chair: Patrycja Pochwatka, Konstantinos Tsamoutsoglou	11:45 - 13:15
Traditional and Indigenous Knowledge and Conservation Practices of Forest Food Species in Guinean Forests of West Africa (GFWA) Biodiversity Hotspot A. E. Assogbadjo, G. Avakoudjo	11:45 - 12:00
Optimization of microwave assisted delignification of apple tree pruning using deep eutectic solvents A. Morales, X. Erdocia, J. Labidi, L. Sillero	12:00 - 12:15
Application of remote sensing technologies for sustainable forest fire monitoring and management A. Dudnyk, O. Opryshko, K. Witaszek	12:15 - 12:30
Scalable deployment of IoT systems for sustainable precision olive farming in Laconia, greece G. Panagopoulou, I. Moutsinas, T. Theodosiadis	12:30 - 12:45
The application of Revised Theory of Planned Behaviour (RTPB) model to assess the citizens' attitudes and intentions towards e-waste management in the circular economy. F. Shahudin, R. Ya'cob, L. Yiyang, G. Zihan	12:45 - 13:00
Microplastics an emerging environmental pollutant in agriculture: interaction between microplastics and soil humic substances on an agricultural crop M. Dimitropoulou, A. Ioannidi, I. Zafeiropoulos, K. Bourikas, M. Papadaki, E. Giannakopoulos	13:00 - 13:15
Lunch break	13:15 - 14:45
PLENARY LECTURE: Professor Konstantinos Kakosimos From bench to module scale hydrogen production via artificial and natural light water splitting Chair: Petros Gikas, Paraschos Melidis	14:45 - 15:30
Session 8: Sustainable water management and reuse Chair: Kupryaniuk Karol, Jakub Kostecki	15:30 - 16:15
Beyond Negative Environmental Results: The Critical Role of Risk Assessment in Legionella Control A. Papadakis, A. Roditakis, P. Manetakis, N. Gikas, E. Koufakis, K. Tsamoutsoglou, P. Gikas	15:30 - 15:45

Modification of membranes by using the layer by layer technology for the removal of Cr(VI) anions from groundwaters N. Malouchi	15:45 - 16:00
Sensors selection for real-time recovery of textile dyeing wastewater R. Partal, S. Murat Hocaoglu, M. Kitis, E. Ozturk, E. Simsek Yesil	16:00 - 16:15
Session 9: Biosolids management and valorization Chair: Patrycja Pochwatka, Jacek Dach	16:15 - 18:30
Conversion of sewage sludge to lipids for biofuels A. Jonas-Levi, Y. Gerchman, H. Azaizeh	16:30 - 16:45
Quantifying european biomass flows and certifying residual biomass valorization: a case from the region of central Macedonia, Greece A. Karanasiou, S. Skoutida, M. Batsioulas, G. Banias, S. Patsios	16:45 - 17:00
The role of demand-driven biogas plants in Europe's energy transition: potential for energy system stability and integration with other RES A. Łukomska, J. Dach	17:00 - 17:15
The role of animal biomass in the biogas sector in Poland: current status and potential P. Pochwatka, A. Kowalczyk-Juśko, A. Mazur, W. Czekala, J. Dach	17:15 - 17:30
Influence of the type of straw on the intensity of sewage sludge composting W. Bojarski, W. Czekala, M. Nowak, P. Sołowiej, K. Kupryaniuk, J. Dach	17:30 - 17:45
Spray mode and precursor molarity as key parameters in solution spray pyrolysis of oxygen electrodes for low temperature reversible solid oxide cells (socs) A. Kotsiolis, E. Roditis, C. Ziazias, N. Nikiforidis, A. Krestou, N. Kiratzis	17:45 - 18:00
Optimizing drying kinetics and calorific value assessment of wastewater sludge for enhanced waste-to-energy gasification L. Tshenyego	18:00 - 18:15
Innovative wastewater technologies for the treatment of sewage sludge at overloaded activated sludge plants K. Tsamoutsoglou, P. Gikas	18:15 - 18:30
Session 10: Life cycle assessment Chair: Gregoris Panayiotou, Janah Shaya	18:30 - 19:15
Is Life Cycle Assessment essential for sustainability in heritage? T. Tsoutsos, C. Nikoloudakis	18:30 - 18:45

