IITB-Monash Research Academy

Indian Institute of Technology Bombay | MONASH University

An Indian-Australian research partnership

Project Title:	**Interactions of microbial consortia with microplastics in context of biofilm formation and spread of antibiotic resistance in the environment**	
Project Number	**IMURA1283**	
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IITB Department:	Environmental Science and Engineering Department	

Research Clusters: Research Themes:

Highlight which of the Academy's CLUSTERS this project will address? *(Please nominate JUST **one.** For more information, see www.iitbmonash.org)*		Highlight which of the Academy's Theme(s) this project will address? *(Feel free to nominate more than one. For more information, see www.iitbmonash.org)*	
1	*Material Science/Engineering (including Nano, Metallurgy)*	***1***	*Artificial Intelligence and Advanced Computational Modelling*
2	*Energy, Green Chem, Chemistry, Catalysis, Reaction Eng*	2	*Circular Economy*
3	*Math, CFD, Modelling, Manufacturing*	3	*Clean Energy*
4	*CSE, IT, Optimisation, Data, Sensors, Systems, Signal Processing, Control*	4	*Health Sciences*
5	*Earth Sciences and Civil Engineering (Geo, Water, Climate)*	5	*Smart Materials*
6	*Bio, Stem Cells, Bio Chem, Pharma, Food*	6	*Sustainable Societies*
7	*Semi-Conductors, Optics, Photonics, Networks, Telecomm, Power Eng*	7	*Infrastructure*
8	*HSS, Design, Management*		

The research problem

Define the problem

Microplastics are inert hydrophobic particles either originating from the production process of different industries such as toothpaste, cosmetics, cleanser in personal care products, clothing, cleaning products, textiles, and plastic industries (primary MPs) or from the physical detrition of bigger plastic particles/objects (secondary MPs). Hydrophobic nature of the MPs enables the attachment of organic compounds on such MP surface thus creating a conducive environment for the biofilm growth as such presence of organic matter and hydrophobic surface enables easier attachment of micro-organisms. The surface of the MPs of covered with biofilms is thus a habitat for the growth of microbes which is distinct as compared to the environment. The surface of such a MP covered with biofilm is expected to contain high concentrations of extra-cellular polymeric substance secreted by the microbes in the biofilm in addition to organic compounds that the microbes might utilize as a substrate. The environmentally denuded MPs have high specific surface area and high porosity which makes it further suitable for biofilm formation as such high surface area and roughness promotes better proliferation and adhesion during biofilm formation.

This discussion indicates that the microplastic surface can be a highly active space for the microbial proliferation. Acknowledging the ubiquity of these MPs in the current environment, these small particles, distinct from the rest of the environment collectively form what we now call as 'Plastisphere'. The role of this plastisphere in the harbouring, transport and dissemination of ARB (antibiotic resistant bacteria) and ARGs (antimicrobial resistance genes) is yet to be understood fully. This is because the complex bio-physico-chemical interactions of the substrate, organic molecules, microbes and the denuded MP surface is not only hard to understand but equally hard to model for predicting the nature of the biofilm. In addition to this complex interaction, analytical strategies for studying such MPs and the associated biofilms, predicting the ARB and the ARG concentrations, underpinning the microbial species diversity in such 'Plastispheres', modelling the interspecies interactions for substrate and ARG transfer, evolution of the MP surface physiology owing to presence of such biofilms, transfer of mobile carrier gene elements in such modified MP surface etc. also need to be developed. Figure 1 below outlines potential MP pathways of biofilm formation and AMR spread in the environment.

