BACTERIAL RECOVERY FROM HVAC SYSTEM FILTERS

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BRIEF BACKGROUND

- The 2001 anthrax attacks brought into focus the need to develop methods for sampling and recovering bacteria from unique materials such as air filters
  - Much of the current research focuses on sampling and recovery form non-porous surfaces
  - Burton et al. looked at recoveries from sampling filters (2005)
  - Stanley et al. (2008) looked at background microbes on HVAC filters
  - Farnsworth (2006) investigated MERV 14 filters and *B. subtilis* (*atrophaeus)*
  - High recoveries obtained in these studies
  - What factors are most important?

PURPOSE

- The purpose of this study is to develop a method to recover bacteria from commonly used in Heating, Ventilating, and Air conditioning (HVAC) system air filters.
- Systematically vary factors to identify best conditions
  - Used lower rated filters than previously considered
    - Want to make method more broadly applicable
  - A different surrogate organism was used

EXPERIMENTAL DESIGN OVERVIEW

- In this experiment, a known concentration of bacteria *Bacillus thuringiensis* was used to spike HVAC filters and then extracted using various trial combinations of extraction temperature, growth media, and detergent.
- Cell culturing methods were used to grow extracted bacteria samples and determine the concentration of the extraction.
- To reduce bacteria growth during the experimental procedure, all primary experimental materials were stored and maintained at 10°C throughout the entire procedure.
  - Filters and deionized water were refrigerated over night.
- All bacterial suspensions used in the experiment were diluted using deionized water to a standardized concentration of 2.67 * 10^8 CFU/ML.
  - The suspensions were kept refrigerated or put on ice to maintain the temperature at 10°C or less.

SPORE PREPARATION

- Guiding rationale
  - Eliminate vegetative cells
  - Simple protocol (time, $, easy)
  - Non-invasive: less disruptive to spore features (i.e. exosporium)
- Possible Methods
  - H_2O wash
    - takes longer
  - lysozyme digestion, sonication
    - May disrupt exosporium

SPORE PREPARATION CONT.

- H_2O wash
  - Isolate colony
  - Plate on sporulation agar (2XSG)
  - Collect into ddH_2O when spores are >90% sporulated
  - Store in ddH_2O and wash several times over 2 wks
    - Allows for autolysis of vegetative cells and removal of debris

SURROGATE SELECTION

- Extensive literature review of *Bacillus anthracis* (*Ba*) surrogates
  - Predominant use: *Bacillus atrophaeus (Bacillus subtilis* var. *niger, Bacillus globigii)*
  - Moderate use: *Bacillus cereus*, *Bacillus thuringiensis (Bt)*
  - Low use: Bacillus megaterium, Geobacillus
- We chose *Bacillus thuringiensis* HD 1011
  - Unique genetic fingerprint, not used as pesticide
  - Did not clump, get quantitatively accurate recoveries from culture methods

WHY *BT*

- *Bacillus atrophaeus* +/-
  - + non-pathogen and ubiquitous
  - + Used mostly due to characteristic darker pigment and thus easy identification
  - + Conservative surrogate for disinfection study (i.e. more resistant than *Ba* to chlorine)
  - + history of use (more data)
  - - genetically and morphologically differences
- *Bacillus cereus*
  - +Very closely related to *Ba*
  - - Some pathogenic strains

WHY BT CONT.

- *Bacillus thuringiensis*
  - Also very closely related
  - Ubiquitous, used as a pesticide worldwide
- Since recovery experiments are physical we are concerned with physical characteristics such as size and morphological features (i.e. short nap hairs that surround the spore)
- *Bt* is the candidate with highest physical and genetic similarity to *Ba* and is low risk to release.

FILTER LOADING METHOD

Filter Loading Method

- All filters were loaded with 1mL/1000ul of bacterial suspension with concentration 2670 CFU/ML . The bacterial suspension was poured onto a 1 inch by 1 inch piece of filter using a 100ul-1000ul pipette.

