Plastics Symposium 2024

Programme

CREATE Tower, 1 Create Way, Singapore 138602 22-23 July 2024





School of Chemistry, Chemical Engineering and Biotechnology College of Engineering - College of Science

Supported by









NTU Sustainability Office

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About Plastics Symposium 2024

Dear Colleagues:

Plastic wastes have been a concern to the world, especially in the Asia Pacific. The amount of global plastics production in 2022 reached an alltime high of 400 Mt and is projected to reach 470 Mt by 2030. With the ever-increasing amount of plastics produced, so is the waste which may affect our environment. New innovations are required to address this issue.

The Plastics Symposium aims to showcase the latest innovations in the academic and industry in plastic designs, waste processing (physical, chemical, and biological approaches), and techno-economic analysis. It will serve as a forum to foster collaborations between industry, academia and government, as well as community organizations. Featuring talks by academics, industry leaders, and government stakeholders, the Symposium will highlight gaps in infrastructures and investments needed to commercialize new technologies in addressing plastic waste.

The key priorities include:

- Promote discussions between industry and academia to identify key challenges and strategies in the current plastics waste management.
- Enhance industry and academic partnerships to address key challenges in translating innovations into practice.
- Explore biological approaches as complementary methods in future plastics recycling technology.

We look forward to your support of the symposium to enable our collective actions toward a sustainable future.

Thank you, Sierin Lim Chair, Plastics Symposium 2024 Associate Professor, School of Chemistry, Chemical Engineering and Biotechnology Nanyang Technological University, Singapore

Chair



Sierin Lim

Purpose and Outcome

Purpose

This symposium aims to bridge the gap between research innovations and practical applications by **convening experts from academia, industry, and government** to discuss current trends, challenges, and solutions in plastic waste management.



The inaugural Plastics Symposium in Singapore will explore opportunities to **launch a Plastics Consortium**. This Consortium is envisioned to be a hub for plastics innovation in South-East Asia (SEA) and the Pacific, connecting regional advancements to the world and attracting global research and business opportunities to SEA

Event Venue and Schedule



CREATE Tower Seminar Room and Theatrette L2 1 Create Way, Singapore 138602 Google maps

22-23 July 2024

Directions to CREATE TOWER by MRT

- Alight at Kent Ridge MRT Station (CC24, Circle Line) and proceed to Lower Kent Ridge Road Exit
- Proceed to Bus Stop No. 18221 (right after NUH entrance)
- Take bus service "D2" to University Town. Alight at University Town Bus Stop

Directions to CREATE TOWER via AYE (towards Tuas)

- Along AYE towards Tuas, exit at Exit 9
- Turn left on Clementi Road
- Turn left onto Kent Ridge Crescent (Landmark: University Cultural Centre on the left)
- Turn left onto College Link at the roundabout
- Proceed straight along Create Way
- At the roundabout, turn right and proceed down the road ramp
- Proceed to CREATE Tower carpark, turn right

Directions to CREATE TOWER via Dover Road

- Head onto Dover Road from Clementi Road
- Turn right into University Town (Turn left if you are coming from North Buona Vista Road onto Dover Road)
- Keep left and continue onto College Avenue East
- Turn right after the roundabout onto Create Way
- Proceed down the road ramp
- Proceed to CREATE Tower carpark, turn right

Download a print copy of the directions with maps.

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Schedule Overview

Day 1 – 22 July 2024 : 13.00-19.30 SGT

Time	Event	Location
14.00 14.15 16.30	Registration Starts Symposium Starts Session 1 – Plastic Waste to Value Session 2 – Polymer Design and Challenges Reception & Poster Session	CREATE Tower 1 CREATE Way, UTown Singapore 138602 <u>Google maps</u>

Day 2 – 23 July 2024 : 08.30-18.00 SGT

Time	Event	Location
	Registration Starts - Symposium Starts - Session 3 – Bio-based Plastic - Degradation	
	- Plenary Talk Session 4 – Enzymes for Plastics Degradation: New and Engineered	CREATE Tower
	Session 5 – Organisms for Plastics Conversion: Native and Engineered Session 6 – Enabling Technologies	1 CREATE Way, UTown Singapore 138602 <u>Google maps</u>
	Panel Discussion - LIM Yee Hwee - Alain Marty - Keats Nelms - Daniel Tay - Moderator: Sierin Lim	
18.00	Symposium Ends	

Symposium Agenda

Day 1 – 22 July 2024 : 13.00-19.30 SGT

- 13.00-14.00 Registration opens
- 14.00–14.05Welcome remarksSierin Lim, Chair, Plastics Symposium 2024
- 14.05-14.15 Opening remarks Warren Chan, Dean, College of Engineering, NTU

Session 1 – Plastic Waste to Value

- **14.15-14.35 Keynote Speaker:** <u>Sally McArthur</u>, Deakin University, Australia *"Tailoring polymer and composite recycling solutions for different industry sectors"*
- **14.35-14.50** Invited Speaker: <u>DUONG Hai Minh</u>, National University of Singapore *"From Plastic Waste to Wealth"*
- **14.50-15.05** Invited Speaker: <u>LI Hong</u>, Nanyang Technological University, Singapore "Co-upcycling of PET Plastic and Glyerol Wastes"
- **15.05-15.20** Invited Speaker: <u>Christopher Hurren</u>, Deakin University, Australia "Developing Sustainable Textile Fibres from Sea Grass and Food Waste"
- **15.20-15.45 Keynote Speaker:** <u>HU Xiao</u>, Nanyang Technological University, Singapore "Chemical Recycling of Plastics: Recent Case Study and Initiatives"
- 15.45-16.30 Coffee/Tea break and Poster Session

Session 2 – Polymer Design and Challenges

- 16.30-16.50 Keynote Speaker, Atsushi Goto, Nanyang Technological University, Singapore "Sustainable and Functional Polymers and Materials Using Biobased and Degradable Monomers"
 16.50-17.05 Invited Speaker: LAM Yeng Ming, Nanyang Technological University Singapore "Polymer Recycling Some Considerations"
 17.05-17.20 Invited Speaker: Joanne Ngeow, Nanyang Technological University Singapore "Plastics and Healthcare"
- **17.20-17.40 Keynote Speaker:** <u>CAO Bin</u>, Nanyang Technological University, Singapore *"Plastisphere Biofilm: a Natural Hazard or an Engineering Solution?"*

17.45-19.30 Reception and poster session

Symposium Agenda

	Day 2 – 23 July 2024 : 09.00-18.00 SGT
08.30-09.15	Registration opens
S	ession 3 – Bio-based Plastic Degradation
09.15-09.45	Plenary Speaker: <u>Alain Marty</u>, Carbios, France "PLA Composting & PET Recycling: from Enzymes and Process Optimizations to Industrial Plants"
09.45-10.00	Contributed Speaker : <u>Sukanya Punthambaker</u> , Breaking Inc., USA "Plastic Degradation Using Enzymes and Microbes"
10.00-10.20	Keynote Speaker: <u>Karine Auclair,</u> McGill University, Canada "Plastic Deconstruction by Enzymes in Moist-solids"
10.20-10.40	Keynote Speaker: <u>Vanessa Vongsouthi</u> , Samsara Eco, Australia "Scalable Chemoenzymatic Recycling of Plastics"
10.45-11.15	Coffee/Tea Break and Poster Session
Se	ession 4 – Enzymes for Plastic Degradation: New and Engineered
11.15-11.35	Keynote speaker: <u>YEOM Soo Jin</u> , Chonnam National University, Korea "Exploring a New Biocatalyst for Plastic Biodegradation"
11.35-11.50	Invited speaker: <u>YEW Wen Shan</u> , National University of Singapore "Repurposing Biology through Synthetic Enzymology for Human and Planetary Health"
11.50-12.05	Invited speaker: <u>Farid Ghadessy</u> , A*STAR Singapore "Thermostabilising PETase for Increased Robustness"
12.05-12.20	Contributed speaker: <u>Subha Kalyaanamoorthy</u> , University of Waterloo, Canada <i>"Transforming Plastic Waste: Computational Advances in Enzyme</i> <i>Engineering"</i>
12.20-12.40	Keynote speaker: <u>CUI Yinglu</u> , Chinese Academy of Science "Computational Redesign of a Hydrolase for Nearly Complete PET Depolymerization at Industrially Relevant High-solids Loading"
13.00-14.00	Lunch and Poster session

Symposium Agenda

Day 2 – 23 July 2024 : 09.00-18.00 SGT

Se	ession 5 – Organisms for Plastics Conversion: Native and Engineered
14.00-14.20	Keynote speaker: Federica Bertocchini, Plasticentropy, France
	"Exploring a New Biocatalyst for Plastic Biodegradation"
14.20-14.35	Contributed speaker: <u>Bodhi Dharma</u> , Mulawarman University, Indonesia" Several Promising Actinobacteria-Plastics-Degraders Isolated from East Kalimantan, Indonesia"
14.35-14.50	Invited speaker: <u>ZHOU Kang</u> , Singapore Institute of Technology
	"Engineering Escherichia coli to utilize diols derived from plastic wastes"
14.50-15.05	Contributed speaker: <u>CHUA Yuen Siong</u> , National University of Singapore "Upcycling of PET Monomers through Metabolic Engineering of Pichia pastoris"
15.05-15.20	Contributed Speaker: <u>Jasmine Ikraminaningsih</u> , Universitas Indonesia "Isolation and Performance Evaluation of Polypropylene-Degrading Bacteria from the Gut Microbiome of Tenebrio molitor Larvae"
15.20-15.40	Keynote speaker: <u>LIM Yee Hwee</u> , A*STAR Singapore "Plastics and Sustainability"
15.40-16.00	Coffee/Tea Break and Poster Session
Se	ession 6 – Enabling Technologies
Se 16.00-16.20	ession 6 – Enabling Technologies Keynote speaker: <u>Soichiro TSUDA</u> , bitBiome, Japan
	Keynote speaker: <u>Soichiro TSUDA</u> , bitBiome, Japan "Discovery of PET-degrading Enzymes from Microbial Ssingle-cell
16.00-16.20	Keynote speaker: <u>Soichiro TSUDA</u> , bitBiome, Japan "Discovery of PET-degrading Enzymes from Microbial Ssingle-cell Genome Database"
16.00-16.20	 Keynote speaker: Soichiro TSUDA, bitBiome, Japan "Discovery of PET-degrading Enzymes from Microbial Ssingle-cell Genome Database" Invited speaker: Tristan Bepler, OpenProtein.AI, The USA "Engineering a Plastic Degrading Enzyme with Protein Language Models and Bayesian Learning" Contributed speaker: Jean-Baptiste Lena, Nanyang Technological
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Click the speaker's name for more details