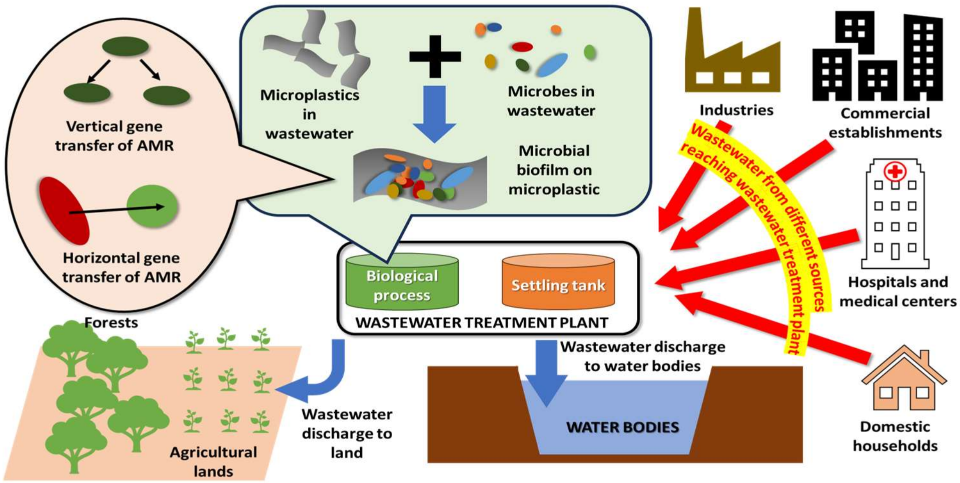

Figure 1: Interaction and propagation of AMR through MP-based biofilms

For example, detection of MPs from environmental samples require a combination of optical microscope assisted visual inspection as well as FTIR or other spectroscopic determination. To achieve a high level of detection accuracy (as much as 90%), the pre-processing of the water and sediment samples for extraction of MPs is another bottleneck towards studying the biofilm growth. The schematic adopted from National Oceanic and Atmospheric Administration (NOAA) as shown below (Figure 2, Masura et al. (2015)). For accurate quantification of MPs, the removal of natural organic matter in the samples is important prior to the next step of density floatation as the organic matter has a specific gravity of 1.2-1.6 and is also prone to floatation. Moreover, the adhesion of such natural organic matter to MPs introduce an error in the gravimetric quantification of such MPs after filtration. However, this step can be counter-productive when the goal of research is to understand and quantify the potential of biofilm formation in the MPs. Also, cross-contamination of environmental MPs and the harboured biofilm by equipment or the sampling machine can lead to reduced accuracy of prediction for microbial diversity and ARG content. The contamination during collection of environmentally present MPs can give a false information about the persistence/presence of ARGs.

Figure 2: Methodology for microplastic characterization

Apart from the methodological bottlenecks in understanding the complex MP-biofilm-ARG interactions, the more challenging task is to establish how the biofilm changes the morphological structure of the MPs which in-turn again contributes to the biofilm proliferation, niche development, speciation and ARG transfer.

For instance, the ARG transmission in such surface property modified will occur distinctly differently than that of the pristine MP surface as the microbial actions on the surface changes the microstructure as well as the stiffness of the surface. Such modifications may incorporate micro-channels facilitating effective substrate and ARGs diffusion and microbial proliferation. To understand how the ARGs and ARBs will be supported by such Plastispheres, the ideal case study would be to collect environmental samples of such plastispheres and study the biofilm development, ARG transfer, new ARB development, microbe-pollutant interactions etc. among other aspects. However, the challenges with identification and quantification of MP, chances of cross-contamination of samples, and the interdisciplinary analytical approach that needs to establish the baseline mechanisms of such MP-biofilm association formation makes it difficult to use the environmental samples for such investigations. The first step towards understanding this complex microcosm of the plasti-sphere is simulating biofilm growth on virgin MP beads and sheets in a controlled environment.

To summarize, the interaction of the MPs with the microbial biofilm and the effect of such biofilm on the MP denudation needs substantial insight. Based this discussion, the following objectives are proposed for this project:

Objective 1: Evaluation, quantification and characterization of MPs from environmental samples.

Objective 2: Detection of microbes including antibiotic resistant strains in such environmental samples in background matrix and on MP surface.

Objective 3: Investigating the development of biofilms on pristine MPs evaluating the intra-biofilm interaction harbored on MP surface

Project aims

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How skills/experience of the IITB and the Monash supervisor(s) support the proposed project

Highlight the purpose of the collaboration and/or the complementary skills/experience that you bring to the project. Do you have any joint or independent publications in the area of the proposed project?