EXTRACTION AND ENUMERATION

- During the extraction process temperature, media, and the use detergents were varied.
- The extraction temperatures used included: 60 degrees Celsius (activation series), 25 degrees Celsius (room temperature), and 10 degrees Celsius (cold series).
- Each extraction temperature series was conducted with the presence Tween 80 (detergent) and also in the absence of detergent (non-Tween 80).
- Both nutrient agar and blood agar were also varied throughout the testing process to determine the best growth media.

VARIABLE LEVELS

Factor	Variable Levels
Media	Blood Agar, Nutrient Agar
Detergent	Tween 80, Non Tween 80
Temperature	60 , 25 , 10 (Celsius)
Filters	Pleated, Polyester

FILTERS

Name	Pore Size	Characteristics
Filtrete™ Micro Allergen Reduction Filter	3mm	Captures microscopic allergens like dust, smoke and smog particles Captures large allergens like mold spores and pet dander Up to 3 months of filtering performance Meets the American Lung Association Health House® program Indoor Air Quality Guidelines
True Blue High Efficiency Polyester Filter	<1 micron	MERV RATING: 8 Specifically designed to protect your family against smaller airborne particles. Filter is also used in industrial applications. Filter is designed to filter contaminants that are one micron or less in size.

COLD SERIES EXTRACTION

- The spiked filters were place on in 200ml of deionized water and placed on a shake table for 30 minutes. The bacteria extracts were then plate on agar (nutrient and blood) and placed in an incubator at 37°C for a 10 hour growth cycle.
  - Note: This series was also with and without the detergent Tween 80 added to the extraction fluid.

60 °C ACTIVATION SERIES EXTRACTION

- The spiked filters were place on in 200ml of deionized water and placed on a shake table for 30 minutes. Filters in extraction fluid are covered and the flasks were placed an in water bath for 25 minutes at 60 ° C. The bacteria extracts were then plate on agar (nutrient and blood) and placed in an incubator at 37°C for a 10 hour growth cycle.
  - Note: This series was also with and without the detergent Tween 80 added to the extraction fluid.

ROOM TEMPERATURE EXTRACTION

- The spiked filters were place on in 200ml of deionized water and placed on a shake table for 30 minutes. Filters in extraction fluid are covered and the flasks were placed on the laboratory countertop for 25 minutes at room temperature. The bacteria extracts were then plate on agar (nutrient and blood) and placed in an incubator at 37°C for a 10 hour growth cycle.
  - Note: This series was also with and without the detergent Tween 80 added to the extraction fluid.