Advanced Thermolysis of Waste Plastics into Sustainable Aviation Fuel Feedstocks and A Mass-Balance Certified Approach for Waste to Energy Solutions Aris Gorogias	18:45 - 19:00
Sustainable extraction of V and Ti from low-grade V-bearing titanomagnetite deposits G. Bartzas, D. Vathi, K. Komnitsas	19:00 - 19:15
Closing ceremony	19:15 - 19:45
DAY 4 - Thursday 4 September Susteng 2025	
Conference excursion at the capital of ancient Macedonia Aegae, in Vergina, and the grave of Philip II, father of Alexander the Great, and the Byzantine town of Veria	09:00 - 17:00

Posters

Aristotle University Research Dissemination Center (KEDEA)

Bioremediation of secondary cheese whey effluent by Pseudomonas sp. phDV1

E. Pappa, A. Lyratzakis, N.C. Stratigakis, G. Tsiotis

Hydrogen-Rich Gas Production From The Steam Gasification Of Olive By-Products With Minimum Carbon Dioxide Emissions

D. Vamvuka, S. Karvounis, K. Kyriakidis, I. Papakyriakopoulos and A. Strataki

Optimal wind power investments and bidding strategies in electricity market considering green certificates trading

M. Kanta, C. Dimitriadis, M. Georgiadis

Copper recovery from printed circuit boards: Alkaline glycine leaching system

J. Matado, L.H. Gomes and S.C. Pinho

Influence of operating parameters on lead hydrometallurgical leaching of lead-acid batteries with NaCl

L. H. Gomes, A. Lourenço, S. C. Pinho, M. J. J. de S. Ponte, H. de A. Ponte, H. de Santana

Adsorption of Reactive Red 120 dye by oxidized biochar from palm tree fibers

P. Haskis, F. Krommida, M. Vlastou, I. Varampoutis, D. Kyvrikoseos, D. A. Giannakoudakis, I. Ioannidis, I. Pashalidis, P. Mpeza and I. Anastopoulos

Valorization of industrial waste into silica based adsorbents for water OPPs removal

G. Silva, O. Freitas, V. Fernandes, F. Gomes

Assessment of marine fuels stability: a storage perspective

I. Kosma, L. Chysikou, S. Bezergianni

Immerse virtual training on critical raw materials: preparing responsible professionals for a sustainable future

E. Machairas, E. Varouchakis, B. Lottermoser, S. Hajinia Leilabadi

Analysis of Phospholipid Fatty Acids (PLFAs) as a soil bioindicator: the effect of vineyard abiotic stress on the soil microfauna as a case study

E. Ieronymaki, M. Drosos, I. Zafeiropoulos, M. Papadaki, E. Giannakopoulos

The use of coffee grounds as fuel

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Anaerobic production of biosurfactants from contaminated soils a waste valorization approach to soil remediation

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keywords: Biosurfactants, Bioemulsifiers, Soil remediation, Food waste valorization

Introduction

Microorganisms capable of degrading hydrocarbons are often producers of biosurfactants (BSs) and bioemulsifiers (BEs), which increase the bioavailability of hydrophobic organic pollutants and enhance their biodegradation. Leveraging this natural metabolic versatility, particularly in hydrocarbon-contaminated soils, offers promising opportunities for the development of sustainable remediation strategies.