Speaker List

Plenary Speaker: Alain Marty, Carbios, France

Keynote Speakers

- Karine Auclair, McGill University, Canada
- <u>Federica Bertocchini</u>, Plasticentropy, France
- <u>CAO Bin</u>, NTU Civil and Environmental Engineering
- <u>CUI Yinglu</u>, Chinese Academy of Science, Institute of Microbiology, China
- <u>Atsushi Goto</u>, NTU School of Chemistry, Chemical Engineering and Biotechnology
- HU Xiao, NTU School of Materials Science & Engineering
- <u>LIM Yee Hwee</u>, A*STAR Institute of Sustainability for Chemicals, Energy and Environment (ISCE²)
- <u>Sally McArthur</u>, Institute for Frontiers Materials, Deakin University, Australia
- <u>Soichiro TSUDA</u>, bitBiome, Japan
- Vanessa Vongsouthi, Samsara Eco, Australia
- YEOM Soo Jin, Chonnam National University, Korea

Invited Speakers

- <u>Tristan Bepler</u>, OpenProtein.Ai, The USA
- DUONG Hai Minh, NUS Mechanical Engineering
- Farid Ghadessy, ASTAR IMCB
- <u>Christopher Hurren</u>, Fibre Science and Technology, Institute for Frontier Materials, Deakin University, Australia
- LAM Yeng Ming, NTU School of Materials Science & Engineering
- <u>LI Hong</u>, NTU School of Mechanical & Aerospace Engineering
- Joanne Ngeow, NTU LKC School of Medicine
- <u>YEO Zhiquan</u>, A*STAR Institute of Manufacturing and Technology (SIMTech)
- YEW Wen Shan, NUS Yong Loo Lin School of Medicine
- <u>ZHOU Kang</u>, Singapore Institute of Technology (Food, Chemical and Biotechnology)

Panelists

- <u>LIM Yee Hwee</u>, A*STAR Institute of Sustainability for Chemicals, Energy and Environment (ISCE²)
- Alain Marty, Carbios, France
- <u>Keats Nelms</u>, Samsara Eco, Australia
- Daniel Tay, Temasek
- Moderator: <u>Sierin Lim</u>, NTU School of Chemistry, Chemical Engineering and Biotechnology

Session 1 – Plastic Waste to Value



<u>Sally McArthur</u> Director, Institute for Frontier Materials Deakin University, Australia

SHORT BIOGRAPHY

Dr. Sally McArthur serves as the Director of the Institute for Frontier Materials at Deakin University, spearheading research and development initiatives in sustainable materials and manufacturing across various sectors. Her expertise spans biomedical engineering, surface engineering, and biointerfaces. Prior to her current role, Dr. McArthur held leadership positions at Swinburne University, including Director of the Manufacturing Futures Research Institute and co-Director of MedTechVic. She has been instrumental in bridging the gap between academia and industry, leading initiatives such as Swinburne's Innovation Precinct and the ARC Training Centre in Biodevices. Dr. McArthur's contributions to academic-industry collaboration earned her a fellowship with the Australian Academy of Technology and Engineering in 2021. She holds degrees from Monash University and UNSW, and served as a CSIRO Science Leader in Biomedical Manufacturing from 2017 to 2021. Dr. McArthur continues to foster innovation through partnerships with industry stakeholders.

ABSTRACT

Tailoring polymer and composite recycling solutions for different industry sectors

Sucessful recycling solutions require systems based thinking to create solutions that are useful and usable. Within the Institute for Frontier Materials at Deakin University we seek to build solutions that are manufacturing sector specific, ie composites manufacturing and cross sectoral like textile recycling. This talk will highlight how we are working with industry partners to translate R&D insights into industrial and societal change.

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Session 1 – Plastic Waste to Value



DUONG Hai Minh Mechanical Engineering National University of Singapore

SHORT BIOGRAPHY

Dr. Hai M. Duong received his Ph.D. at Melbourne University. He was awarded four postdoctoral fellowships at world-class laboratories: University of Oklahoma, USA; University of Tokyo, Japan; Massachusetts Institute of Technology (MIT), USA and University of Cambridge, UK. Currently, as an Associate Professor at National University of Singapore (NUS) and visiting professor at Stanford University, USA, his research interests are of recycling various wastes, aerogels and carbon-based nanomaterials in science and engineering applications through experiments and computational modeling. He has awarded 6 competitive global innovation awards, 16+ granted patents and published 18+ book chapters, 200+ journal papers and conference proceedings. He is the key member of Functional Material Society in Singapore.

ABSTRACT

From Plastic Waste to Wealth

Of the nearly 350 million tons of plastics that are produced annually, about 70 percent end up in managed landfills or are incinerated, about 10 percent is recycled and the remaining 20 percent simply escape into the environment including 24.4 trillion pieces of microplastics in the oceans, 92 million tonnes of textiles and old clothes ended up in landfills and 1.2 billion tonnes of food and agriculture waste are mostly left to be rotten or burned. This wastefulness could release harmful chemicals and greenhouse gases, which harm our planet and human health tremendously. To overcome these environmental problems and to benefit farmers, households, communities and businesses, as well as Earth itself, our team have created successfully cost-effective large-scale technologies to recycle almost all common wastes into high-value eco-products, called eco-aerogel materials with a dozen of useful purposes. Our solutions can bring many advantages to existing technologies and waste management and lead to the cheaper production with energy efficiency and far fewer harmful emissions. Compared to standard aerogel methods, our aerogel technologies using chemical bonding, mechanical bonding and thermal bonding methods can produce continuously the aerogels with the rate of 1.0m x 1.0 m x 1cm aerogel products per minute. They are also very cost-effective (due to 70% of energy saving), faster (18 times) and environmentally friendly (no toxic usage and much less CO2 emission with 0.1 kg CO2 emission/aerogel m2). A waste-based aerogel production has positive impacts on consumption, production, environmental restoration, innovation and climate change. We have recycled the wastes of plastic bags and drinks bottles, agricultural waste products (pineapple, areca, coconut etc), food waste, old clothes, old car tyres, fly ash, metals etc. Our eco-products produced from these wastes can be used for CO2 absorption, fruit preservation, wastewater treatment, oil spill cleaning, cultured meat, human fat removal, and heat and sound insulation of buildings or clothing or material for treating wounds and defence uses. The affordable products can be reused, recycled and disposed safely imposium 2024 | Page 12

Session 1 – Plastic Waste to Value



LI Hong School of Mechanical and Aerospace Engineering Nanyang Technological University, Singapore

SHORT BIOGRAPHY

Dr. Hong Li is currently an Associate Professor in Nanyang Technological University (NTU) Singapore. Before he joined NTU in 2016, he was a postdoc in Stanford University in United States. Dr. Hong Li received a few awards including the prestigious Singapore Millennium Foundation postdoc fellowship and Nanyang Assistant Professorship. Dr. Li's current research focus on renewable energy and sustainability including waste-to-value, green hydrogen generation, passive cooling, etc. Dr. Li has published more than 100 peer-reviewed papers in international journals that have received more than 18, 000 citations.

ABSTRACT

Co-upcycling of PET Plastic and Glycerol Wastes

Upcycling plastic wastes for chemicals and fuels production is a promising solution for ending the plastic waste problem. One of the main obstacles for plastic recycling is contamination (by chemicals, food residue, etc.). In this talk, I will report a one-pot method to reclaim terephthalic acid and derive formate from contaminated PET plastic and glycerol (main biodiesel by-product) wastes with simultaneously green hydrogen generation. Critical facilitating mechanism of glycerol and electrochemical pathways are studied with elaborate testing and DFT calculations. Life-cycle and technoeconomic analyses are also performed to show the great environmental advantage and economic practicality.

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Session 1 – Plastic Waste to Value



Associate Professor Christopher Hurren*

Fibre Science and Technology, Institute for Frontier Materials Deakin University, Australia

SHORT BIOGRAPHY

A. Prof. Hurren's expertise is in textile research. His key areas of research are the development of protective and performance textiles and a circular approach to sustainable textiles. He manages the Future Fibres Facility that enables Deakin Universities "Lab to Label" approach to textile research. He specialises in the measurement and development of protective textile structures for motorcycle apparel. He led the team that developed the Motorcycle Clothing Assessment Program (MotoCAP) that was introduced in Australia and New Zealand in 2018. A. Prof. Hurren spent eight years in industry as a textile colourist, spinner and fabric finisher and has a strong understanding of the textile manufacturing chain.