RESULTS

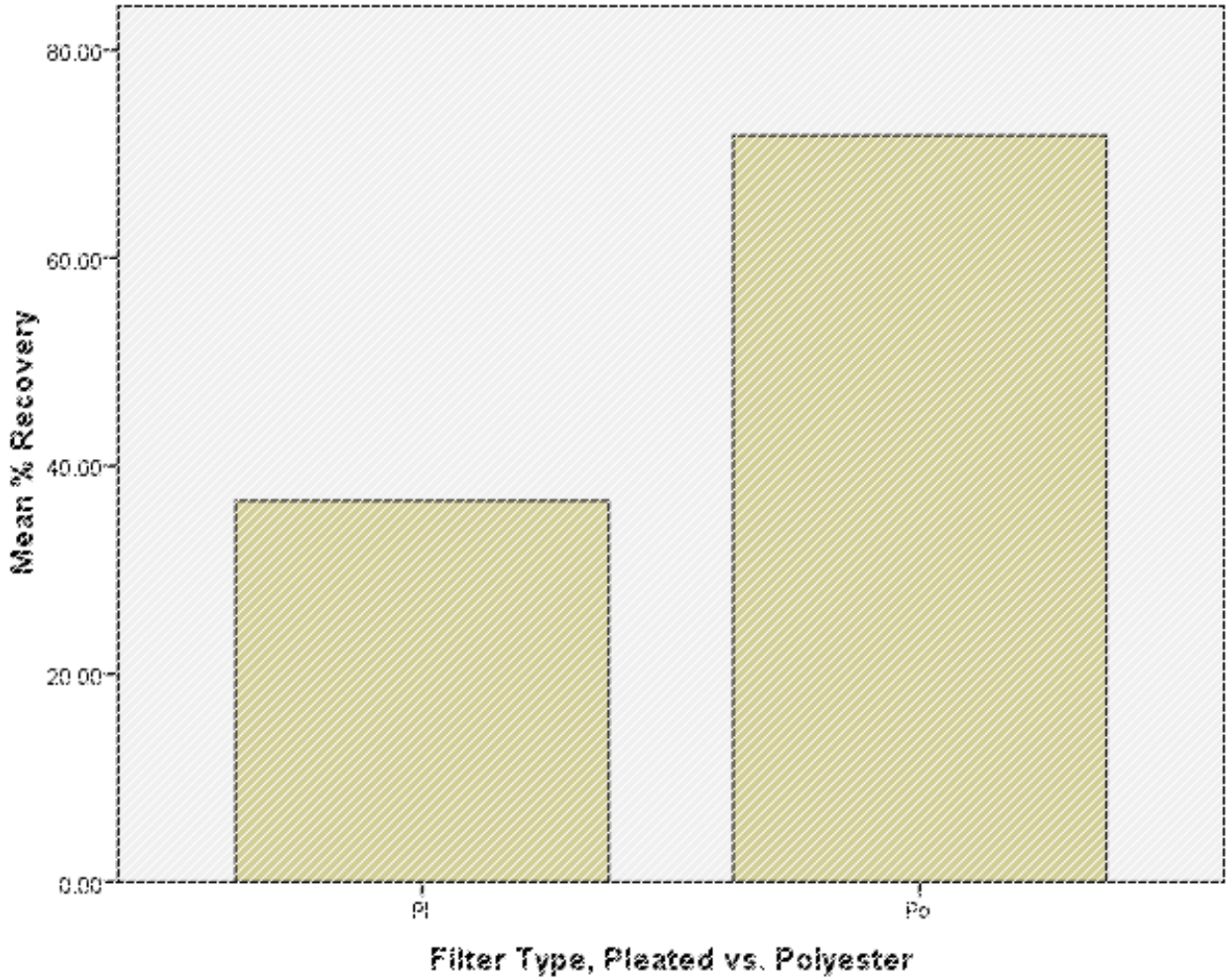
PLEATED FILTER RECOVERY PERCENTAGES

Filter Type	Media Type	Temperature	Detergent	Percent Recovery
Pleated	Nutrient Agar	10° C	Tween	26.2%
Pleated	Nutrient Agar	25° C	Tween	65.5%
Pleated	Nutrient Agar	60° C	Tween	40.5%
Pleated	Nutrient Agar	10° C	No Tween	42.9%
Pleated	Nutrient Agar	25° C	No Tween	34.5%
Pleated	Nutrient Agar	60° C	No Tween	48.2%
Pleated	Blood Agar	10° C	Tween	24.4%
Pleated	Blood Agar	25° C	Tween	21.4%
Pleated	Blood Agar	60° C	Tween	32.7%
Pleated	Blood Agar	10° C	No Tween	15.5%
Pleated	Blood Agar	25° C	No Tween	29.2%
Pleated	Blood Agar	60° C	No Tween	58.9%

