Microbial Degradation of Polymers

"Fungi are the grand recyclers of the planet and the vanguard species in habitat restoration."

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Aim/ Objective

- Plastic is an integral part of our life.
- 5 million tones are consumed every year. Only 24% is recycled resulting in 3.8 million tones left unrecycled in the landfills.
- They are a major contribution to soil, air and water pollution.
- Commonly used methods were inadequate.
- Bioremediation – Environment safe and cost effective method.

Aspergillus niger

Aspergillus niger

- Most common species of Aspergillus

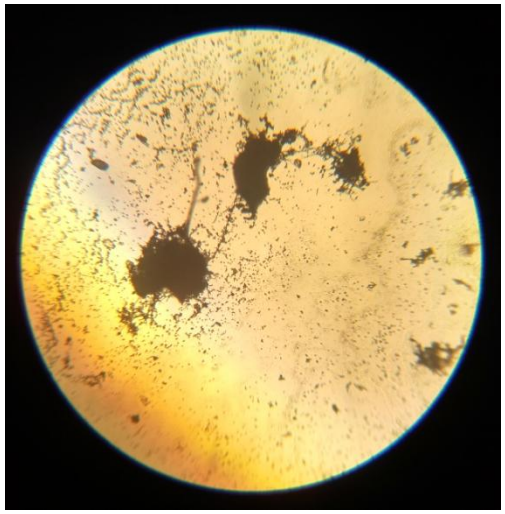
- Found as a black mould on Onions, Peanuts, Apricots, Grapes, etc.

- *A. niger* is not only a xerophilic fungi (mold that doesn't require free water for growth, can grow in humid environments), but is also a thermotolerant organism.

- Many of the enzymes produced by ***A. niger***, such as citric acid, amylases, lipases, cellulases, xylanases, proteases are considered GRAS (generally recognized as safe) by the United States Food and Drug Admin.

- In addition to producing extracellular enzymes and citric acid, *A. niger* is used for waste management and bio-transformations.
- ***Aspergillus niger*** is a haploid filamentous fungi produce colonies. Mycelial, or threadlike, hyphae are divided by a septum and transparent.
- Chitin, makes up the cell wall of *A. niger*.
- Evans blue an azo dye binds with chitin present in cell wall of fungi.

Onion from which sample was taken



A. niger under compound microscope.

Experiment Procedure

Preparation of Media

- PDA:
  - 1000 ml of water
  - 4 g of infused potato (unpeeled) – from 200 g of potato
  - 15 g of Agar
  - 20 g Dextrose

  Conditions:
  - Temperature: 25°C
  - pH: 5.6 ± 0.2

  Sterilization: Autoclave
  - 15 psi
  - 15- 20 minutes
  - 120°C

Experiment Procedure

- POTATO INFUSION:

  5 ml is diluted to 50 ml of distilled water

- PEG:

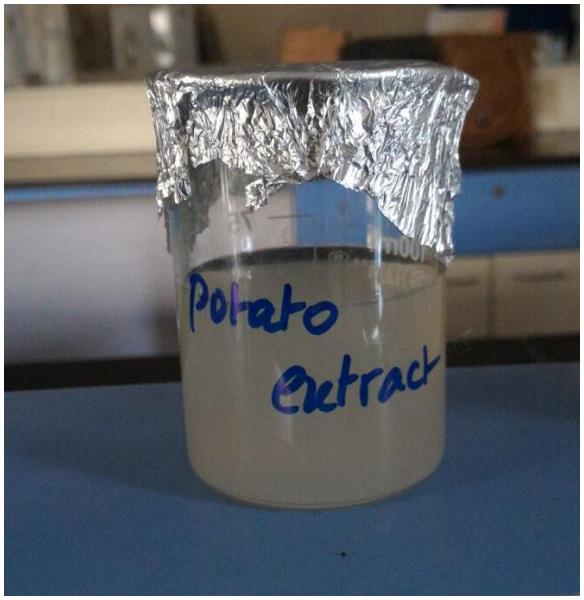
  5 ml solution diluted to 50 ml of distilled water

- PVC:
  - PVC pipe is heated.
  - The molten PVC is immersed in Benzene (5 ml). PVC dissolves in aromatic organic solvents and methylethylketone.
  - After a few hours, Acetic acid (25 ml) and Acetone (25 ml) are added and filtered.
  - Filtrate is centrifuged and a clear solution is obtained.