ABSTRACT

Developing Sustainable Textile Fibres from Sea Grass and Food Waste

Christopher Hurren, Bin Tang, Lucas Rosson

Textiles are one of the major leakage points of plastic into the environment accounting for 14% of environmental plastic contamination. Most synthetic fibre is sourced from crude oil posing future supply issues for more than 70% of textiles. Utilising renewable feedstocks and creating fibres that will biodegrade at end of life is one of the ways forward to achieving sustainable textile production.

Deakin University works with Nanollose to convert their microbial cellulose into textiles. Their food waste derived feedstock enables future sustainability. Their cellulose based fibres break down in soil and water at end of life or if lost to the environment. Initial research focused on creating staple fibres for use in traditional spinning. Ongoing research aims to create continuous filaments for use in advanced textiles like sports clothing.

Uluu research helps them convert their PHA based polymer chip into textiles. Their polymer is naturally synthesised from sea grass and does not use arable land for polymer production. The polymer is naturally occurring, melt extrudable and able to decompose in soil and water at end of life. Deakin research is concentrating on optimisation in melt extrusion combined with quantification of fibre, yarn and fabric properties.

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Session 1 – Plastic Waste to Value



<u>Hu Xiao</u> School of Materials Science & Engineering Nanyang Technological University, Singapore

SHORT BIOGRAPHY

Professor Hu gained his PhD degree from the University of Manchester in the UK. In 1992, he joined NTU as one of the founding member of the Materials Science and Engineering programme. He is currently the Programme Director of Environmental Chemistry and Materials at the Nanyang Environment and Water Research Institute (NEWRI) and Co-Director of the RGE-NTU Sustainable Textile Research Centre. He served as the past President of the Pacific Polymer Federation and is the President Designate (from 2025) of the Federation of Asian Polymer Societies (FAPS). In the past decade, he has dedicated his research to the cross-disciplinary field of materials science, nanotechnology, environmental engineering, water technology and sustainable agriculture. Being an established polymer scientist, tackling plastic waste challenges is part of the DNA of his research and innovation work.

ABSTRACT

Chemical Recycling of Plastics: Recent Case Study and Initiatives

Recent studies on plastic recycling and upcycling in our group at the School of Materials Science and Engineering, Nanyang Technological University (NTU) will be presented. Discussion will first be on how to turn waste PET into branched oligomeric, instead of the usual monomeric, building blocks via controlled partial depolymerization. These oligomeric building blocks can be subsequently converted into conducting iongels for functional applications or covalent adaptable dynamic network polymers, e.g., vitrimer, for structural applications. Example will also be given on non-pyrolytic liquefaction of polyurethane (PU) waste such as PU foams. Some of the key challenges, opportunities and the rationale of the approaches will be highlighted. Finally, the presenter will introduce one of the recent initiatives at the Sustainable Textile Research Centre (SusTex) at NTU, which is a university-industry partnership between NTU and Royal Golden Eagle (RGE).

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Session 2 – Polymer Design and Challenges



Atsushi Goto

School of Chemistry, Chemical Engineering, and Biotechnology Nanyang Technological University, Singapore

SHORT BIOGRAPHY

Atsushi Goto is a professor in Nanyang Technological University (NTU), Singapore, since 2020. He received his Bachelor degree (1996), Master degree (1998), and Ph.D (2001) from Kyoto University, Japan. He was an instructor (2001), an assistant professor (2002-2010), and an associate professor (2010-2015) in Kyoto University. He was an associate professor (2015-2020) in NTU. He was Provost's Chair in Chemistry and Biological Chemistry (2019-2021) and is currently Nippon Shokubai Professor in Chemistry (2021-current). His research interests include polymer chemistry and polymer materials, particularly controlled syntheses of polymers.

ABSTRACT

Sustainable and Functional Polymers and Materials Using Biobased and Degradable Monomers

Biomass and novel chemically recyclable degradable monomer, 4,4-dimethyl-2-methylene-1,3-dioxolan-5-one (DMDL), were used to prepare polymers and functional materials such as degradable capsules. The capsule degraded under a basic condition and gradually released the encapsulated oils and dyes.

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Session 2 – Polymer Design and Challenges



LAM Yeng Ming

Chair, School of Materials Science and Engineering Nanyang Technological University, Singapore

SHORT BIOGRAPHY

Prof Yeng Ming LAM, a distinguished academic at Nanyang Technological University (NTU), holds a Ph.D. from the University of Cambridge and currently chairs the School of Materials Science and Engineering. As Director of FACTS, she oversees electron microscopy and x-ray analysis facilities. Her research concentrates on functional nanostructured materials for sustainable applications. Prof Lam is the founder of FytoSol Pte Ltd, which addresses horticultural and agricultural needs. She serves on governing boards for ISPAC and the National Committee on Measurement and Characterisation. With over 150 refereed publications and numerous patents, her work has been licensed internationally. Prof Lam has held concurrent positions at RWTH University in Germany and IMRE, A*Star. Her achievements include the Nanyang Award for Excellence in Teaching, the L'Oréal Unesco For Women in Science National Fellowship, and the Nanyang Outstanding Young Alumni Award.

ABSTRACT

Polymer Recycling – Some considerations

Polymers are ubiquitous for many applications such as packaging, medical implants, electronic devices and its global production exceeds 9 billion tons at present. This class of materials has many attractive properties such as versatility in terms of shapes and mechanical/chemical properties, light weight, cost-effectiveness, etc. Most traditional polymers such as polyethylene, polypropylene, PET, PVC, and nylon, are made from petrochemicals. The environmental impact in the production of such polymers (energy consideration) and the recycling process can be difficult and expensive, and many products end up in landfills or the environment. Despite the understanding of these negative impact of these materials, it will be challenging to replace all with a sustainable option. A more reasonable approach would be how to effectively recycle and upcycle these materials. In this talk, I will share with you some work that we have been doing on recycling and upcycling using enzymatic degradation and how the properties of the polymer impact the design of the platform for screening enzyme library for more effective degradation. To understand the interplay between substrate crystallinity and microstructures on the efficiency of PET degradation which can also impact other polymers degradation, I will also present the degradation behaviour of PET, PET poly(ethylene terephthalate-co-isophthalate) copolymers (e.g., or poly(ethylene terephthalate-co-butylene terephthalate)), and branched PET. In this talk, I will also share some work that may help with more effective recycling of polyamide.

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Session 2 – Polymer Design and Challenges



Joanne Ngeow

Lee Kong Chian School of Medicine Nanyang Technological University, Singapore

SHORT BIOGRAPHY

Associate Professor Joanne Ngeow is a distinguished medical professional at Nanyang Technological University's Lee Kong Chian School of Medicine and a Senior Consultant at the National Cancer Centre Singapore. She leads the Cancer Genetics Service, specializing in hereditary cancer syndromes and translational clinical cancer genomics. With extensive training from institutions in Singapore, Australia, and the United States, A/Prof Ngeow has made significant contributions to cancer research and clinical practice. Her work has been recognized with numerous awards, including the Early Investigator Award from the Endocrine Society and Merit Awards from the American Society of Clinical Oncology. A/Prof Ngeow has published extensively in prestigious journals and serves on various advisory panels. She is also co-PI of the HELIOS Study, exploring gene-lifestyle contributors to chronic diseases in Singapore. Her achievements in research, clinical practice, and mentorship have earned her the 2020 SkillsFuture Fellow Award.

ABSTRACT

Plastics and Healthcare

Joanne Ngeow, Audrey Koh

Healthcare as an industry is the fifth largest contributor towards carbon emissions globally. There is a great attention on Singapore's net zero green plan but the types of plastic waste in Singapore remain a huge issue be it in the form of pill packs and/ or nurses uniform. We will present on various problem statements from the hospital setting and hope to engage with symposium participants for collaboration.

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Session 2 – Polymer Design and Challenges



<u>CAO Bin</u> School of Civil and Environmental Engineering Nanyang Technological University, Singapore

SHORT BIOGRAPHY

Dr. Cao, an Associate Professor at the School of Civil and Environmental Engineering and Principal Investigator at Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, specializes in biofilm engineering. With a Ph.D. in Chemical and Biomolecular Engineering from the National University of Singapore and postdoctoral experience in U.S. Department of Energy projects, Dr. Cao's research focuses on the intersection of engineering and microbiology. His work explores fundamental mechanisms in biofilm-mediated environmental processes and develops novel approaches for harnessing beneficial biofilms and mitigating detrimental ones in environmental biotechnology applications. Dr. Cao's expertise is recognized through his editorial roles in several prestigious journals, including Water Research, Environmental Research, Critical Reviews in Biotechnology, and Frontiers in Cellular and Infection Microbiology. His interdisciplinary approach contributes significantly to the field of environmental engineering and microbiology.

Talk Title

Plastisphere Biofilm: A Natural Hazard or An Engineering Solution?

Plenary Speaker

Session 3 – Bio-based Plastic Degradation



<u>Alain Marty</u> Chief of Scientific Officer Carbios, France

SHORT BIOGRAPHY

Professor Alain Marty was appointed as Chief Scientific Officer of Carbios in 2015. He holds a degree in engineering and a doctorate in biochemical engineering from INSA Toulouse. He began his career in 1992 as a senior lecturer at the same institution. He progressed to Associate Professor and then Professor, directing research at INSA's LISBP (Laboratory for the Engineering of Biological Systems and Processes). His expertise spans biotechnology, bio-catalysis, enzymology, molecular engineering of enzymes, and developing intensified enzymatic reactors and metabolic engineering. Throughout his career, Professor Marty has seamlessly integrated cutting-edge research with industrial applications. In addition to his teaching and research roles, he serves on the LISBP Laboratory Advisory Board. He evaluates for AERES (Agency for the Evaluation of Research and Higher Education) and ANR (France's National Research Agency).