The Faculty at IIT Bombay, Dr. Indrajit Chakraborty, has research experience of working with microbial systems in both his Ph.D. at IIT Kharagpur and Postdoc at University of Washington. In his doctoral research, Dr. Indrajit worked on the degradation of complex organic contaminants with anaerobic microbial consortia. Further, in his postdoctoral research, Indrajit focussed on the community changes in a height wise profile in up-flow anaerobic sludge blanket reactors. In this work, Indrajit focussed on what is the speciation along the height of the reactor as well as the effect of introducing inert media for biofilm growth. At IITB, Indrajit's research spans on understanding the different material and microbe interactions in built and modified environments. For instance, one of the projects focuses on how biochar-microbe-rhizosphere interaction works. In another project, metallic nanoparticles are being implemented for effective disinfection strategy (This is a previous IITB-Monash project) Dr. Indrajit's lab is equipped with all basic and advanced setup required for understanding the microbial community development in such a plastisphere. Indrajit also has experience in wastewater and water quality analysis which will be crucial for understanding the nutrient-substrate-biofilm-MP interactions. In brief, Indrajit has the expertise to drive the biofilm related experimental work required in this project.

The Co-Supervisor at IIT Bombay, Dr. Amritanshu Shriwastav, has expertise on both bio-processes for resource recovery and bioremediation as well as on MP fate and degradation. In his past research, Dr. Amritanshu has worked on algal biorefinery concept for bioremediation and valuables such as protein, sugar and biofuel recovery. Amritanshu's group is currently working on detection, fate and transport and removal of microplastics from wastewater and other contaminated media. A recent graduate from Dr. Amritanshu's lab has done extensive experimental and modelling work on MP fate and removal in wastewater treatment plant. Prof. Amritanshu's work also spans on understanding the possibility and mechanism of in-vitro digestion of microplastics. In this project, Amritanshu's expertise will be vital for identification and characterization of microplastics. His contribution will also be vital for understanding the biofilm mediated biochemical and environmental factors mediated physico-chemical modification of the MP surface properties.

The Supervisor at Monash University, Dr. Simone Li heads the Microbiome Systems lab at the Biomedicine Discovery Institute (Dept of Microbiology) and Research Leader at the Centre to Impact Antimicrobial Resistance, Monash University Australia. She is a trained bioinformatics engineer and has extensive experience in microbial biotechnology and microbiome data science. She leads a data-oriented research program with a focus on comparative metagenomics and other 'omics technologies to study the ecology and evolution of microbial communities in anthropogenic and health-related settings with direct translational outcomes, such as the proliferation of ARB and ARGs. Dr. Simone has led successful international multi-disciplinary collaborations that revealed first insights into the dynamic impact of interventions on complex microbiomes such as that of the human gut (*Science* 2016, *Nature Medicine* 2022) and industrial bioethanol production (*Nature Microbiology* 2024). Simone's contribution as the Monash Lead will be vital for deciphering the diversity of microbes in the 'Plastisphere', interactions between MPs and biofilm, mechanisms of antibiotic resistance proliferation, as well as the impact of MP denudation on these unique microbiomes.

What is expected of the student when at IITB and when at Monash?

Highlight how the project will gain from the students stay at IITB and at Monash

The student at IITB will conduct experiments with MP samples and study the growth of biofilm over that. In doing so, the student will conduct metagenomic analysis of the biofilm developed on the surface of the MP under different nutrient and environmental conditions in controlled laboratory settings. In addition to this, the student will also study the degradation of MPs exposed to such biofilm during his stay at IITB. The lab of the IITB PI will facilitate the infrastructure set up for the microbial experiments. The Environmental Science and Engineering Department has excellent facilities in terms of LC-MS-MS, IC, biosafety cabinets, dedicated incubators (also available in PI's lab) and other necessary instrumentation, equipment and experimental setup fabrication facility (if required) relevant to the project. The Co-PI's lab will provide the support for the protocol, data collection on the MP denudation and characterization experiments.

In the 3rd year, the student will be well-equipped with acute knowledge of their experimental setup and generated data. This is an optimal time to join the lab of the Monash PI and gain experience in advanced bioinformatics and large-scale data integration. They will perform analysis of their generated data and compliment their findings with data and software maintained by Dr Li's lab. High-performance computing resources and infrastructure will be available at Monash BDI, including the M3 supercomputer that is housed at Monash University.