Aspergillus niger

- Obtained from *Allium cepa.*
- Fungi scraped using scalpel.
- Transferred to a petri dish.

Boiled potato for PDA



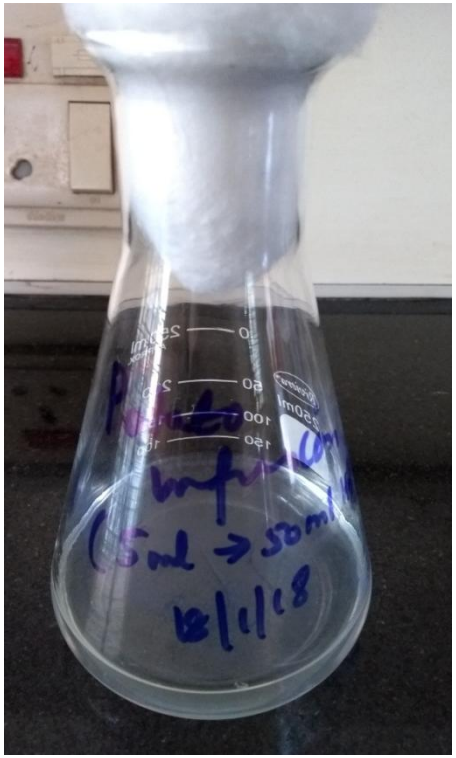
Potato extract

PVC with Benzene and Water

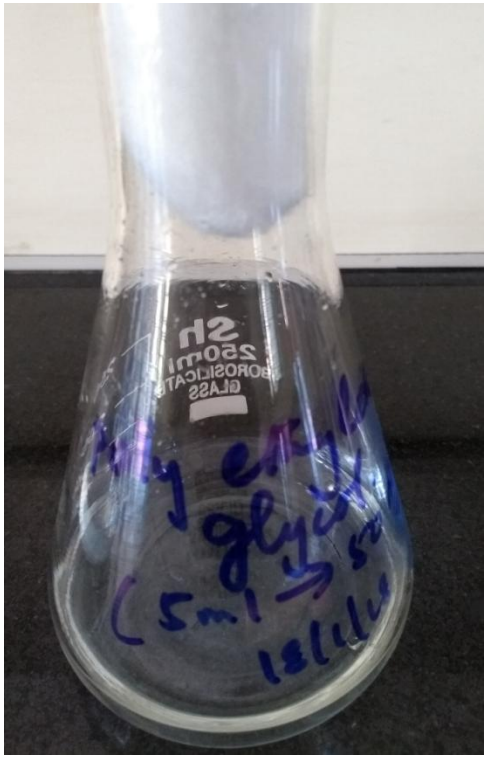
Day 1

Inoculation:

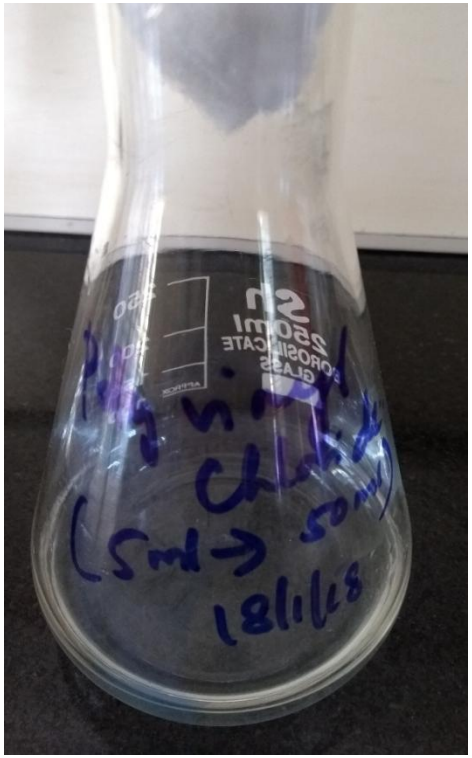
- All the media are transferred into a 250 ml conical flask and labelled with date.
- The fungi are inoculated onto the three media using different cotton swabs.
- After inoculation the conical flasks are placed inside the incubator at 30 °C for overnight incubation.
- Apart from this , PDA Agar Slants are prepared to see the growth of A. niger in solid media.
- These are then placed in the incubator for overnight incubation at 30°C.