Talk Title

PLA Composting & PET Recycling: from Enzymes and Process Optimizations to Industrial Plants

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Contributed Speaker

Session 3 – Bio-based Plastic Degradation

Dr. Sukanya Punthambaker Breaking Inc., The USA

ABSTRACT

Plastic Degradation using Microbes and Enzymes

Sukanya Punthambaker, Vaskar Gnyawali, George Church, Don Ingber

Plastic waste management is a global challenge with more than 390 million tons of plastic waste generated around the world every year. There is presently little or no way for ecosystems to decompose most commercial plastics, with more than 75% of waste plastic remaining either in landfills or in the ocean for decades: they mostly break into small pieces (microplastics and nanoplastics) and are increasingly found to contaminate all environments and forms of life posing unknown risks to human health and the environment. Using bioprospecting, we have identified and isolated a novel microbe, X-32, that can use multiple major types of plastic (polyesters, polyolefins and polyamides) as the sole carbon and energy source for its growth. Further, X-32 can also degrade various commercial polyamides, such as fishing wires, dental floss, etc., with no prior treatment to the samples. X-32 degrades these plastic particles into relatively harmless substances: carbon dioxide, water, and decayed biomass. We at Breaking Inc., a spin-off from the Wyss Institute at Harvard University and partnered with Colossal Biosciences, are applying genetic and synthetic biology techniques to accelerate the evolution of faster and more robust plastic-eating microbes and enzymes that can help decompose all waste plastics globally.

Session 3 – Bio-based Plastic Degradation



Karine Auclair Department of Chemistry McGill University, Canada

SHORT BIOGRAPHY

After graduate studies focusing on a Diels-Alderase at University of Alberta, Canada and a postdoctoral fellowship at UC San Francisco, USA, to work on heme proteins, Auclair joined the McGill chemistry department in 2002 as an Assistant Professor. She was promoted Associate Professor with Tenure in 2009, and Full Professor in 2016. She is currently on the Board of Directors and member of the Scientific Board at Carbios. Among several awards, Auclair holds the Canada Research Chair in Antimicrobials and Green Enzyme, and is recipient of the CIC Clara Benson award, two McGill Professorships (Fessenden and Tomlinson), and the Leo Yaffe teaching award. Her success is further reflected in >100 invited seminars around the world, several patents, and ~100 peer-reviewed publications. Auclair is a bioorganic chemist recognized internationally for her contributions to the field of antibiotic resistance, and more recently, for the innovative concept of mechanoenzymology in moist-solid reaction mixtures.

ABSTRACT

Plastic Deconstruction by Enzymes in Moist-solids

In recent years, many enzymes have been reported that can depolymerize plastics. Enzymes are typically manipulated as dilute aqueous solutions, which generates wastewater, leads to solubility issues and is remote from enzymes' natural environment. Whereas cellular enzymes operate in a highly concentrated milieu, with little bulk water available, other enzymes are naturally secreted into the environment by microorganisms and have evolved to operate on a surface exposed to air moisture, conditions that can be emulated using moist-solid reaction mixtures and accelerated with mechanical mixing (mechano-enzymology). Our main technique, coined RAging, consists of brief, intermittent periods of mixing between longer periods of aging (i.e. static incubation). This led to an unconventional and highly efficient biocatalytic method that is especially promising for poorly soluble and chemically recalcitrant substrates such as biomass and plastics. Remarkably, when applied to PET, this technique not only proceeds with higher yields than in bulk water, but also enables the direct depolymerization of highly crystalline contents, which normally requires an initial energyintensive amorphization step. Other advantages of this unorthodox approach range from avoiding solubility issues and minimal wastewater production, to avoiding the need for pH control. This presentation will summarize our latest results for plastics enzymatic depolymerization.

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Session 3 – Bio-based Plastic Degradation



Vanessa Vongsouthi Head of Science and Operations and Research Co-Founder Samsara Eco, Australia

SHORT BIOGRAPHY

Vanessa Vongsouthi is a Research Co-Founder of Samsara Eco. Vanessa worked with Matthew Spence on the engineering of plastic-degrading enzymes at the beginning of her PhD at the Australian National University (ANU). This research evolved into the seed technology of Samsara's infinite recycling process. Completing her PhD at the ANU in engineering proteins for industrial and medical applications, her previous research has ranged from the design of fluorescent biosensors to study neurological disease, to the enhancement of enzymes for industrial applications. As Head of Science Operations (and formerly Head of Protein Engineering), Vanessa leverages her technical background in enzyme engineering to provide leadership on the strategy and direction of Samsara's Science program, including the continuous improvement of enzymatic processes and the development of new enzymes.

ABSTRACT

Scalable Chemoenzymatic Recycling of Plastics

Samsara Eco's chemoenzymatic technology for the depolymerisation of various plastics, specifically polyester (PET) and nylon 6,6, addresses significant environmental challenges. Vanessa Vongsouthi, a co-founder of Samsara Eco, will discuss the global plastic waste crisis and the limitations of current recycling methods. The presentation will highlight Samsara's innovative approach using engineered enzymes, designed through deep learning algorithms, to efficiently break down different plastics into monomers that can be repolymerised into virgin-like resins. Samsara's collaboration with industry partners exemplifies the practical applications and potential of this technology to revolutionise plastic recycling and reduce global plastic waste and emissions.

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Session 4 – Enzymes for Plastic Degradation: New and Engineered



<u>YEOM Soo Jin</u> School of Biological Sciences and Technology Chonnam National University, Korea

SHORT BIOGRAPHY

Dr. Yeom is currently an associate professor in School of Biological Sciences and Technology of Chonnam National University, Korea. Yeom was a senior researcher at Synthetic Biology and Bioengineering Research Center, KRIBB, Korea from January 2013 to August 2019. She has focused on screening of new biocatalyst and the enzyme engineering for plastic (polyethylene, polystyrene, polypropylene etc,.) biodegradation and C1 gas (methane) bioconversion. Her research field is highly interested in the directed evolution and rational approached based evolution for achieving desired enzyme functions using synthetic biology-based biosensor.

ABSTRACT

Exploring a New Biocatalyst for Plastic Biodegradation

Plastic contamination currently threatens a wide variety of ecosystems and presents damaging repercussions and negative consequences for many wildlife species. Sustainable plastic waste management is an important approach to environmental protection and a necessity in the current life cycle of plastics. In this regard, plastic biodegradation by microorganism is most notable. This presentation includes current plastic pollution trends, screening of plastic biodegradable new bacteria, discovery of new a trigger biocatalyst for polyethylene (PE) biodegradation and the research hypothesis is that our common area is already surrounded with a bunch of plastics, so it could be possible to screen plastic-degrading bacteria, although a trash-contaminated uncommon environment might have many plastic-degrading bacteria. Furthermore, we propose adopting P450 into plastic-eating synthetic bacteria for PE biodegradation. This strategy can be applicable to other dense plastics, such as polypropylene and polystyrene.

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Session 4 – Enzymes for Plastic Degradation: New and Engineered



YEW Wen Shan

Head of Department, Biochemistry Yong Loo Lin School of Medicine National University of Singapore (NUS) Deputy Director and Principal Investigator, NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI)

SHORT BIOGRAPHY

Associate Professor Wen Shan YEW heads the Department of Biochemistry at the National University of Singapore's Yong Loo Lin School of Medicine. Trained in mechanistic enzymology under Professor John A. Gerlt at the University of Illinois at Urbana-Champaign, Prof Yew's research focuses on "synthetic enzymology," bridging enzymology, biological chemistry, and synthetic biology. His work applies structural and mechanistic enzymological knowledge to protein engineering, biocatalysis, biotransformation, and therapeutic development. Prof Yew's research addresses metabolic diseases, infectious diseases, cancer, ageing, and urban sustainability. Aligning closely with industrial needs, he contributes to the Wilmar@NUS Corporate Laboratory, HH@NUS SINERGY Laboratory, and the National Centre for Engineering Biology of Singapore. His innovative approach to enzymology aims to advance both scientific understanding and practical applications in various fields.

ABSTRACT

Repurposing Biology through Synthetic Enzymology for Human and Planetary Health

Synthetic Enzymology has been increasingly used to engineer enzymes within biological systems for the repurposing of biology for purposeful function. The brief seminar will touch on the use of Synthetic Enzymology to achieve sustainable biomanufacturing and urban sustainability. Using mechanistic and engineering principles in enzymology, we have constructed a glycerol-utilizing pathway in a non-canonical 1,3-Propanediol (PDO) producer. Using combinatorial engineering approaches, we optimized 1,3-PDO production strains and achieved 1,3-PDO production in the engineered microbes at pilot scale. Together with our industry partner, we scaled up 1,3-PDO production, and have embarked on an industrialization journey. Traditional production of polyester feedstock is dependent on crude oil, is one of the largest contributors to carbon emissions worldwide and is unsustainable. Through fermentation, modified microbes can be grown using industrial waste feedstocks to produce polymer feedstocks, and together with plastic degradation feedstocks, a tractable route towards sustainable biomanufacturing can be envisioned. Biomanufacturing using this method, instead of using crude oil feedstocks, is a step closer to sustainability and planetary health, as it shifts the reliance away from traditional industrial petrochemistry.

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Session 4 – Enzymes for Plastic Degradation: New and Engineered



Farid Ghadessy Institute of Molecular and Cell Biology A*STAR, Singapore

SHORT BIOGRAPHY

Farid Ghadessy is a Principal Investigator in the Protein and Peptide Engineering and Research Group (P2ERL) in the Institute of Molecular and Cellular Biology (Singapore). His research interests cover engineering/discovery of novel biotherapeutics and molecular diagnostics enzymes using directed evolution approaches. He is the co-founder of AbAsia Pte Ltd, a reagents and protein expression company.