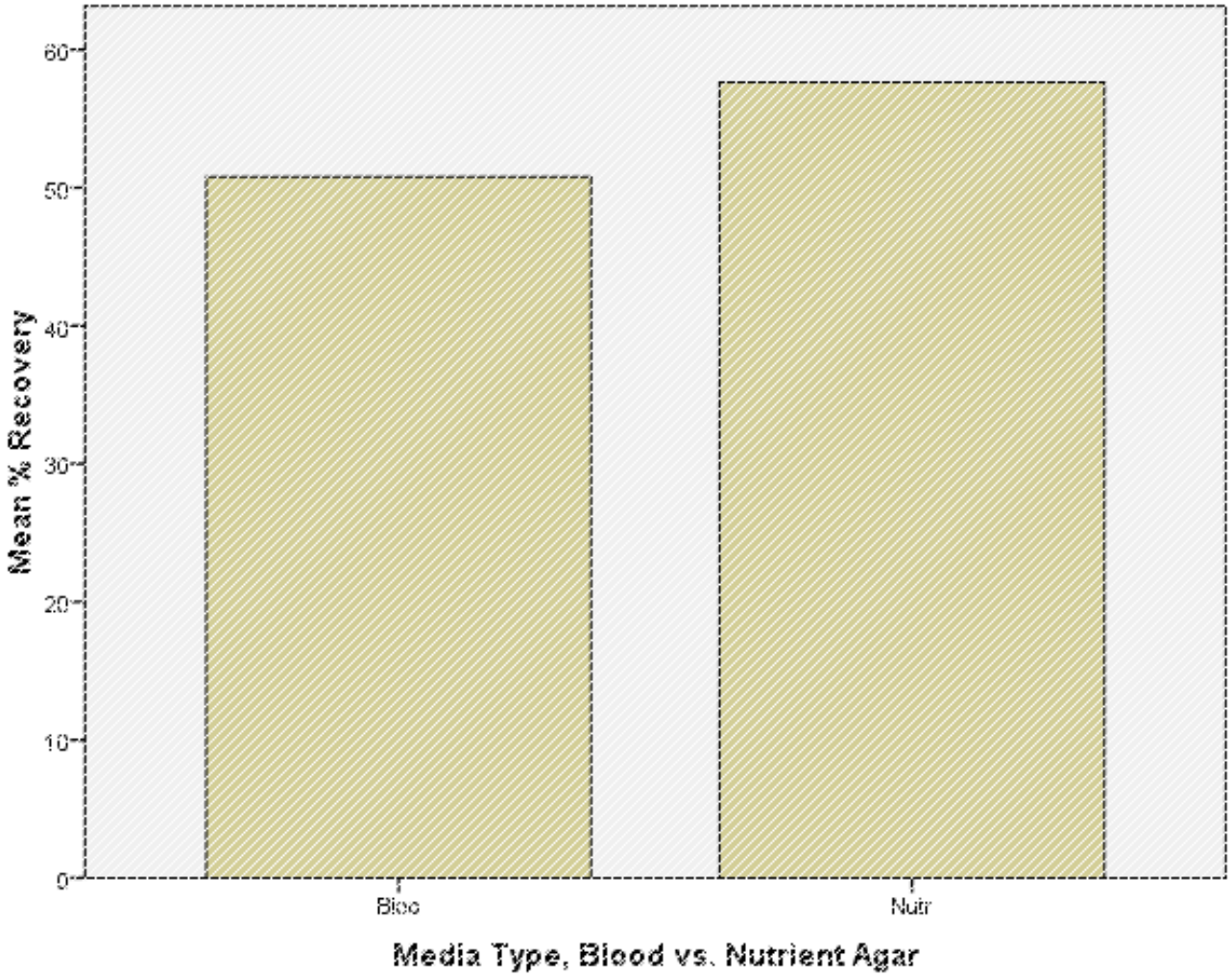
POLYESTER FILTER RECOVERY PERCENTAGES

Filter Type	Media Type	Temperature	Detergent	Percent Recovery
Polyester	Nutrient Agar	10° C	Tween	93.4 %
Polyester	Nutrient Agar	25° C	Tween	57.7 %
Polyester	Nutrient Agar	60° C	Tween	97.0 %
Polyester	Nutrient Agar	10° C	No Tween	56.0 %
Polyester	Nutrient Agar	25° C	No Tween	75.6 %
Polyester	Nutrient Agar	60° C	No Tween	54.2 %
Polyester	Blood Agar	10° C	Tween	99.4 %
Polyester	Blood Agar	25° C	Tween	48.2 %
Polyester	Blood Agar	60° C	Tween	98.2 %
Polyester	Blood Agar	10° C	No Tween	67.9 %
Polyester	Blood Agar	25° C	No Tween	58.3 %
Polyester	Blood Agar	60° C	No Tween	55.4 %

