

**Indian Science Technology and Engineering facilities Map
(I-STEM)**

Invites application for

1. Partial financial support for using R&D facilities in other institutions through the I-STEM web portal to Users from Academic Institutions and Start-ups (Format-1)

Format for requesting support for accessing and using facilities through the I-STEM web portal.

(i) Details of the Personal Information

- a. Name of the Researcher/Faculty Member : Dr. Kishalay Paria
- b. Name of the Institution/Start-up: Oriental Institute of Science and Technology under Vidyasagar University
- c. Name of the Department: Department of Biotechnology
- d. Degree registered for/Product being developed (for start-up) Ph.D
- e. Mob. No. 7384634260
- f. Email ID: kishalayparia@gmail.com

(ii) Project/Research topic Details:

- a. **Project Title/Research Title:** Development of low cost eco-sustainable fish preservation technique for development of rural economy of India with special emphasis on coastal area of West Bengal
- b. **Sub area:** Rural development
- c. **Area Description:** Fish preservation
- d. **Aims & Objective (in Bullets):**
 - To survey traditional technique for fish preservation in coastal region of West Bengal.
 - Isolation and identification of fish spoilage microbes.
 - To develop new technique for reducing the microbial spoilage by organic nanomaterials
 - To evaluate the efficacy of anti-biofilm agents after their application on fish surface in enhancing the self-life without affecting the major characteristic features of the fish.
- e. **Duration (MM/YYYY):** 10 months
- f. **Project Summary (1000 words):**

Development of low cost eco-sustainable fish preservation technique for development of rural economy of India with special emphasis on coastal area of West Bengal

Background

Fish is a globally consumed protein source in our diet providing 20% of the protein intake of one-third of the world's population. India is the second largest fish-producing country in the world with almost 8% of the global production and it's contributing 1.07 % of India's total GDP. According to FAO, global seafood consumption has increased dramatically from 9.9kg per capita in the 1960s to 20kg in 2016. In addition to this, the exports of fishery

products in developing countries deposited an income of \$80 billion and were estimated to be \$148 billion on world-scale exports in 2014. Fish is one of the best proteins, omega-3 (ω -3) polyunsaturated fatty acids (PUFAs), vitamin D and different minerals rich foods that need careful handling as it spoils easily after capture due to the high tropical temperature which accelerates the activities of bacteria, enzymes and chemical oxidation of fat. Though India has a large area and also a large fish-consuming population, transportation of fish is very costlier and risky for the perishable character of Fish. Most of the rural economy of the coastal region in West Bengal depends upon hilsa, pomfret, shrimp, lobster and several types of valuable fishes. Spoilage begins at the time of fish catch. This process begins as soon as the fish dies. The rate of spoilage is accelerated in warm climates. The fish's gut is a rich source of enzymes that allow the living fish to digest its food. Once the fish is dead, these enzymes begin digesting the stomach itself. Eventually, the enzymes migrate into the fish flesh and digest it too. This is why the fish becomes soft and the smell of the fish becomes more noticeable. There are a huge number of bacteria and sometimes fungi naturally present on the skin of the fish, in the gills, and in the intestines. The aim of fish processing and preservation is to slow down or prevent this enzymatic, bacterial, and chemical deterioration, and to maintain the fish flesh in a condition as near as possible to that of fresh fish.

Justification of proposal

Traditional knowledge of the local people is very important to document our heritage and India is a well-known country for its traditional knowledge over the years. Even though, ice is used as a traditional preservative. But salting, drying and a few special kinds of preservation techniques have been employed in rural areas. In India about 17% of fish catch is sundried. Fish processing and preservation is first priority of fishermen and fish shopkeepers before and after the marketing of catch. Sun drying is the most ancient and cost-effective method of fish processing all over the world. Though due to sun drying, there are losses of nutrients like fibers, carbohydrates and essential amino acids, still it is used as one of the cheap methods.

During drying as well as ice preservation microbial bio-film should be generated on the upper surface of the fish. Biofilm being a syntrophic microbial association can be extremely problematic when grown on some biotic surface. The fish surface is found to be an attractive place for the growth and development of biofilm. Many bacteria are capable of colonizing

fish surfaces as micro-aggregates and forming biofilms. This biofilm in turn enhances the chances of diseases that require the adoption of new controlling measures. The use of chemicals and antibiotics for controlling fish biofilm increases the chance of induction of antibiotic resistance and hence, is not recommended. Since biofilm is a major constraint for fish preservation and is a significant setback for the fishing industry. To determine the traditional method of sun-drying of fishes and the way of life practiced by fisher tribes living along the coast of the Bay of Bengal of Sankarpur, West Bengal State, fishermen were visited repeated times in 2022. The interviews of individuals or a group of fishermen (age group 19-50 years) were conducted. It was also observed that these low-income group poor fisher tribes live happily on the bank of river in the plane sandy coastal areas for 3-4 months. During their stay, they use the available natural resource in the river basin area to fulfill their basic needs of survival like food, shelter, and water.

