ECOFRIENDLY AND COST-EFFECTIVE APPROACH FOR THE BIODEGRADATION OF MICROPLASTICS USING WATER AS A SOURCE

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Introduction:

Microplastic, defined as particles <5mm in diameter, are emerging environmental pollutants that pose a threat to eco-system and human health (Xiang et al., 2022). These particles are ingested by a variety of marine organisms from invertebrates to fishes with various consequences and there evidence that particles smaller than the current level of detection in the environment are also ingested by the aquatic invertebrates (Medarno,Thompson et al,.2015). Microplastics are divided into two types: Primary microplastic found in cosmetics, plastic pellets used in industrial manufacturing. Secondary formed from the breakdown of larger plastics undergo a weathering process e.g. wind, wave, and sunlight (Mohammad, 2020).

Fish receive bacteria in the digestive tract from the aquatic environment through water and food that are polluted with bacteria. Being rich in nutrient, the environment of the digestive tract of fish confers favorable conditions for the microorganisms (Shivasubramanian et al,. 2012). The use of gut microbes that can withstand the harsh environments for plastic degradation (Pikoli, Astuti et al,). The gut of the fresh water is expected to have bacteria that can degrade the microplastic present in the fresh water. Degradation of microplastic by bacteria is known as biodegradation. Microorganisms can degrade the plastic over 90 genera from bacteria and fungi among them Bacillus megaterium, Psudomonas spp. Azatobacter, Ralstonia eutropha, Halomonas Spp. etc (Chee et al 2010). Recently a novel bacterium was isolated,Ideonella sakaiensis 201-F6, that is able to use poly(ethylenetetraphthalate) as its major energy and carbon source(Yoshida et al,.2016). So this article focus on biodegradation of microplastic using water as a source and the bacteria extracted from the fresh water fish gut (Labeo rohita, Catla catla). Humic and clear lake water has been used for the degradation of the microplastic.

Objectives:

There are millions of plastic particles in lakes, soils, and oceans all throught the world. The microbial degradation of plastic backbone is regarded as a long process due to its high molecular weight, hydrophobicity, and lack of functional groups. This long procedure makes it challenging to evaluate by standard methods such as weight loss or CO2 evolution. This project tackles the root of both challenges by putting forward a new, sustainable approach based on water as a source for microplastics (MP's) mainly polyethylene (PE) degradation. The aim is to prove effective degradation of MP's (PE) at feasibility research using invitro conditions, and to test prototypes with fresh water. Specific objectives are:

1. Isolation of gut bacteria from fresh water fishes.
2. Biodegradation of MP's (PE) in artificial medium and natural water.
3. Determining the quantity of microplastic degraded

Methodology:

WP 1. Isolation of gut bacteria from fresh water fishes

Task 1a. The Labeo rohita (Rohu), Catla catla fresh water fishes were collected and gut was isolated. The fish was surface sterilized by immersion in 70% ethanol for 30 seconds. The gut was aseptically dissected from the animals musculature. Gut was weighed and placed into 10ml sterile 1% saline (1 gram of Sodium Chloride in 100ml of distilled water).

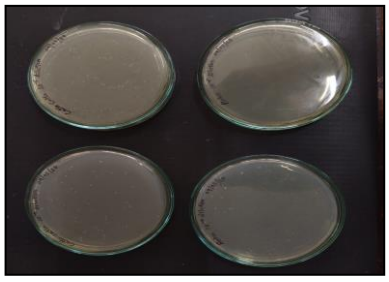

Figure 1: 10^{-4}, 10^{-5} Serial dilution *Catla catla* and *Labeo rohita* without

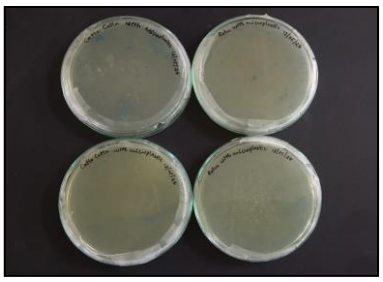

Figure 2: 10^{-4}, 10^{-5} Serial dilution Catla catla and Labeo rohita with microplastic

The gut was then centrifuged by centrifuging at 3000rpm for 20 minutes. The fish guts were serially diluted up to 10-5 in sterilized saline, and 100µl from each dilution was spread on nutrient agar plates (NA) and then incubated at 30 ºC.

WP 2. Biodegradation of microplastics in artificial medium and natural water

Task 2a. The Micro plastic mainly PE was purchased from Ambika industries Pune, Maharashtra, India. 10mg of MP (PE) sample are added to 100ml of basal medium, sterilized water, humic and clear lake water was collected from Sadanakeri and Kelageri lake, Dharwad, Karnataka. These flasks were placed in incubator shaker at 30ºC in 100 rpm. Parallelly, fish gut micro flora is utilized to grow in the presence of MP's (PE) in artificial and natural media.

Task 2b. Isolated bacterial strains will be screened and the bacterial morphology with and without MP was studied, growth of bacteria in humic and clear lake water will assessed by standard plate count method.

Task 2c. Periodically, oxygen will be provided to humic and clear lake water for better aeration and to increase the process of degradation of polymer.

Figure 3: Degradation of MP'S in Natural and artificial media

WP 3. Determining the quantity of microplastics degraded

Task 3a. The degraded Micro plastics were collected using sterile plastic syringes; the samples will be subjected to Gas chromatography.

Task 3b. After the degradation process, samples need to subject to FE-SEM analysis (surface analysis) for morphology differentiation. The FTIR analysis of MP's (PE) for induced surface polymers. Differential scanning calorimetry (DSC) will be studied to investigate the thermal gravity of PE after degradation will be analyzed in the University Scientific and Instruments Center (USIC), Karnatak University Dharwad.

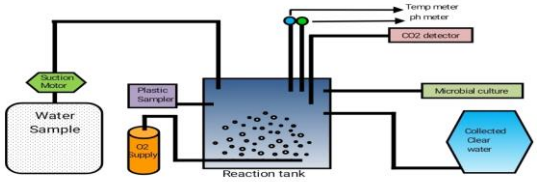

Figure 4: Proposed model showing workflow of MP's (PE) degradation in humic or clear lake water

Conclusion:

Since the project is under progress we are expecting the gut bacteria of the fresh water fish which is inoculated into humic lake water will degrade more amount of microplastic(PE) than in the clear water. The degradation of microplastic can estimated by subjecting to FE-SEM analysis for morphology differentiation and FTIR analysis, differential scanning calorimetry (DSC). The model that has been designed will give a clear evidence of the degradation of microplastic in the cost-effective and ecofriendly manner.

Scope for future work:

The metabolic diversity of bacteria makes them a useful resource for remediation of pollution in the environment. Biodegradation is an attractive alternative to current practices for waste disposal, as it is generally a cheaper process, potentially much more efficient and does not produce secondary pollutants, such as those associated with incineration and landfill. In some cases, it may even be

possible to obtain useful end products with economic benefit from bacterial metabolism of pollutants, for example, ethanol for use in biofuels.

This project provides the ecofriendly and cost-effective approach for the biodegradation of microplastic using water as a source. The Model provides an innovative approach of degradation of microplastic not only in lab scale but used in industrial scale. Use of this model in the water treatment areas provides a great benefit for the purification of water, which is free from microplastics.