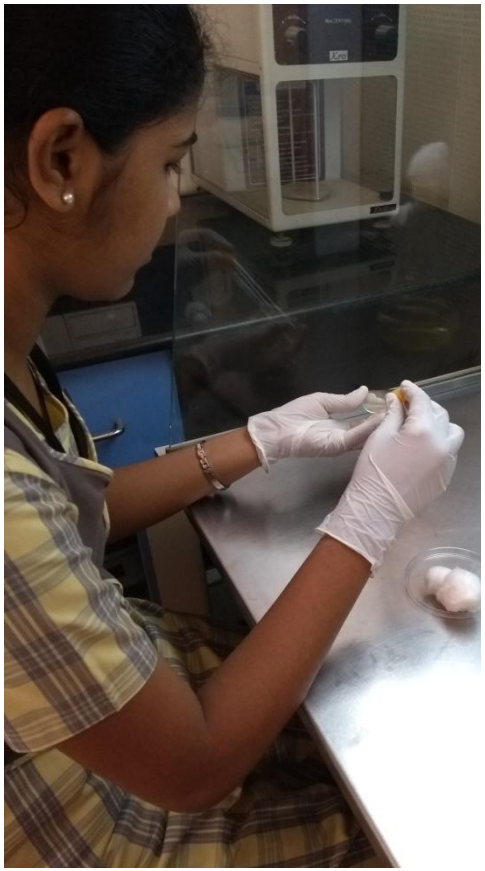
Inoculation of *A. niger* in Potato infusion



Inoculation of *A. niger* in PEG



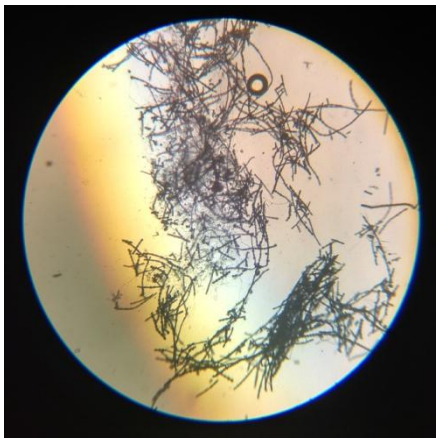
Inoculation of *A. niger* in PVC

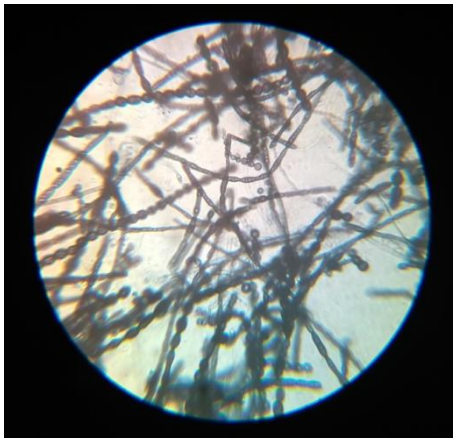

Inoculation of *A. niger* in the PDA slants.