Research gap

After preliminary observation in coastal region of West Bengal we assume that some fish preservation related researches are required. On the basis of field visit few research gaps are observed which are highlighted here under

1. Lack of ideal preservation technique, safe for human health.
2. Sun drying process needs to be optimized
3. Proper awareness programs are required for the fisher men and fish cultivators.

Hypothesis

As a solution to this problem, we have introduced an organic technique instead of harmful chemical preservatives. During the preliminary study of Hilsa fish, several types of bacteria, and fungi were found to produce biofilm. The microbial biofilm are responsible for the fish spoilage. After analysis of spoil-causing microorganisms, different types of natural products are tested as preservatives. A new organic preservative will be formulated with phytocompounds and organic nano based emulsion as effective anti-biofilm agent. This will be economically sustainable and safe for human health. The alternative strategies, such as the use of some phytocompounds and the application of organic nano-based emulsion may be proved effective in eradicating fish biofilm and thereby increasing the self-life of the fish in a nontoxic way.

Keywords: Biofilm, spoilage, nanoemulsion, antimicrobial, shelf life

Expected result

The expected outcome will be to remove of biofilm on fish by using natural compounds and organic nano emulsion that will nonhazardous, safe and can be made available at a nominal cost. The shelf life of fish will be enhanced without the risk of development of antibiotic resistance.

(Separate page attached to justify the requirements)

g. Technical Details

a. **TRL** Active research and development is initiated. This includes analytical laboratory studies to field application.

b. Methodology

1. Study the physico-chemical parameters of fish sample

From the study sites, fish samples will be collected and their morphometric characterization will be done. The morphometric and biochemical study of fish samples will be examined in the laboratory. For doing this process, each fish's carbohydrate, protein, fat, triglyceride, mineral and moisture content have to be measured. All analyses will be done in triplicate and taken the average value.

The techniques are as follows:

The anthrone method is followed for carbohydrate analysis.

For protein analysis, Lowry method is best. It is a biochemical assay for determining the total level of protein in a solution.

The most common analytical techniques used in the triglyceride analysis are gas chromatography (GC) and mass spectrometry (MS).

The mineral content analysis will be processed by atomic absorption spectrometer (AAS) and flame photometer.

2. Identification of biofilm producing microbes

Biofilm forming microbes will be cultured in congo red added nutrient agar plate for bacteria and potato dextrose agar media for fungal culture. On the other hand biofilm can be estimated by crystal violet method.

3. Development of anti biofilm nano materials

For controlling the biofilm formation, some anti-biofilm agents will be developed. Although previously reported that several classes of natural compounds that have high anti-biofilm properties e.g. phenolics, essential oils, terpenoids, lectins, alkaloids, polypeptides, and

polyacetylenes. But we will try to use curcumin based organic nano materials that will better anti-biofilm agent than traditional ones. The Curcumin sample will be subjected to mix up with old bacterial culture broth .The mixture will shake by shaker in incubator at normal temperature. After certain time curcumin nano will separated by what man filter paper and then will dry in desiccators for about 24 h.

4. Characterization of isolated nanomaterials:

4.1.1. UV –Visible spectroscopic study

The changing of color will be indication of the generation of nanoparticle that will be further identified by UV– visible spectroscopy (pyne et al., 2022). UV-Vis studies will be performed using a quartz cell with a thickness of 1 cm. The synthetic nano will measure by UV-Visible spectrophotometer at 400-700 nm wavelengths (Doner et al., 2019).

4.1.2. Fourier transforms infrared (FTIR) spectrometer study:

According to the method of paria et al 2018, the FT-IR studies have been performed. The potassium bromide (KBr) pellet will be added to the sample. Then the sample will be placed in a transparent disc of thickness 1mm and diameter 13mm and subjected to about 5×10^6 Pa pressure in an evacuated die. IR spectra region of $4000\text{--}500\text{ cm}^{-1}$ will be recorded on a FT-IR Nexus TM 870 spectrophotometer (PerkinElmer).