This study explored the potential of stimulating the indigenous microbial community in real contaminated soils to produce BSs and BEs under anaerobic conditions, using low-cost, food- and agricultural-derived carbon sources. A circular economy perspective underpins this approach, which aims to valorize waste materials while minimizing the use of synthetic additives. In particular, this study investigated whether biosurfactant and bioemulsifier production can be enhanced under electron-acceptor-limited and mild oxidative stress, with the goal of developing low-toxicity formulations for remediation.

These preliminary experiments were designed as exploratory tests, intended to gather foundational data on biosurfactant productivity, efficacy, and environmental compatibility in real soil systems prior to more extensive process optimization and application studies.

Materials and methods

Sixty anaerobic microcosms were prepared using a weathered hydrocarbon-contaminated soil (Total Petroleum Hydrocarbon = 4500 mg/kg d.w.) as the microbial inoculum and a microemulsion of waste frying oil and chickpea powder as the main carbon source to investigate the native microbial community's potential for BS and BE biosynthesis under oxygen-deprived and static conditions over a period of nine months. Hydrogen peroxide was added to induce mild oxidative stress and to stimulate enzymatic activity. The experimental design included different concentrations of nitrate and sulfate as electron acceptors (EAs), and salinity as potential regulatory factors for microbial biosynthetic pathways.

All microcosms (250 mL total volume) contained 40 g dried soil and 200 mL amendment solution (Table 1). Methanogenesis was inhibited using 50 mM 2-bromoethanesulfonate to steer microbial metabolism toward alternative anaerobic pathways, which is more conducive to biosurfactant production.

Table 1: Amendment solution

	Concentration
NaCl	0.0 or 0.5 g/L
MgSO ₄	0.1 or 0.5 g/L
KNO ₃	5 or 40 mg/L
Urea	0.1%
Chickpea Powder	6.0%
Waste Frying Oil	1.5%
H ₂ O ₂ 3%	0.35g/1g TPH
2-Bromoethanesulfonate	50 mM

The crude and microfiltered supernatants were analyzed using oil displacement tests and emulsification index (EI_{24h}) assays with various hydrocarbon substrates (fuel oil, gasoline, diesel, and n-hexadecane). The

BS and BE activities were evaluated in terms of surface activity and emulsification stability. Phytotoxicity tests (germination index) were performed using *Zea mays*, *Lepidium sativum*, and *Medicago sativa*.

Results and Discussion

Oil displacement (Table 2) and emulsification index tests indicated that the biosurfactant and bioemulsifier activity was more frequently observed under mild salinity (NaCl 0.5 g/L), suggesting that appropriate osmotic conditions are beneficial for biosynthesis.

In microcosms amended with high nitrate and sulfate concentrations, more than 40% of oil displacement tests produced a clearing zone ≥ 1 cm, and emulsification indices above the 50% cutoff were observed in both crude and filtered extracts. These results suggest that even minimally processed biosurfactant-rich mixtures may provide sufficient surface activity to enhance hydrocarbon mobilization, without requiring purification or bioaugmentation.

These findings support the feasibility of biosurfactant production directly within the contaminated matrices, reducing reliance on exogenous chemical surfactants that often pose toxicity and persistence concerns. Moreover, the use of readily available waste materials as substrates makes this approach potentially scalable and compatible with circular-economy principles.

Importantly, this work should be considered as the first exploratory step, with several open questions remaining, including the kinetics of BS/BE production, the identity and stability of active compounds, and their effects on microbial community dynamics, pollutant bioavailability, and plant health.

Table 2: Results of oil displacement tests performed with gasoline, diesel oil, mineral and fuel oil, 12 tests for each condition as number of tests with no detected oil displacement (NEG), with oil displacement < 1 cm (in diameter) (PART), and oil displacement ≥ 1 cm (in diameter) (POS).