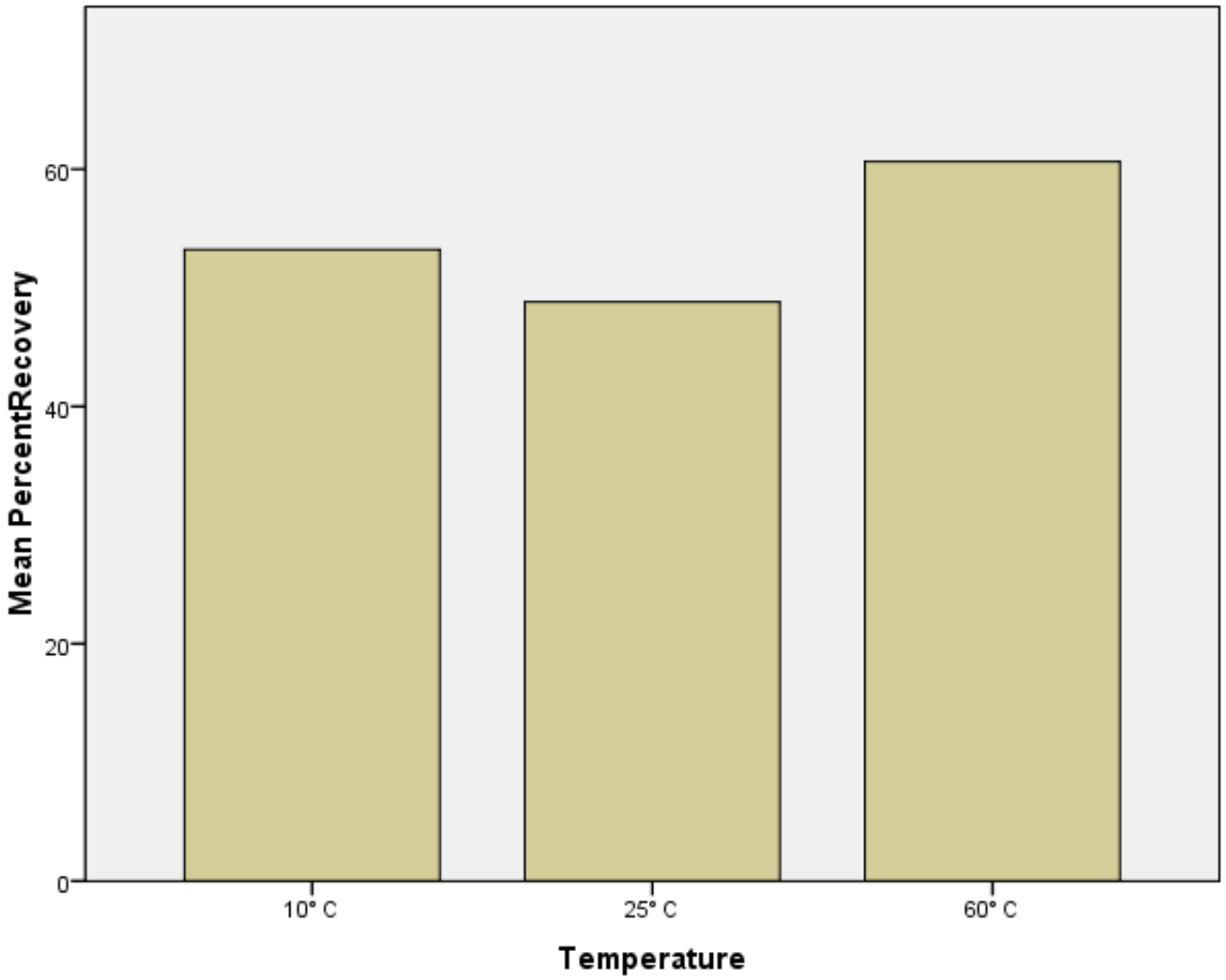
Filter Type (p<0.01)