4.1.3. TEM and SEM analysis:

The sample with concentration of 1 mg/ml will be drop casted on a Cu-grid. After drying at room temperature, the samples will be directly viewed under TEM following the guidelines as described earlier by researchers. The HR-TEM (JEOL JEM 2100 instrument) will be used to analyze the samples and capture the images. The structural investigation of sample will be performed through SEM (OCTANE PLUS, ZEISS, Merlin, UK). The samples will be carbon coated to increase the conductivity. After that the sample will be placed onto graphite stub for scanning the surface morphology.

4.1.4. Nuclear magnetic resonance (NMR) spectroscopy analysis:

^1H NMR can be used to study the presence of hydroperoxy groups as well as of hydrogen, carbonyl and carbon atoms associated in conjugated-dienoic systems. Data will be recorded on a DPX200 Bruker (200 MHz for ^1H and 500 MHz for ^{13}C) in D_2O with tetra methylsilane (TMS) as an internal standard. Every signal will be compared within ± 0.1 ppm to standard solution.

4.1.5. Evaluation of antimicrobial activity:

Antimicrobial activity of the isolated compound will be carried out by the well diffusive method against isolated microbes. The 0.2 ml of test bacterial solution will be evenly spreaded in sterile luria–bertani (LB) broth agar. Then the sample (50 µl) will be poured on the inoculated agar plates, but one of them contained no extract. They were incubated at 37°C for 24 h in a culture incubator. After incubation, the diameter of each inhibition zone will be measured with a millimeter scale. On the other hand potato dextrose agar and broth will be use for antifungal activity assessments. All experimental assessments will be conducted in triplicate.

5. Evaluation the efficacy of anti-biofilm agents in enhancing the self-life without affecting the major characteristic features of fish sample

Finally, a total two sets of studies will be conducted by varying storage temperature viz. refrigerated (4 ± 2 °C) and ambient storage (room temperature) at specific time interval (0, 7, 15, 30, 45, 60 days). The total bacterial and fungal plate count of samples will be performed on nutrient agar and potato dextrose agar medium as per standard protocol. After that the petri plates will be incubated at 37 °C for 24-48 h for bacterial growth whereas 28°C for 96 h during fungal growth respectively. The bacterial growth will be reported as number of colony forming unit per milliliter of samples. The biofilm assay will be done according to the method of Lahiri et al, 2021.

References

1. Doner, N., Pargan, A. R., Aygahoglu, A., Liu, F., & Sen, F. (2019). Radiative properties of hematite particles in the UV-visible spectrum. *International Journal of Thermal Sciences*, 139, 79-87.
2. Paria, K., Pyne, S., & Chakraborty, S. K. (2022). Optimization of heavy metal (lead) remedial activities of fungi *Aspergillus penicillioides* (F12) through extra cellular polymeric substances. *Chemosphere*; 286, 131874.
3. Pyne, Smritikana, Kishalay Paria, Santi Mohan Mandal, Prem Prakash Srivastav, Paramita Bhattacharjee, and Tarun Kumar Barik (2022)."Green microalgae derived organic nanodots used as food preservative." *Current Research in Green and Sustainable Chemistry* 5: 100276.
4. Lahiri, D, Moupriya N, Hassan I. S, Sarkar T, Edinur H. A, Pati S, and Ray R. R. (2021). "Microbiologically-synthesized nanoparticles and their role in silencing the biofilm signaling cascade." *Frontiers in microbiology* 12: 636588.
5. Mishra, R, Panda A K, Mandal De S, Shakeel M, Bisht Singh S, and Khan J. (2020)"Natural anti-biofilm agents: strategies to control biofilm-forming pathogens." *Frontiers in Microbiology* 11: 566325.

c. Design & development

Months	Work to be done
1 st and 2 nd month	Survey on existing fish preservation method and collection of fishes from different coastal area of West Bengal. Compilation of biometric characteristics of collected fish specimens.
3 rd and 4 th month	Isolation of biofilm producing microbes from fish specimens and their characterization
5 th and 6 th month	Synthesis of organic nano emulsion and characterization. Estimation of its anti –biofilm activity of nanomaterials
7 th and 8 th month	Evaluation the efficacy of anti-biofilm agents in enhancing the self-life of fish sample
9 th and 10 th month	In-situ application of nonmaterial by self-help group of fisher man

d. Facilities and Instrumentation required :

Microbiologically facilities are not required from external sources but the proximate analysis of fish sample, characterization of nano materials through SEM, TEM, DLS, NMR, FTIR etc. will be accessed from IIT Kharagpur. Besides that if there is necessary any instrumentation facilities, these will be accessed from different research institutes of west Bengal.

e. Consumables (Scientific materials)