ABSTRACT

Thermostabilising PETase for Increased Robustness

Farid Ghadessy, Ding Ke, Zarina Levitskaya, Barindra Sana, Rupali Reddy Pasula, Abdurrahman Adam, Chandra Verma, Sierin Lim

Thermostable enzymes are often desirable for industrial applications. They can facilitate higher temperature catalysis if desired, and are often more stable in organic solvents. Stable enzymes are also more amenable to solid support coupling and re-use. Expression yields of thermostable proteins are also often higher than their mesophilic counterparts, resulting in production cost savings. We have implemented various strategies to increase thermostabilty of the PETase enzyme which hydrolyses polyethylene terephthalate (PET) and has a melting temperature of around 42 degrees C. Of these, a scaffolding approach using the Very Green Fluorescent Protein (vGFP) has shown promising results, increasing product yields on pre-processed bottle grade PET substrate by up to 3 fold.

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Contributed Speaker

Session 4 – Enzymes for Plastic Degradation: New and Engineered

Subha Kalyaanamoorthy University of Waterloo, Canada

ABSTRACT

Transforming Plastic Waste: Computational Advances in Enzyme Engineering

Subha Kalyanamoorthy

Plastics are versatile materials used worldwide in applications such as packaging and biomedical industries. However, only about 9% of the plastic produced globally is recycled, with the rest accumulating in landfills or oceans. This creates an urgent need for next-generation technologies to address this challenge. Recently, enzyme-based biocatalysts have emerged as a promising alternative to conventional recycling methods, being clean, safe, and environmentally friendly. Traditional protein engineering methods like directed evolution and random mutagenesis are widely used but involve exploring vast numbers of protein sequences and mutations to identify suitable modification hotspots. Additionally, understanding the mechanistic processes of enzymatic catalysis is crucial for designing efficient biocatalysts. With rapid advancements in high-performance computing and sophisticated software, computational methods are now well-suited to tackle these challenges. This talk will highlight different computational techniques our team has adopted for understanding the structure-activity relationships and engineering novel enzyme variants with improved properties.

Session 4 – Enzymes for Plastic Degradation: New and Engineered



<u>CUI Yinglu</u> Institute of Microbiology Chinese Academy of Sciences, China

SHORT BIOGRAPHY

Yinglu Cui, Ph.D., Associate Professor at the Institute of Microbiology, Chinese Academy of Sciences. Her primary research interests lie in the computational design of enzymes. To date, she has published over 40 SCI papers, with 16 as the first or corresponding author, including Nature Catalysis, Nature Communications, and ACS Catalysis. In 2023, she received the "Young Enzyme Engineer Award" from the Enzyme Engineering Committee of the Chinese Society of Microbiology. She currently serves as a member of the Computational Toxicology Committee of the Chinese Society of Toxicology, a member of the Youth Scholars Working Committee of the Biochemical Engineering Specialized Committee of the Chemical Industry and Engineering Society of China, and a youth editorial board member of the journal Green Carbon.

ABSTRACT

Computational Redesign of a Hydrolase for Nearly Complete PET Depolymerization at Industrially Relevant High-Solids Loading Yinglu Cui, Yanchun Chen

Biotechnological plastic recycling has emerged as a suitable option for addressing the pollution crisis. A major breakthrough in the biodegradation of poly(ethylene terephthalate) (PET) is achieved by using a LCC variant, which permits 90% conversion at an industrial level. Despite the achievements, its applications have been hampered by the remaining 10% of nonbiodegradable PET. Herein, we address current challenges by employing a computational strategy to engineer a hydrolase from the bacterium HR29. The redesigned variant, TurboPETase, outperforms other well-known PET hydrolases. Nearly complete depolymerization is accomplished in 8 h at a solids loading of 200 g kg-1. Kinetic and structural analysis suggest that the improved performance may be attributed to a more flexible PETbinding groove that facilitates the targeting of more specific attack sites. Collectively, our results constitute a significant advance in understanding and engineering of industrially applicable polyester hydrolases, and provide guidance for further efforts on other polymer types.

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Session 5 – Organisms for Plastics Conversion: Native and Engineered



Federica Bertocchini Principal Investigator Plasticentropy, France

SHORT BIOGRAPHY

A molecular biologist by training, F.B. specialized in the study of early development of amniote embryos, working on basic questions related to the molecular mechanisms driving early development of chick and reptile embryos. At the same time, F.B. developed a parallel line of research driven by the interest for environmental bioremediation by biological methodologies. F.B discovered that the larvae of Lepidoptera Galleria mellonella (wax worms) are capable of fast degradation of polyethylene (PE) the most resilient and also produced plastic material in the world. Recently, in F.B laboratory the first PEases were discovered: these are enzymes produced by the larvae and capable of degrading PE within a few hours from exposure. The study of these new enzymatic activities, in view of potential future applications, together with the understanding of their role within the invertebrate physiology and ecology, constitute her current topics of research.

ABSTRACT

Insects and Bioremediation:

Wax Worm Enzymes versus Plastic Waste Accumulation

Plastic production is steady increasing, causing the growth in plastic waste accumulation all over. Environmental damages and health risks to animals are already here, while solutions to this urgent issue are not even at the horizon. Degradation of polyolefins, such as polyethylene (PE), polystyrene (PS) and polypropylene (PP) by biological means, with recovering and utilization of degradation products, presented itself as a potential solution for the past few decades. Nonetheless, the identification of enzymes as biocatalysts to degrade such sturdy compounds has remained elusive. Recently, insects appeared on the scene as polyolefin degraders, opening another path to the quest for enzymes. It is within this new niche that the first PE degrading enzymes have been identified: the larvae of lepidopter Galleria mellonella produce hexamerins that localize in the saliva. These proteins, as well as the saliva, can oxidize and degrade PE within a few hours from exposure. Could these newly discovered PEases provide a solution for the plastic pollution plague?

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Contributed Speaker

Session 5 – Organisms for Plastics Conversion: Native and Engineered

Bodhi Dharma Mulawarman University, Indonesia

ABSTRACT

Several Promising Actinobacteria-Plastics-Degraders Isolated from East Kalimantan, Indonesia

Bodhi Dharma, Maudy Rahayu, Nur Ulmi, Subur Pasaribu, Imam Rosadi, Muhammad Fathurrahman, Ali Budhi Kusuma

Plastic waste is abundant in the environment, causing many problems. The aim of the research is to use bacteria in the biodegradation of PCL and PET plastic wastes. We successfully isolated Actinobacteria from two sites in East Kalimantan province. From more than 90 isolated strains, we obtained 10 potential strains that were able to degrade PCL film. Those strains were treated to grow in two different substrates, i.e., PCL film and commercial PET (AQUA-TM). This biodegradation experimental work was designed with a CRD factorial. The result showed that the strain designated KT05C3 has superior capacity in biodegradation of PCL with the percentage of biodegradation reaching 97.778% after 30 days of incubation and 98.33% after 45 days. The second strain with the ability to biodegradation PCL is designated KT01C5, with a percentage of PCL biodegradation at 8.347% after 30 days of incubation. The strain KT05C3 also has strong activity against PET, with a biodegradation percentage of 4.747% after 45 days of incubation. The strain KT05C3 has 97.81% genetic similarity to Streptomyces cavourensis W192, respectively. This is the first report on the isolation of several plastic-degrader indigenous strains from East Kalimantan, Indonesia, with a high percentage of plastic biodegradation.

Session 5 – Organisms for Plastics Conversion: Native and Engineered



ZHOU Kang Food, Chemical and Biotechnology Cluster Singapore Institute of Technology

SHORT BIOGRAPHY

Dr. Zhou Kang is an Associate Professor at Singapore Institute of Technology. He received his Bachelor's degree from Tianjin University in 2007. After obtaining his PhD from National University of Singapore (NUS) in 2012, he spent three years as Postdoctoral Associate at Massachusetts Institute of Technology. He was an Assistant Professor at NUS from 2015 to 2023 and start to hold the current appointment since 2023. Dr. Zhou's research focuses on producing valuable small molecules from low-cost feedstocks through metabolic engineering. He has also been passionate about developing new tools and methods for engineering and monitoring microbial fermentation. Dr. Zhou received the 2020 SIMB Young Investigator Award and served as Guest Editor for many journals including Current Opinions in Biotechnology and Current Opinions in Green and Sustainable Chemistry.

ABSTRACT

Engineering Escherichia coli to Utilize Diols Derived from Plastic Wastes

My research group has focused on engineering Escherichia coli to utilize new C2-C3 feedstocks. In the context of plastic waste utilization, we have primarily worked on ethylene glycol (EG), a diol that can be derived from polyethylene terephthalate after hydrolysis. Through metabolic engineering, we not only enabled the bacterium to grow on EG as a major carbon source, but also manage to produce valuable aromatic amino acids at a yield that is even higher than using glucose, a conventional feedstock. I will share this story in the presentation and will also report our latest progress in utilizing 1,3-propanediol.

