DEVELOPMENT AND TESTING OF DEVICE FOR H_2S REMOVAL FROM ANAEROBIC CHAMBERS

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ABSTRACT

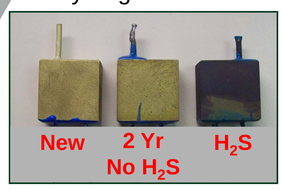
One challenge encountered when working with organisms in anaerobic chambers is management of hydrogen sulfide. H_2S poisons palladium catalysts, damages expensive electronics, and can also lead to sulfuric acid corrosion. Existing H_2S management approaches, such as bubbling the atmosphere through a silver sulfate solution, can be inefficient, lack capacity, be difficult to use, and/or lack indication of when H_2S removal capacity is reached. Therefore, we developed a convenient hydrogen sulfide removal column (HSRC) that removes H_2S by recirculating the chamber atmosphere through removal media at a controlled rate and coupled this column with a simple, sensitive H_2S indicator at the column outlet to indicate the column's effectiveness and H_2S binding capacity. After examining various media, we determined that a trilayer design of carbon (activated specifically for H_2S absorption) sandwiched between two layers of permanganate impregnated media provided the best H_2S removal capacity. We initially deployed the HSRC in a chamber used for cultivating large volumes of *Clostridium difficile* where H_2S levels reached at least 10 ppm. Within 10 minutes of operation, the HSRC could remove all detectable H_2S. We then deployed the HSRC in chambers used for cultivating human fecal microbial communities. Although the non-remediated concentration of H_2S in these chambers was lower (< 1 ppm), H_2S accumulation over time was sufficient to significantly reduce the lifetime of the palladium catalysts. These mixed communities also produced other volatile compounds that interacted with the HSRC. Therefore, using the same media, we developed a second bilayer HSRC design used for increased non-specific removal of volatile compounds. These two HSRC designs are currently deployed within the chambers reducing H_2S to undetectable levels at the column outlet. Although we are continuing to test these columns for their long-term utility and function, they are currently available from Coy Laboratory Products.

BACKGROUND

H_2S CAN INHIBIT ANAEROBIC CHAMBER FUNCTION AND USAGES

Poisons Palladium Catalysts

Effect of H_2S on Hydrogen Sensor



Damages Expensive Electronics (monitors, pumps, plate readers, etc.)

EXISTING H_2S REMEDIATION APPROACHES CAN BE CUMBERSOME AND INEFFICIENT

Static Containers of Activated Charcoal

Bubbling Atmosphere through Silver Sulfate

Repeated Purging of Chamber Atmosphere

METHODS

DEVELOPED H_2S REMOVAL COLUMNS (HSRC)

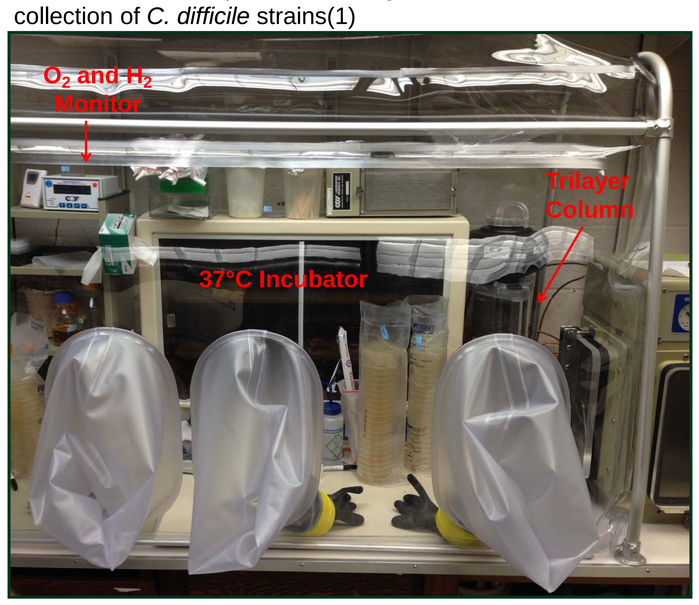
- **Trilayer Media Format:**
  - Layer of carbon activated specifically for H_2S binding sandwiched between two layers of permanganate impregnated media
  - Function primarily via:
    - Chemisorption
    - Adsorption
- **Bilayer Media Format:**
  - Layer of activated carbon proximal to air intake above layer of permanganate impregnated media
  - Additional activated carbon for increased removal of volatile organic compounds in addition to H_2S
- **H_2S Indicator Strip:**
  - Lead acetate designed for gaseous use
  - Lead sulfide deposition (brown → black precipitate) is sensitive to low levels of H_2S [50-60 ppb can cause deposition after 1 hr of exposure (2)] and deposition is cumulative
  - Positioned at outlet of column to detect media reaching removal capacity