Test conditions	EAs	Oil displacement test				
			Gasoline	Diesel Oil	Fuel Oil	Mineral Oil
0.5 g/l NaCl ("saline")	MgSO ₄ 0.5 g/L + KNO ₃ 40 mg/L	NEG (%)	8.3	16.7	25.0	8.3
		PART (%)	33.3	25.0	33.3	16.7
		POS (%)	58.3	58.3	41.7	75.0
	MgSO ₄ 0.1 g/L + KNO ₃ 5 mg/L	NEG (%)	8.3	8.3	25.0	8.3
		PART (%)	0.0	25.0	25.0	8.3
		POS (%)	91.7	66.7	50.0	83.3
No added NaCl ("non saline")	MgSO ₄ 0.5 g/L + KNO ₃ 40 mg/L	NEG (%)	75.0	75.0	75.0	75.0
		PART (%)	16.7	8.3	0.0	0.0
		POS (%)	8.3	16.7	25.0	25.0
	MgSO ₄ 0.1 g/L + KNO ₃ 5 mg/L	NEG (%)	25.0	25.0	33.3	25.0
		PART (%)	0.0	8.3	33.3	0.0
		POS (%)	75.0	66.7	33.3	75.0

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**Anaerobic production of biosurfactants from contaminated soils
a waste valorization approach to soil remediation**M. Puddu¹, G. Beretta¹, S. Saponaro¹ and E. Sezenna¹¹Department of Civil and Environmental Engineering, Politecnico di Milano, Milan, ItalyCorresponding author email: marta.puddu@polimi.it**keywords:** *Biosurfactants, Bioemulsifiers, Soil remediation, Food waste valorization***Abstract**

This study investigated the anaerobic production of biosurfactants (BSs) and bioemulsifiers (BEs) by indigenous microbial communities in hydrocarbon-contaminated soils using waste-derived carbon sources as part of a circular economy approach. Sixty microcosms were prepared with real polluted soil, a microemulsion of waste frying oil and chickpea powder, and varying concentrations of nitrate, sulphate, and salinity to explore the influence of environmental conditions on BS/BE biosynthesis under oxygen-limited conditions. Mild oxidative stress was induced by hydrogen peroxide to enhance enzymatic activity. After nine months of incubation, the surface activity of the supernatant was assessed using oil displacement and emulsification index assays, and phytotoxicity was evaluated using germination tests. The results showed that mild salinity and sufficient electron acceptors significantly improved biosurfactant activity, with promising implications for in situ remediation. This low-cost, low-toxicity method valorizes food waste and supports sustainable soil remediation, providing a scalable alternative to synthetic surfactants. Further research is required to optimize production and assess field performance.

Introduction

The use of biosurfactants (BSs) and bioemulsifiers (BEs) in environmental remediation has gained considerable interest in recent years owing to their natural origin, biodegradability, and effectiveness in enhancing the solubility and bioavailability of hydrophobic organic pollutants. Produced by various microorganisms, BSs, reducing surface tension and promoting oil spreading, and BEs, stabilizing emulsions without necessarily lowering surface tension, serve as metabolic by-products or extracellular compounds that facilitate hydrocarbon degradation by improving contact between microbial cells and hydrophobic contaminants.

In hydrocarbon-contaminated soils, especially those affected by petroleum-based pollution, leveraging indigenous microbial communities for in situ production of these compounds presents a promising and sustainable remediation strategy. Traditional bioremediation methods often rely on aerobic microbial processes and the addition of purified biosurfactants or synthetic surfactants, which can be costly, environmentally persistent, and occasionally toxic to non-target organisms.

The approach explored in this study diverges by focusing on anaerobic conditions, which are more representative of contaminated subsurface environments, and by using food-derived waste as carbon sources. This study investigated whether the microbial consortia present in real contaminated soils can be stimulated to produce BSs and BEs using a circular economy framework that emphasizes resource recovery and waste minimization.