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Contributed Speaker

Session 5 – Organisms for Plastics Conversion: Native and Engineered

CHUA Yuen Siong National University of Singapore

ABSTRACT

Upcycling of PET Monomers through Metabolic Engineering of *Pichia pastoris*

Chua Yuen Siong, Lim Yan Ping, Elvis Chua, Han Ping, Yew Wen Shan

The enzyme PETase is known to biodegrade poly(ethylene terephthalate) (PET) into ethylene glycol (EG) and terephthalic acid (TPA). However, studies on the utilisation and valorisation of its monomers are comparatively scarce. In this study, we genetically engineered Pichia pastoris towards the utilisation of PET monomers as non-conventional carbon sources. We successfully engineered a strain that converts EG to glyceric acid, which is part of the glycolysis pathway. Our TPA engineered strain also showed the capability to convert TPA to protocatechuic acid, an intermediate in central metabolism pathways. In addition, we have established analytical methods to detect intracellular metabolites from the conversion of EG or TPA. As a logical progression of the studies, labelled monomers will be used to verify the metabolism of the PET monomer feedstock in our engineered strains. This work will serve as a basis for future bioremediation of PET into specialty chemicals.

Contributed Speaker

Session 5 – Organisms for Plastics Conversion: Native and Engineered

> Jasmine Ikraminaningsih Universitas Indonesia, Indonesia

ABSTRACT

Isolation and Characterization of Polypropylene-Degrading Bacteria from the Gut Microbiome of *Tenebrio molitor*

Jasmine Ikraminaningsih

Post-COVID-19, the accumulation of non-degradable medical waste, particularly single-use polypropylene (PP) face masks, has become a significant issue. This study explores bacteria from the digestive tract of Tenebrio molitor (T. molitor) larvae for their ability to degrade PP. Six bacterial species were identified: Staphylococcus succinus, Staphylococcus edaphicus, Pantoea anthophilia, Metabacillus niabensis, Pseudomonas aeruginosa, and Pantoea ananatis, with Staphylococcus succinus and Staphylococcus edaphicus showing the best growth. PP sheets were fermented in minimal salt media inoculated with these isolates in a 500 mL batch reactor. After 65 days, bacterial growth was indicated by increased optical density (OD) values. The isolates effectively degraded the PP sheets, with mass reductions of 20% and 27.3%, and notable changes in morphology and functional groups, as observed through SEM and FTIR-Spectroscopy. These results suggest that bacteria from T. molitor larvae possess potential for biodegrading single-use PP face masks, offering a sustainable solution to medical waste.

Session 5 – Organisms for Plastics Conversion: Native and Engineered



LIM Yee Hwee Director, Chemical Biotechnology & Biocatalysis Institute of Sustainability for Chemicals, Energy and Environment (ISCE²) A*STAR, Singapore

SHORT BIOGRAPHY

Yee Hwee LIM leads the Chemical Biotechnology and Biocatalysis division at the Institute of Sustainability for Chemicals, Energy and Environment (ISCE2), A*STAR. Her team focuses on developing integrative technologies at the interface between chemistry, biology, informatics and engineering for sustainable chemical manufacturing. As a chemist by training trained in the UK and US, she is passionate about advancing chemistry frontiers and harnessing Nature's catalytic powers to solve molecular challenges.

Beyond her primary role at ISCE2, Yee Hwee also serves as deputy programme director for SIBER 2.0, an A*STAR central initiative program in sustainable chemicals biomanufacturing. Concurrently, she is also the deputy director for SINERGY, the Singapore Consortium for Synthetic Biology and an adjunct Associate Professor at the Synthetic Biology Translational Research Program, Yong Loo Lin School of Medicine (NUS).

ABSTRACT

Plastics and Sustainability

Plastics has changed the way we live today. However, low recycling rates coupled with increased production have resulted in an alarming rapid accumulation of a widely used plastic material, polyethylene terephthalate (PET). With the build-up of plastics in our environment, there is an urgent need for more sustainable solutions to process them. This talk will briefly explore the key research strategies for plastics in the area of sustainability. Further discussion on the emerging biobased solutions involving the use of enzymes for plastics recycling or bioprocessing will be discussed. By leveraging on a A*STAR strain collection, the discovery of a new family of PET degrading enzymes with ~60% similarity to LCC will be shared. Enzyme activity can be further enhanced through a variety of rational engineering and modern Alassisted accelerated enzyme engineering methods.

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Session 6 – Enabling Technologies



<u>Soichiro TSUDA</u> Chief Technology Officer bitBiome, Japan

SHORT BIOGRAPHY

Soichiro (So) Tsuda has a Ph.D. from Kobe University Graduate School of Natural Science and Technology. He joined bitBiome in October 2020 and assumed his current role in September of 2022. He has over 15 years of expertise in interdisciplinary research across biology, engineering, and mathematical modeling. Previously, he worked as a Lord Kelvin-Adam Smith Research Fellow (2012) at the University of Glasgow in the UK, a Japan Society for the Promotion of Science Outstanding Research Fellow (2019), and the Director of Application Development at On-Chip Biotechnologies Co., Ltd. At bitBiome he is leading new application developments using microbial single-cell genome sequencing and enzyme discovery and engineering from our database.

ABSTRACT

Discovery of PET-degrading Enzymes from Microbial Single-cell Genome Database

Soichiro Tsuda, Hideaki Mabashi, Makoto Hirai, Shigeru Sakurai, Masato Kogawa

Environmental microbiota are a vast, untapped resource for novel biocatalytic enzymes. Using the proprietary microbial genome database created via the bit-MAP(R) single-cell sequencing method, we have efficiently recovered unique microbial genes from complex environmental samples. Our database, enriched with enzyme genes absent in public databases, supports the discovery of enzymes capable of novel reactions. To capitalize on this, we developed bit-QED, a computational pipeline for enzyme identification. This pipeline has been effective in identifying biocatalytic enzymes, which we confirmed experimentally. At the workshop, we report the discovery of a new PET hydrolase, bbPET0069, from a soil microbial genome. This enzyme resembles a cutinase-like Type I PET-degrading enzyme but lacks disulfide bonds. Remarkably, bbPET0069 demonstrated significant synergy with Candida antarctica lipase B (CALB), achieving rapid and efficient PET degradation. By employing bitQED's 3D structural modeling for protein engineering of the enzyme, it resulted in a 12.6-fold increase in PET degradation activity with CALB compared to the wild-type enzyme alone. Our integrated approach enabled the efficient degradation of PET to terephthalic acid monomers up to 95.5%, showcasing the potential for enhancing PET-degradation enzymes through protein engineering.

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Session 6 – Enabling Technologies



<u>Tristan Bepler</u> Co-founder and CEO OpenProtein.AI, Singapore

SHORT BIOGRAPHY

Tristan Bepler, PhD, is the CEO and co-founder of OpenProtein.AI, a company building ML/AI solutions for protein engineering. He is also a group leader in the Simons Electron Microscopy Center at New York Structural Biology Center where he develops machine learning methods for analyzing microscopy images and experimentally determining protein structures. He received his PhD from MIT under the supervision of Bonnie Berger and completed a short post-doc with Tim Lu prior to starting OpenProtein.AI and his academic research group.

ABSTRACT

Engineering a Plastic Degrading Enzyme with Protein Language Models and Bayesian learning

Tristan Bepler, Timothy Truong Jr, Zarina Levitskaya, Sierin Lim, and Farid John Ghadessy

Plastic degrading enzymes are of increasing interest for recycling and mitigating the environmental impact of plastics worldwide. Recently, enzymes capable of degrading Polyethylene terephthalate (PET) have been discovered in natural environmental microbial communities. However, these enzymes have insufficient activity and stability for industrial use. Here, I'll discuss our efforts to engineer plastic degrading enzymes with increased catalytic rates using our generative protein language model, PoET, and Bayesian machine learning platform at OpenProtein.AI. We demonstrate that by combining representations from protein language models with data-efficient fine-tuning procedures, we are able to learn sequence-activity mappings from just tens of experimentally characterized variants and propose new variants with enhanced activity.

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Contributed Speaker

Session 6 – Enabling Technologies

Jean-Baptiste Henri Lena Nanyang Technological University, Singapore

ABSTRACT

Screening the Enzymatic Degradation of Semi Aromatic Polyesters

Jean-Baptiste Lena, Keerthi Mohan Pinchi, Cho Sung Ju, Sharad Kharel, Ke Ding, John F. Ghadessy, Yeng Ming Lam, Sierin Lim

Global plastic production exceeds 9 billion tons. PET is one of the most widely used plastics thanks to its good thermal and mechanical resistance. Its main applications are in the packaging and clothing industries. Nowadays, only 10 % of plastic is recycled and 12 % is incinerated, while 78 % escapes to the waste management system. The main recycling method is mechanical, which lowers the properties of materials. Alternative methods include chemical recycling which uses toxic solvents and is highenergy consuming and biodegradable PET, whose long degradation leads to residual microplastics. An alternative method is enzymatic depolymerization which is more sustainable (low temperature, mild pH, aqueous solution). However, enzymatic activity strongly depends on substrate physical properties and sometimes leads to a low degradation rate (a few percent per day). Consequently, it is important to identify enzyme mutants with enhanced activity. In this work, we developed a high-throughput screening platform to quickly identify mutants of PETase from Ideonella sakaiensis. This platform relies on fluorescent dye encapsulation into PET microspheres and shows good efficiency. Moreover, it was successfully applied to other semi-aromatic polyesters (PBT and PEN).

Invited Speaker

Session 6 – Enabling Technologies



YEO Zhiquan R&D Deputy Director (Sustainability & Life Cycle Management) Acting R&D Division Director Institute of Manufacturing and Technology (SIMTech) A*STAR, Singapore

SHORT BIOGRAPHY

Dr YEO Zhiquan is the Group Manager of the Sustainability and Life Cycle Management Group at A*STAR SIMTech. He has been actively involved in conducting Life Cycle Assessment (LCA) studies in various sectors, including agri-food, manufacturing, and the built environment for more than 12 years. Dr Yeo also serves as the Convenor for the Working Group on Greenhouse Gas and Product Life Cycle Assessment under the Singapore Standardisation Programme.