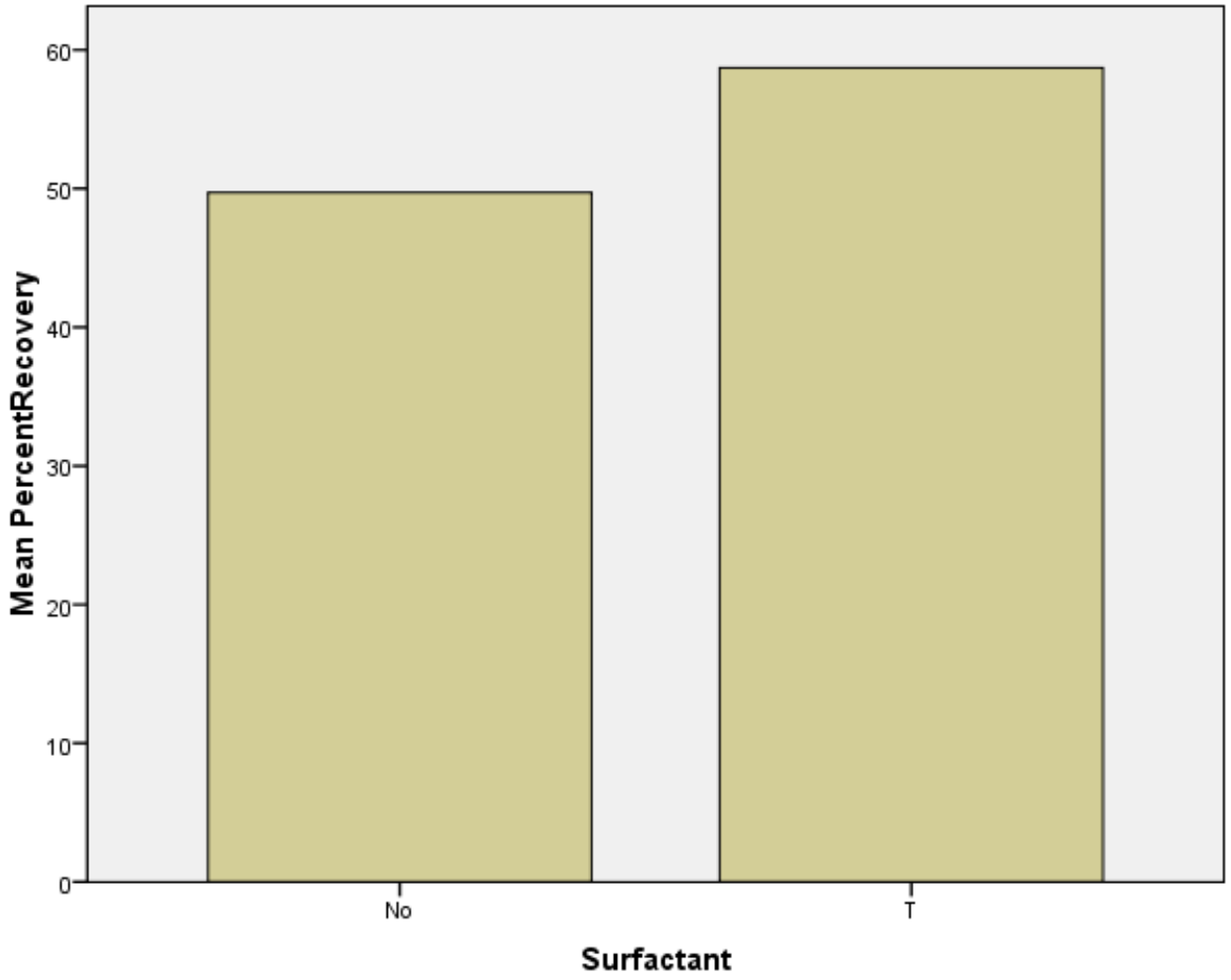
Growth Media (p=0.51)