In particular, this study evaluated the influence of electron acceptor (EA) availability, salinity, and mild oxidative stress on biosurfactant and bioemulsifier productivity. These factors are hypothesized to modulate microbial metabolism and stress responses, potentially enhancing the synthesis of surface-active compounds in the process. The ultimate goal is to develop low-toxicity, scalable formulations that can be directly applied to contaminated matrices without extensive downstream processing.

Materials and methods

The experimental system was designed to simulate anaerobic bioremediation conditions using microcosms constructed with weathered hydrocarbon-contaminated soil (total petroleum hydrocarbons (TPH) = 4500 mg/kg dry weight) as the microbial inoculum. Sixty microcosms were established in 250 mL

flasks, each containing 40 g of air-dried soil and 200 mL of amendment solution tailored to stimulate biosurfactant and bioemulsifier production under controlled conditions.

To mimic realistic low-cost amendment strategies, a microemulsion composed of waste frying oil (1.5%) and chickpea powder (6.0%) was used as the main carbon source. Urea was added as a nitrogen source (0.1%), while nitrate and sulphate were supplied at low (5 mg/L KNO₃, 0.1 g/L MgSO₄) and high (40 mg/L KNO₃, 0.5 g/L MgSO₄) concentrations to explore the impact of electron acceptor abundance on microbial metabolic pathways. Methanogenesis was selectively inhibited using 50 mM 2-bromoethanesulfonate to redirect metabolism toward non-methanogenic, potentially BS/BE-producing pathways. Additionally, hydrogen peroxide (0.35 g/g TPH) was added to induce mild oxidative stress, which was hypothesised to stimulate the enzymatic activity involved in hydrocarbon degradation and biosurfactant synthesis.

Salinity was investigated as a regulatory factor by comparing systems amended with and without NaCl (0.5 g/L). The final composition of the amendment solution is presented in Table 1.

Microcosms under anaerobic conditions were maintained for nine months in the dark at a controlled temperature (18 ± 2 °C). Samples were collected periodically to monitor the pH, redox potential, and concentrations of nitrate, sulphate, and dissolved organic carbon.

Table 6: Amendment solution

	Concentration
NaCl	0.0 or 0.5 g/L
MgSO ₄	0.1 or 0.5 g/L
KNO ₃	5 or 40 mg/L
Urea	0.1%
Chickpea Powder	6.0%
Waste Frying Oil	1.5%
H ₂ O ₂ 3%	0.35g/1g TPH
2-Bromoethanesulfonate	50 mM

The supernatants (both crude and microfiltered) were analysed for the presence of biosurfactants and/or bioemulsifiers using standard oil displacement tests and emulsification index (EI_{24h}) assays using four representative hydrocarbon substrates: gasoline, diesel oil, fuel oil, and n-hexadecane. These tests evaluated the ability of the culture fluids to reduce surface tension and form stable emulsions.

Oil displacement experiments were conducted following the procedure described by Morikawa et al. (2000). In summary, for each combination of hydrocarbon mixture and supernatant, glass beakers with a diameter of 50 mm were filled with 20 ml of distilled water, and 1 ml of hydrocarbon substrate was added to the water's surface. Subsequently, 100 µl of the supernatant was carefully introduced at the centre of the oil layer on the water. The presence of biosurfactants in the supernatant would cause the oil to be displaced, creating a clear zone without oil. The diameter of this zone was measured to indicate surfactant or oil displacement activity. Negative controls were performed using distilled water, where no oil displacement or clear zone was observed.

The emulsification index at 24 hours (EI_{24h}), used to evaluate emulsification capacity, was determined by vortexing equal volumes (5 mL each) of the biosurfactant solution—either microfiltered or in its crude extract form—and hydrocarbons in a 15 mL Falcon tube. After 24 h of static incubation, the ratio of the emulsion layer thickness to the total height of the solution was calculated.

The phytotoxicity of the most promising biosurfactant solutions was assessed using germination index (GI) assays on *Zea mays* and *Lepidium sativum* to determine whether the biosurfactant-rich extracts were compatible with plant growth and suitable for environmental application.