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Click the speaker's name to see their profile page

Panelists

Session 6 – Enabling Technologies

Opportunities for Bio-based Plastics Degradation



LIM Yee Hwee Institute of Sustainability for Chemicals, Energy and Environment A*STAR, Singapore



<u>Keats Nelms</u> Executive Director of Science Operations Samsara Eco,



<u>Alain Marty</u> Chief of Scientific Officer Carbios, France



Daniel Tay

Australia

Vice President Temasek , Singapore

Moderator



Sierin Lim

School of Chemistry, Chemical Engineering and Biotechnology Nanyang Technological University, Singapore

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- 2. <u>Jingyu DENG</u> and <u>Ahmad Albar</u>, Nanyang Technological University, Singapore
- 3. <u>Barindra Sana</u>, Singapore Institute of Food and Biotechnology Innovation (SIFBI), A*STAR, Singapore
- 4. <u>Raden Himan Haryo Teguh</u>, Nanyang Technological University, Singapore
- 5. <u>Jingyuan WANG</u>, Nanyang Technological University, Singapore
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- 11. <u>Wei Xin CHAN</u>, Nanyang Technological University, Singapore
- 12. <u>Yan Chun CHEN</u>, Nanyang Technological University, Singapore
- 13. <u>CHUA Yuen Siong</u>, National University of Singapore, Singapore
- 14. <u>Zarina Levitskaya</u>, Institute of Molecular and Cell Biology, A*STAR, Singapore

Abdurrahman Adam Nanyang Technological University, Singapore

ABSTRACT

The Effect of Pre-treatment in Enhancing Enzymatic Degradation of Pc-PET Bottle

Abdurrahman Adam, Rupali Reddy Pasula, Raden Himan Haryo Teguh, Sierin Lim

Enzymatic degradation of PET plastics has been gaining interest and has led to the reporting of many novel variants of PET degrading enzymes. Nonetheless, each study used different substrates to report each new enzyme's performance, which led to an inconclusive comparison of each enzyme. To accelerate the stage of translation, a real Post-consumer (Pc-) PET should be used as a testing substrate, although its polymer properties inhibit the degradation mechanism. Thus, this study investigated the effects of substrate pre-treatment and enzyme loading on the degradation of post-consumer polyethylene terephthalate (pc-PET) using engineered PETase variants. Lab-scale pretreatment methods were employed to reduce substrate crystallinity and particle size. Results demonstrated that these pre-treatments enhanced the degradation rate of pc-PET by STAR PETase, achieving up to 50% degradation yield after 72 days. In contrast, the wild-type Ideonella sakaiensis PETase (IsPETase) showed less than 20% improvement under similar conditions. Furthermore, the enzyme-to-substrate loading ratio was identified as a critical factor in optimizing the degradation yield of treated pc-PET bottles. These findings contribute valuable insights to the development of bioprocesses for the enzymatic depolymerization of pc-PET using engineered PETase variants, potentially advancing sustainable recycling strategies for plastic waste.

Jingyu DENG Nanyang Technological University, Singapore

Ahmad Albar Nanyang Technological University, Singapore

ABSTRACT

Microplastics from Food Containers Inhibit Lysosomal Activity in Murine Macrophages

Jingyu Deng, Ahmad Albar

The pervasive use of plastic products, exacerbated by the COVID-19 pandemic, has raised serious concerns regarding microplastic ingestion and its impact on human health. This study investigates the direct threat posed by microplastic contamination from plastic packaging. We analyzed plastic packaging demand and usage in Asia-Pacific, North America, and Europe, focusing on commonly used plastic food containers. Our results indicate that food containers exposed to high temperatures release over 10 million microplastics per milliliter of water. Moreover, recycled plastic packaging continuously leaches micro- and nanoplastics. In vitro studies demonstrated that murine macrophages readily engulf both micro- and nanoplastic leachates without preconditioning. Microplastic exposure led to inflammation, while nanoplastic exposure significantly suppressed lysosomal activities in macrophages. These findings suggest that ingestion of micro- and nanoplastics from food containers exerts differential adverse effects on macrophage function. Our research highlights the significant health risks posed by the increasing use of plastic packaging and underscores the need for rigorous evaluation of plastic materials to protect consumer health.

Barindra Sana

Singapore Institute of Food and Biotechnology Innovation (SIFBI), A*STAR

ABSTRACT

Developing Thermostable *Is*PETase Using Protein Scaffolding Approaches

Ke Ding, Jia Wei Siau, Rupali Reddy Pasula, Sharon Chee, Sharad Kharel, Jean-Baptise Henri Lena, Eunice Goh, Lakshminarayanan Rajamani, Yeng Ming Lam, Sierin Lim, John F. Ghadessy

Polyethylene terephthalate (PET) hydrolase enzymes show promise for enzymatic PET degradation, which is crucial for addressing global plastic pollution through green recycling of single-use PET. Their full potential can be unlocked with enzyme engineering to achieve thermostability and improved activity on recalcitrant PET substrates. Industrial enzymes benefit greatly from thermostability, as it enhances robustness and significantly reduces the amount required. To date, most engineered PET hydrolases show improved thermostability over their parental enzymes. We developed two thermostable variants of Ideonella sakaiensis PET hydrolase (IsPETase) using different scaffolding strategies. The first strategy utilized SpyCatcher-SpyTag technology to covalently cyclize IsPETase, resulting in increased thermostability but reduced turnover of PET substrates compared to native IsPETase. The second approach involved using a GFP-nanobody fusion protein (vGFP) as a scaffold, which produced the engineered IsPETase with a melting temperature of 80°C. The melting temperature was further elevated to 85°C when a thermostable PETase variant (FAST PETase) was scaffolded into vGFP. Enhancing thermostability using the vGFP scaffold did not affect the PET-degrading activity of the parental enzymes, as observed following thermostability enhancement with SpyCatcher-SpyTag. These contrasting results highlight potential topological and dynamic constraints imposed by scaffold choice as determinants of enzyme activity.

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ABSTRACT

Process Development for PETase Production and Purification

Raden Himan Haryo Teguh, Abdurrahman Adam, Keerthi sundar Mohan Pinchi, Nitish Jeyaraj Suja, Sierin Lim

Plastic pollution has become a critical global issue, and polyethylene terephthalate (PET) is a significant component of plastic waste. Recently, an engineered enzyme has been explored to depolymerize PET into terephthalic acid (TPA) and ethylene glycol (EG). One such enzyme is STARPETase. However, most PETase production is still done in lab-scale shaking flasks. This study aims to scale up the production of STARPETase using a 5 L bench-scale fermenter. This study uses the SHuffle T7 E. coli strain, which was transformed using a pET-22b plasmid containing STARPETase and ampicillin resistance genes. A benchtop bioreactor with a total volume of 5 L was filled with 2 L of various media, shown in Table 1. Oxygen saturation was maintained above 20% by adjusting the airflow from 2 L/min to 4 L/min and stirring speed from 600 rpm to 1000 rpm. By fed-batch fermentation, up to 37 mg/L of STARPETase can be produced in a day, up from 11.62 mg/L in shake flasks.

Jingyuan WANG Nanyang Technological University, Singapore

ABSTRACT

Bio-cycling to Convert Plastic Waste Monomers into Bacterial Cellulose

Jingyuan Wang, Rupali Reddy Pasula, Sierin Lim

The development of efficient process for the conversion of ethylene glycol from plastic waste into bacterial cellulose represents a significant opportunity for plastic management. Despite there are several EG-utilizing bacterias reported, our work is trying to realize using EG as carbon source for bacterial cellulose production. We succeeded in engineering the cellulose-producing bacteria with novel EG metabolic pathway, which has the ability to consume EG and produce bacterial cellulose at the same time. this work could be a promising approach towards biological upcycling of PET plastic.

Keerthi sundar Mohan Pinchi Nanyang Technological University, Singapore

ABSTRACT

Screening Platforms for Plastic Degrading Enzymes

Keerthi sundar Mohan Pinchi, Rupali Reddy Pasula, Sierin Lim

Persistent production and use of synthetic polymers have generated a critical challenge in the form of waste accumulation, thereby deleteriously impacting the environment. The current mechanical and chemical modalities employed for the recycling or degradation of PET plastics holds inherent limitations, contributing to the prevailing crisis. The discovery of PET hydrolysing enzymes within microbes that have adapted to plastic-contaminated environments offers a prospect for enzymatic degradation.

Several synthetic biology tools have been utilized to make strides in enhancing stability and efficiency of PETase. Random mutagenesis followed by high-throughput screening entails one such powerful approach. However, this approach relies on the ability to efficiently screen a large enzyme library which is currently lacking for these screens. This research is directed towards developing multiple screening methodologies – solid and liquid phase. These screening platforms are based on fluorescence measurements of enzymatic activity, employing micro- or nano-sized PET for high-throughput screening of PETase mutants.

The elucidated screening methodologies present an advancement in an efficient process to identify the most proficient mutant from an extensive library of variants, thus extending potential resolutions in enzymatic PET biodegradation.

