# Applications of Fungal Extracts as Sterilants to Biofilm Growth on Medical Implantable Devices

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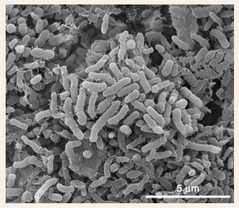
## Biofilm Infections

- 50-70% nosocomial infections are biofilm infections on medical implantable devices
- Over $100 billion spent annually on biofilm infection treatment in the USA
- Biofilms are more antibiotic resistant, which poses a global issue
- 929,000 deaths annually from biofilm infections

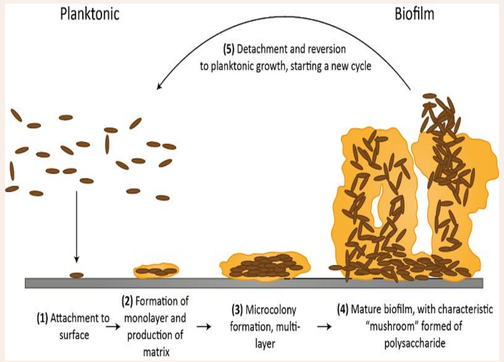



E. coli under scanning electron microscopy

## Mushrooms

- Contain secondary bioactive metabolites
- Exhibit antimicrobial, antiviral, immunomodulatory, anti-cancerous, antioxidant, and anti-inflammatory properties
- The mushrooms studied are globally accessible and sustainable

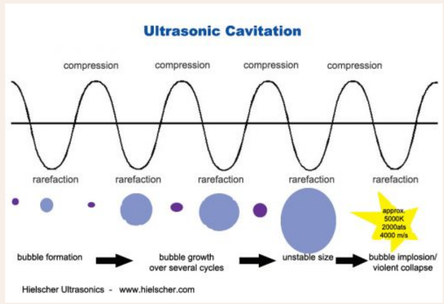
## Biofilm Growth




Stages of Biofilm Growth (Giorgi et al., 2022)

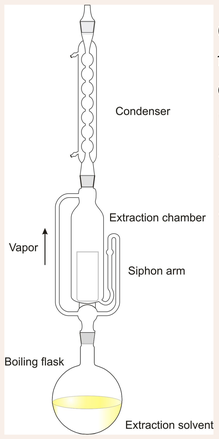
## Extraction Techniques

**1. Ultrasonic Assisted Extraction** (UAE)




High-intensity ultrasound energy waves allows for destruction of cell membrane + extraction of intracellular and cell surface-bound material including the polysaccharide

**2. Soxhlet Extraction**




Current Extraction technique of bioactive compounds in mushroom extracts:

- 18-24 hrs extraction time
- 0.5g of ground mushroom (TT or SH) in ~42 mL of organic DI water solvent heated in a silicone oil bath with stir bar

## Methodology

### Fungal extract evaporation

- Extract vacuum filtered and dried via rotovap

### Inoculation

- Inoculated autoclaved 20mL TSB culture with glycerol stock, stored overnight at 37°C on shaker at 200 RPM

### Extract Resuspension

- Dried fungal extract resuspended in 3.5 mL PBS buffer

### Plating

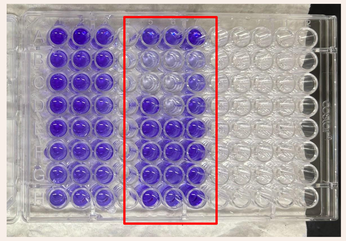
- Extract serially diluted with PBS buffer into overnight culture following dilution scheme below

### Biomass Staining

- Fixed cells to plate, added CV stain, and analyzed via plate reader

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | PBS | EMPTY | TT Extract | TT Extract | TT Extract | EMPTY | 40 µL PBS + 200 µL sterile Media | EMPTY | EMPTY | EMPTY | EMPTY | EMPTY |
| B | PBS | EMPTY | 1:5 Extract | 1:5 Extract | 1:5 Extract | EMPTY | 40 µL PBS + 200 µL sterile Media | EMPTY | EMPTY | EMPTY | EMPTY | EMPTY |
| C | PBS | EMPTY | 1:25 Extract | 1:25 Extract | 1:25 Extract | EMPTY | 40 µL PBS + 200 µL sterile Media | EMPTY | EMPTY | EMPTY | EMPTY | EMPTY |
| D | PBS | EMPTY | 1:125 Extract | 1:125 Extract | 1:125 Extract | EMPTY | 40 µL PBS + 200 µL sterile Media | EMPTY | EMPTY | EMPTY | EMPTY | EMPTY |
| E | PBS | EMPTY | 1:625 Extract | 1:625 Extract | 1:625 Extract | EMPTY | 40 µL PBS + 200 µL sterile Media | EMPTY | EMPTY | EMPTY | EMPTY | EMPTY |
| F | PBS | EMPTY | 1:3125 Extract | 1:3125 Extract | 1:3125 Extract | EMPTY | 40 µL PBS + 200 µL sterile Media | EMPTY | EMPTY | EMPTY | EMPTY | EMPTY |
| G | PBS | EMPTY | 1:15625 Extract | 1:15625 Extract | 1:15625 Extract | EMPTY | 40 µL PBS + 200 µL sterile Media | EMPTY | EMPTY | EMPTY | EMPTY | EMPTY |
| H | PBS | EMPTY | 1:78125 Extract | 1:78125 Extract | 1:78125 Extract | EMPTY | 40 µL PBS + 200 µL sterile Media | EMPTY | EMPTY | EMPTY | EMPTY | EMPTY |

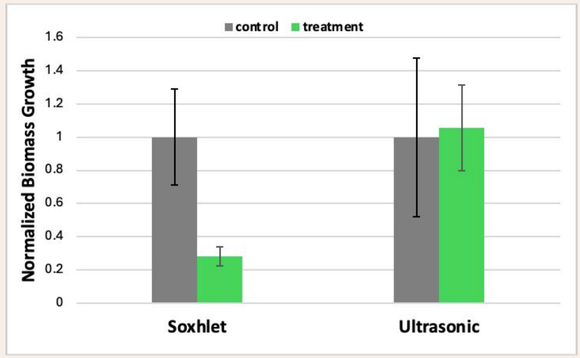
96 well plate diagram for biofilm assay



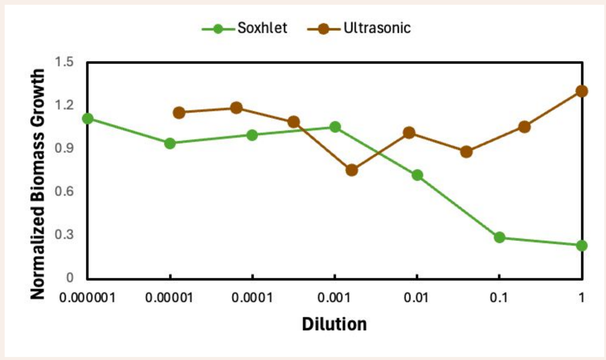
Finished plate stained with crystal violet

## Biofilm Assay Results

- The Soxhlet extract showed significant inhibition
  - ~72% inhibition in biomass growth
- UAE extract had no inhibition




- Observed dose-dependent inhibition for soxhlet extract
- UAE extract has no defining trend for inhibition




## Future Directions

- Utilize organic-based extracts in the future to maximize our yield and acquire pure sample
- Apply inhibitory properties of mushrooms to treat biofilm extractions that form on medical devices such as catheters, pacemakers, and prosthetics

## Acknowledgements & References

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