Results and Discussion

The results of the oil displacement tests are reported in Table 2 and indicate that biosurfactant activity was more frequently observed under mild salinity (NaCl 0.5 g/L), suggesting that appropriate osmotic conditions are beneficial for BS/BE biosynthesis. However, oil dependency is relevant, with fuel oil consistently showing lower displacement performance than gasoline and mineral oil, potentially due to its higher viscosity, which could limit the spread of the displacement halo.

Table 7: Results of oil displacement tests performed with gasoline, diesel oil, mineral and fuel oil, 12 tests for each condition as number of tests with no detected oil displacement (NEG), with oil displacement <1 cm (in diameter) (PART), and oil displacement ≥1 cm (in diameter) (POS).

Test conditions	EAs	Oil displacement test				
			Gasoline	Diesel Oil	Fuel Oil	Mineral Oil
0.5 g/l NaCl ("saline")	MgSO ₄ 0.5 g/L + KNO ₃ 40 mg/L	NEG (%)	8.3	16.7	25.0	8.3
		PART (%)	33.3	25.0	33.3	16.7
		POS (%)	58.3	58.3	41.7	75.0
	MgSO ₄ 0.1 g/L + KNO ₃ 5 mg/L	NEG (%)	8.3	8.3	25.0	8.3
		PART (%)	0.0	25.0	25.0	8.3
		POS (%)	91.7	66.7	50.0	83.3
No added NaCl ("non saline")	MgSO ₄ 0.5 g/L + KNO ₃ 40 mg/L	NEG (%)	75.0	75.0	75.0	75.0
		PART (%)	16.7	8.3	0.0	0.0
		POS (%)	8.3	16.7	25.0	25.0
	MgSO ₄ 0.1 g/L + KNO ₃ 5 mg/L	NEG (%)	25.0	25.0	33.3	25.0
		PART (%)	0.0	8.3	33.3	0.0
		POS (%)	75.0	66.7	33.3	75.0

Table 3 summarises the results of the 24-hour emulsification index (EI_{24h}) assays under different test conditions across various hydrocarbon substrates. The data include the percentage of extracts showing any emulsifying activity (POS%) and the mean EI_{24h} ± standard deviation for both crude and microfiltered extracts. These indicators provide insights into the frequency, strength, and consistency of bioemulsifier production.

Under saline conditions, emulsifying activity was frequently detected, with POS% values as high as 100% (e.g. high EA microfiltered extract with gasoline) and generally above 80% for fuel and mineral oil. This suggests that salinity (NaCl 0.5 g/L) also supports the production of emulsifying compounds.

In contrast, under non-saline conditions, the POS% values were significantly lower (e.g. only 8–25% in high-EA systems and diesel), indicating less consistent emulsification. However, some non-saline-low-EA combinations still produced high POS% for certain hydrocarbons (e.g. 67% for gasoline and fuel oil), suggesting that limited EAs may trigger emulsifier synthesis in specific microbial subpopulations.

Regarding the emulsifying capacity measured by the EI_{24h} index, crude extracts from saline conditions generally yielded higher values than those from non-saline conditions, exceeding 30% for both mineral and fuel oils. Most saline microfiltered extracts demonstrated similar or even improved performance in comparison to crude extracts. Preservation of emulsifying capacity after filtration in these extracts indicates that likely the active emulsifying agents are predominantly extracellular and soluble, rather than being cell-associated.

Conversely, under non-saline conditions, particularly with low low-EA availability, a notable reduction in EI_{24h} was observed after microfiltration, probably because of the removal of larger, cell-associated, or colloidal emulsifying structures during filtration. These findings highlight the importance of environmental conditions, particularly salinity, in influencing not only the quantity but also the physical nature of the produced emulsifiers.