Expected outcomes

Highlight the expected outcomes of the project

Based on the above discussed objectives and project aim, the following outcomes are expected from the project:

1. Enhanced understanding of the microplastic-biofilm interaction, especially in relation to the development of antimicrobial resistance in the microbes
2. Understanding the microbial ecology and its dynamics on microplastic surface
3. Role of microbes and their biofilm in MP degradation
4. Protocol for the quantification of microbial diversity on environmental MP samples

How will the project address the Goals of the above Themes?

Describe how the project will address the goals of one or more of the 6 Themes listed above.

The project focuses on understanding the microplastic-microbe interactions. Such MP harboured microbial biofilms can also propagate antibiotic resistant genes and bacteria apart from providing a protected environment for the pathogenic microbes. Understanding of such threats would be helpful in framing future policies for monitoring and treatment of

MPs. This will be beneficial to the health science theme of research. In addition, the project will help frame policies for further tackling the MP mediated pathogen spread as well as associated environmental impact. This will contribute to building sustainable societies which is one more topic-aligned theme of the cluster.

How well the IITB and the Monash supervisor(s) know each other

Provide details of previous collaborations (if any). For new collaborators, have you had a chance to meet each other in person or through VC or Skype?

Dr. Indrajit and Dr. Simone had a detailed discussion on the project in the past few months through web meetings as well as during Indrajit's visit to Monash University in May 2025 as a part of the delegation. Dr. Amritanshu is Indrajit's colleague from the same department (ESED) at IIT Bombay.

Potential RPCs from IITB and Monash

Provide names of the potential research progress committee members (RPCs) and describe why they are most suited for the proposed project

IITB RPC members:

1. Prof. V. S. Vamsi Botlaguduru: Prof. Vamsi has research experience in advanced oxidation and advanced reduction processes for degradation of emerging contaminants. He has been guiding students in the topics of emerging contaminant fate and transport as well as the degradation of emerging contaminants present in runoff and surface water.
2. Prof. Suparna Mukherji: Prof. Mukherji has extensive experience working with microbes for bioremediation as well as for resource biorecovery from waste. In addition, a past research at Prof. Mukherji's lab also explored the spread of antibiotic resistance in Indian context as a part of the joint project with a UK team under the UKEIRI collaborative grant.
3. Dr Andre Mu is a Lab Head in the Department of Biochemistry and Molecular Biology, Monash University Australia, specialising in integrative microbiome sciences. He has extensive international experience in experimental microbial ecology and bioinformatics, with a focus on environmental and clinical contexts.

Capabilities and Degrees Required

List the ideal set of capabilities that a student should have for this project. Feel free to be as specific or as general as you like. These capabilities will be input into the online application form and students who opt for this project will be required to show that they can demonstrate these capabilities.

Candidates meeting either criteria 1 or 2 as well as **all three criteria (3 & 4)** eligible to apply:

1. M.Sc. in Biochemistry/Microbiology/Environmental Science/Bioinformatics

   OR

2. Masters in Environmental Engineering/Civil Engineering with Environmental Engineering specialization/Chemical Engineering/Biotechnology
3. Should have secured 60% or equivalent CPI in Class 10, 10+2, graduation and post-graduation examinations.
4. The medium of instruction of the candidate's education should have been English for undergraduate and post-graduation degrees.

The student should be motivated and proactive with excellent problem-solving skills. A general proficiency in English is also desirable since the student would have to spend a considerable time at Monash University. The student should also demonstrate experience or a willingness to work in a culturally and socially diverse environment.

Necessary Courses

Name three tentative courses relevant to the project that the student should complete during his/her coursework at IITB (the student will require to secure 8 point in these courses)

Environmental Chemistry, Environmental Microbiology, any molecular biology course

Potential Collaborators

Please visit the IITB website www.iitb.ac.in OR Monash Website www.monash.edu to highlight some potential collaborators that would be best suited for the area of research you are intending to float.

Dr. Simone Li

K**eywords** relating to this project to make it easier for the students to apply.

Biofilm, Microbial consortia, Microplastics, Wastewater treatment