Nutrient Broth, potato dextrose broth, agar, congored, curcumin, filter paper, tissue paper, petri dish, micropipettes, conical, Gram staining kit, cedar wood oil, luria-bertani (LB) broth, ethanol, methanol, protein estimation kit, fat estimation kit, lacto phenol cotton blue, some glass goods.

h. Expected output and outcome

- Chemical-free organic nano based safe value-added product development for fish preservation in a cost effective way.
- Technology may be transferred to local people through self-help group.
- Nano based fish preservative will be used when there will be scarcity of ice in the sea fishing.
- To be reduce secondary medical cost of consumers.

i. Industry/Institute Collaboration No

If, yes

- (i) Name of the Collaborating Industry/Institute

- (ii) Collaboration Details
- (iii) Additional Details if any

j. **Funding required from I-STEM (with justification:**

Our research group has already started the proposed research. But major problem is funding for field work as well as biochemical estimations from different research institutions. Although basic instruments for microbial culture are available in our institute but there is required fund for purchasing chemicals and accession of various sophisticated instruments like SEM, DLS, TEM, FTIR, NMR etc.

Details of Requirements & Mode of the Engagement: (Attach the quotation for the consumables)

- (i) Short term Engagement:
 - a. Usage of testing/characterization facilities across the country (Characterization Mode)
 - i. Testing Charges: Rs. 30,000
 - ii. Consumable charges: Rs.40,000
 - iii. Travel Cost (Boarding and Lodging, if any) Rs.28,000
 - b. Usage of fabrication and testing facilities across the country (Fabrication Mode)
 - i. Fabrication and testing Charges
 - ii. Consumable charges (if any)
 - iii. Travel Cost (Boarding and Lodging, if any)
- (ii) Medium term Engagement:
 - a. Execution of project in any one of the Host Institution (Project Mode)
 - a.Fabrication and or testing Charges
 - b.Consumable charges (if any)
 - c.Travel Cost (Boarding and Lodging, if any)
 - b. Execution of project in Multi Host Institutions (Project Mode)
 - a.Fabrication and or testing Charges
 - b.Consumable charges (if any)
 - c.Travel Cost (Boarding and Lodging, if any)

(Round-trip to the Host Institution by 2nd class (Non-AC) railway via the nearest route)

Details of User's Bank Account:

(Please send a copy of the cancelled cheque/copy of the passbook carrying all the information needed)

Beneficiary Name (User's name) Kishalay Paria **PAN:** DGQPP2534H

Bank Account No.17480110019153 **IFSC Code:** UCBA0001748

Name of the Branch: UCO bank Vidyasagar University branch

Note: Proposed proposal must be executed within stipulated duration (max. 6 month) to avoid fund availability issues. Travel form will be provided by the I-STEM office. The users will submit the technical report, original invoice (duly signed by the Head of the Institution) raised by the Host Institution through I-STEM portal only along with a copy of the travel forms and original tickets through post to the Nodal Officer, I-STEM, Indian Institute of Science, Bangalore. A scan copy of all the documents may be sent in advance.

Justification of all requirements

Serial number	Items	Justification of requirements
1	Nutrient Broth	Require for cultivation of bacteria
2	Potato dextrose broth	Require for cultivation of fungi
3	Agar	Use for media solidification
4	Tissue paper	Use for cleaning purpose
5	Congored,	Determination of biofilm producing microbes
6	Curcumin	Use for curcumin nano synthesis
7	Filter paper/ syringe filter	Use for purification of chemicals
8	Petri dish, conical,	For microbial growth
9	Micropipettes	For measurement
10	Gram staining kit	Require of identification of bacteria by gram staining
11	Cider wood oil,	Use for better visualization of microbes by microscope
12	Luria-bertani (LB) broth,	Bacterial culture
13	Ethanol, methanol	Sterilization purpose as well as use of chemicals for precipitation of some compounds
14	Protein estimation kit,	Use for protein estimation
15	Lacto phenol cotton blue	Use for fungal staining
16	Some chemicals	Extra chemicals require for at the time of experiments
17	Some glass goods.	Require various purpose
18	SEM,	Visualization of biofilm producing microbes and estimation the structure of nano materials including size
19	TEM,	estimation the structure of nano materials
20	DLS,	Size estimation of nano materials
21	NMR,	Structural analysis of nano materials
22	FTIR	Estimation of proper functional group nano materials those are specific against upon microbes
23	UV –Vis Spectroscopy	To be confirm nano material synthesis
24	GC MS	For estimation of lipid profile
25	Proximate analyzer	For estimation of different biological compounds such as carbohydrate, protein, fat, ash

Kishalay Parida,