Kesya Hanna Rosalie Universitas Indonesia, Indonesia

ABSTRACT

Ex-Situ Biodegradation of Single-Use Masks by Gut Microbial Consortium from *Tenebrio molitor* Larvae

Kesya Hanna Rosalie

The COVID-19 pandemic has led to a surge in single-use mask waste, primarily made of non-decomposable polypropylene. T. molitor larvae can degrade various plastics, but their ability to break down masks ex-situ using gut microbes is under-researched. This study aimed to explore this by feeding larvae exclusively on masks to establish a microbial basis for ex-situ degradation. The larvae's gut microbes were extracted, analyzed through metagenomics, and cultured in Minimum Salt Media with 3x3 cm mask pieces in a 500 mL bioreactor for 52 days. Dominant microbes included Klebsiella aerogenes, Tenebrionicola larvae, Enterobacter, Lactococcus garvieae, and Lactococcus formosensis. Microbial growth was monitored via optical density at 600 nm. The mask layers' consumption was 19.200%±0.031 (outer), 30.333%±0.031 (middle), and 26.400%±0.040 (inner), as indicated by mass reduction. Surface damage and functional group changes were confirmed through SEM and FTIR. These findings suggest the microbial consortium from T. molitor larvae can effectively degrade single-use masks ex-situ, offering a sustainable waste management solution.

Rupali Reddy Pasula Nanyang Technological University, Singapore

ABSTRACT

Towards Carbon-Neutral Plastic Bio-Upcycling

PI: Sierin Lim; Co-PI: Farid John Gadessy, Lam Yeng Ming, and Yew Wen Shan

Biocatalysis of plastics and their subsequent re-utilization is hindered by the enzymes' poor activity, thermostability and lack of screening tools for mutant identification. Our research takes a multi-pronged approach to address these issues. We design PETase mutants with higher activity and thermostability using a rational design approach, develop screening platforms for mutant identification, and utilize peptides fused to PETase to enhance enzyme-substrate affinity for superior PET degradation. Additionally, we employ metabolic engineering tools to convert the monomer EG into valuable products, completing the carbon loop with bacterial and yeast chases.

Sakcham Bairoliya Nanyang Technological University, Singapore

ABSTRACT

Cyanobacteria Drive Formation and Sustenance Plastisphere Microbiome in Tropical Environments

Sakcham Bairoliya, Jonas Koh Zhi Xiang, Zin Thida Cho, Bin Cao

Upon entering the marine environments, plastics are colonized by a plethora of microorganisms to form a plastisphere, influencing the fate and transport of the plastic debris and the health of marine ecosystems. The assembly of marine plastisphere is generally shown to be dominated by stochastic processes. However, it remains elusive whether microbial interaction in the assembly of plastisphere microbial communities is conserved or not. We analyzed 137 plastispheres from intertidal zones at different geographical locations and habitats and compared them with the surrounding sediment and seawater microbiomes. Microbial community structure of the plastisphere from different locations was more similar to each other but differed substantially with that of the surrounding sediment and water microbiomes, implying a common mechanism of plastisphere assembly. We used different machine learning algorithms to classify plastic debris samples with high sensitivity based on the microbiome composition. Eukaryotic and prokaryotic phototrophic organisms such as green algae, diatoms, and cyanobacteria, were found to be enriched on the plastic surfaces. Network analysis revealed the central role of the phototrophic organisms in the formation and sustenance of the plastispheres. Our results explain the stochastic assembly of the plastisphere along with conserved properties driven by the phototroph-heterotroph interactions.

Sara Kabirnataj Nanyang Technological University, Singapore

ABSTRACT

Sustainable PET Degradation with a Solar-powered PETase Production

Parth Patankar, Sara Kabirnataj, Sierin Lim

Plastic pollution, particularly from polyethylene terephthalate (PET), poses a significant environmental threat due to its persistence and widespread use in packaging and textiles. Traditional recycling methods are often inefficient and energyintensive, necessitating innovative solutions. PETase, an enzyme capable of degrading PET into its monomeric components, offers a promising alternative. However, sustainable and scalable production of PETase remains a challenge. This study investigates using genetically engineered cyanobacteria to produce PETase intra and extracellularly, leveraging their photosynthetic capabilities for eco-friendly enzyme production. We successfully integrated the PETase gene into the cyanobacterial genome and optimized its expression and secretion using various promoters and signal peptides. The engineered cyanobacteria demonstrated significant PETase activity, both intracellularly and extracellularly, as confirmed by SDS-PAGE, and enzyme assays. The secreted PETase could degrade BHET in the BHET clear zone assay, visually confirming its secretion and activity. Our findings suggest that cyanobacteria present a viable platform for sustainable PETase production with their low-cost cultivation and minimal energy requirements. This approach offers a potential solution to plastic pollution and enhances environmental sustainability. The goal of future studies will be to scale up this system.

Wei Xin CHAN Nanyang Technological University, Singapore

ABSTRACT

Upcycling of Non-biodegradable Plastics by Base Metal Photocatalysis

Wei Xin Chan, Chenfei Li, Xin Ying Kong, Maoping Lyu, Xiu Ting Tay, Milos Đokic, Kek Foo Chin, Crystal Ting Yang, Erin Ke Xin Lee, Jinfan Zhang, Chun Yuan Tham, Wen Jie Lee, Teik Thye Lim, Atsushi Goto, Michael B. Sullivan, and Han Sen Soo

Plastics are integral to the modern lifestyle, but the accumulation of plastic waste in landfills and in the natural environment has been detrimental to ecosystems. Chemical recycling taps on plastic waste as a cheap and abundant carbon resource for the production of valuable chemicals. Traditional chemical recycling processes such as pyrolysis and hydrogenolysis are capable of achieving high conversions, but typically use high temperatures, often require expensive noble metal catalysts and have limited product selectivity. In contrast, photocatalysis is a promising alternative for the upcycling of plastics under mild reaction conditions, but has thus far been limited to condensation polymers or polystyrene plastics that are easier to valorize. Here, we report a photodriven, vanadium-catalyzed upcycling method that is generalizable to most conventional plastics such as polystyrene, polypropylene, polyethylene, polyvinyl chloride, and polyvinyl acetate. We achieved carbon recoveries of up to 77%, with the selective formation of valuable, isolable products including formic, acetic, and benzoic acids. To demonstrate the scalability of the photocatalyzed plastic upcycling reaction, gram-scale reactions using actual Styrofoam waste and polystyrene-based multilayered packaging were conducted using a flow photoreactor with recirculation, where moderate yields of benzoic acid and formic acid were obtained at ambient temperature and pressures.

CHEN Yan-chun

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China

ABSTRACT

Computational Redesign of a Hydrolase for Nearly Complete PET Depolymerization at Industrially Relevant High-solids Loading

CHEN Yan-chun, CUI Ying-Lu, WU Bian

Biotechnological plastic recycling has emerged as a suitable option for addressing the pollution crisis. A major breakthrough in the biodegradation of poly(ethylene terephthalate) (PET) is achieved by using a LCC variant, which permits 90% conversion at an industrial level. Despite the achievements, its applications have been hampered by the remaining 10% of nonbiodegradable PET. Herein, we address current challenges by employing a computational strategy to engineer a hydrolase from the bacterium HR29. The redesigned variant, TurboPETase, outperforms other well-known PET hydrolases. Nearly complete depolymerization is accomplished in 8 h at a solids loading of 200 g kg–1. Kinetic and structural analysis suggest that the improved performance may be attributed to a more flexible PET-binding groove that facilitates the targeting of more specific attack sites. Collectively, our results constitute a significant advance in understanding and engineering of industrially applicable polyester hydrolases, and provide guidance for further efforts on other polymer types.

Yuen Siong CHUA National University of Singapore, Singapore

ABSTRACT

Upcycling of PET Monomers through Metabolic Engineering of *Pichia pastoris*

Yuen Siong Chua, Lim Yan Ping, Elvis Chua, Han Ping, Yew Wen Shan

The enzyme PETase is known to biodegrade poly(ethylene terephthalate) (PET) into ethylene glycol (EG) and terephthalic acid (TPA). However, studies on the utilisation and valorisation of its monomers are comparatively scarce. In this study, we genetically engineered Pichia pastoris towards the utilisation of PET monomers as non-conventional carbon sources. We successfully engineered a strain that converts EG to glyceric acid, which is part of the glycolysis pathway. Our TPA engineered strain also showed the capability to convert TPA to protocatechuic acid, an intermediate in central metabolism pathways. In addition, we have established analytical methods to detect intracellular metabolites from the conversion of EG or TPA. As a logical progression of the studies, labelled monomers will be used to verify the metabolism of the PET monomer feedstock in our engineered strains. This work will serve as a basis for future bioremediation of PET into specialty chemicals.

Zarina Levitskaya A*STAR, Singapore

ABSTRACT

Modulation of PETase Active Site Flexibility and Activity on Morphologically Distinct Ppolyethylene Terephthalate Substrates by Surface Charge Engineering

Zarina Levitskaya, Ke Ding, Barindra Sana, Rupali Reddy Pasula, Srinivasaraghavan Kannan, Abdurrahman Adam, Vishnu Vadanan Sundaravadanam, Chandra Verma, Sierin Lim, John F. Ghadessy

Surface charge engineering is an effective approach for enhancing stability and activity of enzymes. In this study, we investigate the effects of surface charge mutations on activity of PET-degrading enzymes in the context of different parental enzyme scaffolds and PET substrates. Notably, the vicinal K95A mutation in IsPETase reduced hydrolysis of low crystallinity PET powder and PET film. When the same mutation was introduced to the more potent and thermostable V3 variant, enzyme activity was reduced on PET film, but enhanced on PET powder. Addition of the distal R132N and R280A surface charge mutations further improved V3K95A enzyme activity on all tested PET substrates and enabled complete degradation of pre-processed bottle-grade PET powder at 40°C within 3 days. Molecular dynamics simulations suggest additional impact of surface charge mutations on active site flexibility that contributes to the complex interplay between mutation context and substrate morphology.

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