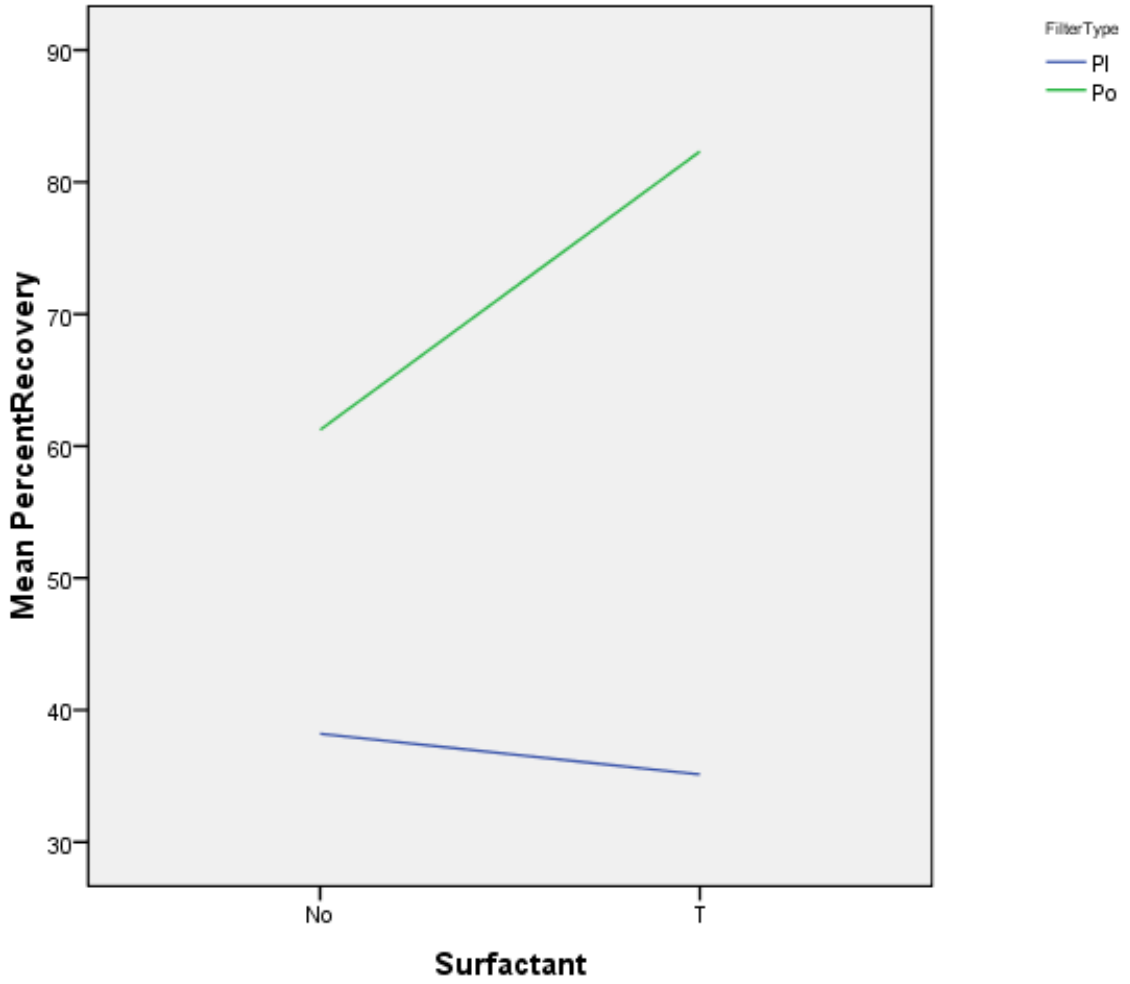
Temperature (p=0.65)



Surfactant (p=0.39)



Interaction (p=0.09)



DISCUSSION

- Filter type influences recovery percentage
- Other variables appear less important based on these preliminary results
  - Tween may be effective on some filter types
- Recoveries for the more commonly used filter are not as high as for the highly rated filter
- Bt recoveries may also be lower than some other surrogates?
  - Morphology, hydrophobicity
- Need for a library of recovery efficiencies or more aggressive recovery techniques that work on all filters

DISCUSSION CONTINUED…..

- Future Directions
  - More filter types
  - Use of different organisms
  - Interferences
  - More aggressive recovery techniques
  - PCR inhibition
- Further work needed on a variety of issues (Skolnick and Hamilton 2004)

- Skolnick, E. B. and R. G. Hamilton (2004). Legacy science suggests improved surface-testing practices for detection of dispersed bioagents in bioterrorism response. 2004 National Environmental Monitoring Conference. Washington, DC.

Acknowledgements

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