A crucial aspect to consider is the relative dispersion of the EI24h results in many tests (as indicated by the high standard deviation, sometimes approaching or exceeding the mean). This high variability suggests that although some samples perform very well, emulsifying capacity is not yet consistent across replicates, possibly due to batch-to-batch variability in substrate availability, microbial activity, or instability of the produced emulsifiers under certain conditions and/or time. This underscores the need for further optimization and replication to ensure consistent production, which is essential for reliable application.

Germination indexes to evaluate the environmental safety of the filtered mixtures, against the two selected model species, provided restrictions regarding their application “as made”.

Concentration related effects were evident in the 15% mixtures of both biosurfactant mixtures tested where inhibition of the germination and growth of the specimens were assessed, while in 5% concentration mixtures the inhibition was lower and transitory.

The observed influence of concentration and stabilization highlights key formulation parameters and suggests avenues for optimization through improved preparation and component characterization; future research should further investigate concentration-dependent effects and assess the mixture’s potential interactions with environmental pollutants to better understand its applicability.

Table 8: Results of emulsification index on 24 hours (EI24h) assays performed with gasoline, diesel oil, mineral and fuel oil, 12 tests for each condition. The results are reported as the percentage of tests in which the extract (crude or microfiltered) showed emulsifying activity (POS) and the average EI24h ($\pm$ standard deviation).

Test conditions	Extract	Emulsification Index				
			Gasoline	Diesel Oil	Fuel Oil	Mineral Oil
0.5 g/l NaCl (“saline”) MgSO ₄ 0.5 g/L + KNO ₃ 40 mg/L	crude	POS (%)	83	17	83	92
		EI24h	16.9 (21.9)	20 (7.8)	30.5 (23)	35.5 (32.4)
	microfiltered	POS (%)	100	50	83	83
		EI24h	17.5 (18.8)	29.4 (21.5)	35.1 (13.6)	25 (28.8)
0.5 g/l NaCl (“saline”) MgSO ₄ 0.1 g/L + KNO ₃ 5 mg/L	crude	POS (%)	83	33	83	50
		EI24h	13.1 (19.5)	30.7 (24.4)	30.3 (18.6)	13 (22.6)
	microfiltered	POS (%)	83	50	83	50
		EI24h	14.7 (14.6)	11.1 (11)	23.9 (20.4)	29.3 (32.7)
No added NaCl (“non saline”) MgSO ₄ 0.5 g/L + KNO ₃ 40 mg/L	crude	POS (%)	25	8	33	25
		EI24h	14.3 (15.2)	3.3 (0)	30.6 (25.1)	3.2 (2.5)
	microfiltered	POS (%)	25	17	33	17
		EI24h	16.7 (16.1)	10 (6.7)	22.3 (21.4)	20.5 (35)
No added NaCl (“non saline”) MgSO ₄ 0.1 g/L + KNO ₃ 5 mg/L	crude	POS (%)	67	25	67	33
		EI24h	20.5 (12.8)	10 (4.2)	40.7 (21.2)	44.2 (36.1)
	microfiltered	POS (%)	67	25	67	42
		EI24h	8.5 (7.8)	8.1 (6.5)	21.6 (23.9)	18 (29.4)

Conclusions

This exploratory study illustrates that indigenous microbial communities in hydrocarbon-contaminated soils can be effectively stimulated for the production of biosurfactants and bioemulsifiers under oxygen-limited conditions through the application of food waste-derived amendments when methanogenesis is inhibited. This process is influenced by environmental parameters such as salinity and the availability of electron acceptors, with mild oxidative stress induced by H₂O₂ further enhancing the activity. These observed dependencies suggest that careful tuning of environmental conditions can enhance BS/BE yield.

Additional studies are necessary to more thoroughly define the properties and stability of biosurfactants and bioemulsifier compounds, and their effects on microbial community dynamics, pollutant bioavailability,

and plant health. It is important to evaluate production kinetics and refine formulations for practical field use.

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