Day 2

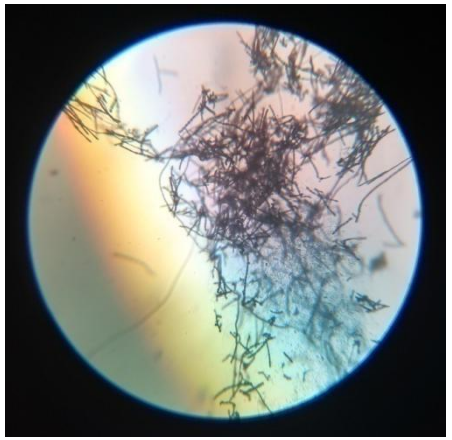
- A drop of sample from each of the conical flasks (PI, PVC, PEG) were placed on a glass slide.
- A drop of Evans blue was added.
- Cover slip was placed and observed under compound microscope.



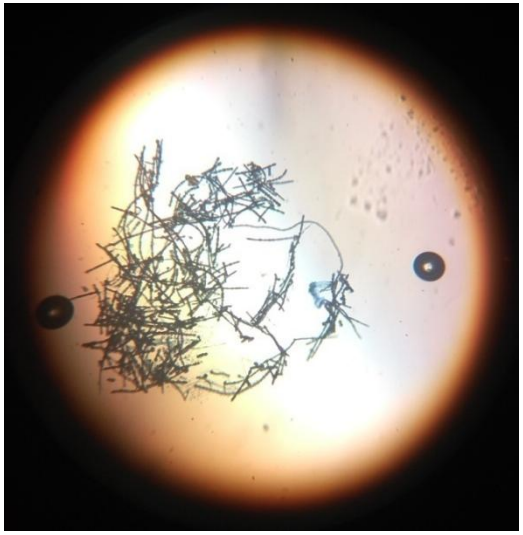
Aspergillus niger growth in Potato infusion- under **low power**



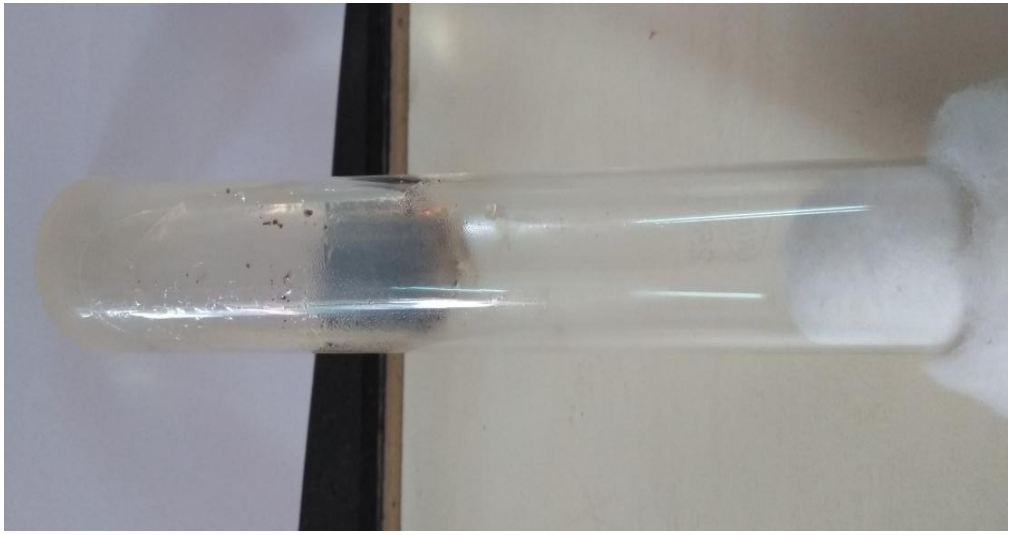
Aspergillus niger growth in Potato infusion- under **high power**



Aspergillus niger growth in PEG



Aspergillus niger growth in PVC



A. niger growth in PDA

Turbidity

Result:

- Fungal growth was observed on all the samples.
- Turbidity was observed in PI, PVC, PEG.
- Visually PI showed more turbidity.

Project Extension :

- Spectrophotometric measurements of enzymic activity.
- Genome analysis.

FURTHER INVESTIGATION