RESULTS

TESTED H_2S REMOVAL CAPACITY UNDER DIFFERENT EXPERIMENTAL OPERATING CONDITIONS

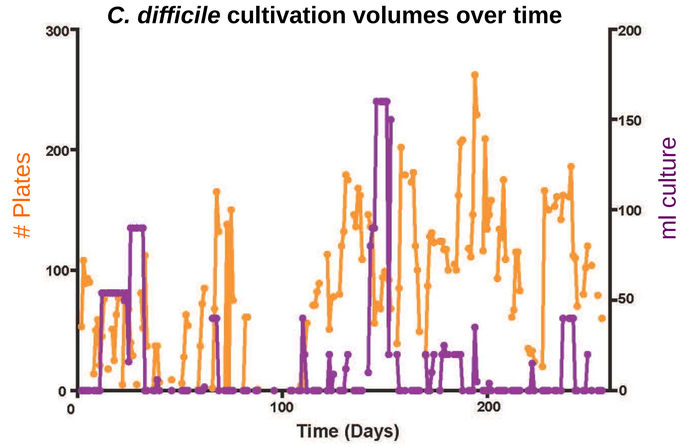
Condition 1: Large volumes of *C. difficile* cultured in complex medium (primarily BHIS broth)

- Carlson examined sporulation and growth characteristics of diverse collection of *C. difficile* strains(1)



- Cultivation was in an unheated, Coy Vinyl Anaerobic Chamber (size A until change to size B on day 166) with one trilayer HSRC
- Average temperature of 29.3 ± 0.9°C, humidity of 72.2 ± 8.8% RH and CO_2 concentration (size A chamber) of ~3.3%

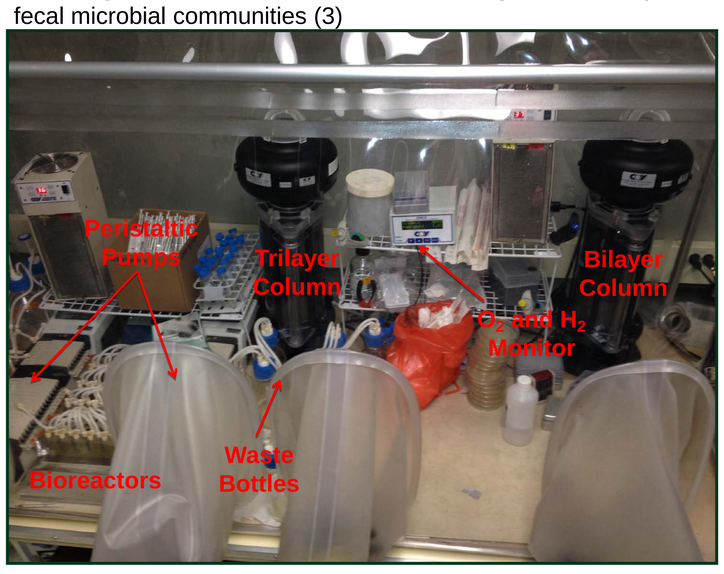
***C. difficile* cultivation volumes over time**



- H_2S concentrations reached at least 10 ppm (size A) without HSRC
- HSRC operation:
  - Decreased H_2S accumulation in chamber
  - Extended life of oxygen sensor in Coy Anaerobic Monitor-12
    - >18 months of full function with sequential HSRC units
  - Did not affect CO_2 levels

Condition 2: Mixed fecal microbial communities cultured in complex medium (BRM)

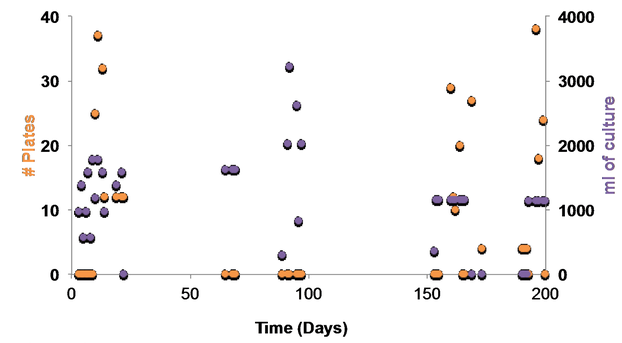
- Auchtung and Robinson examined *C. difficile* growth in complex fecal microbial communities (3)



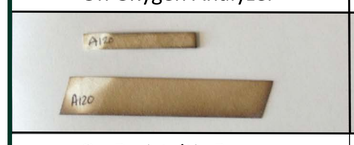
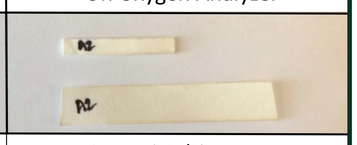
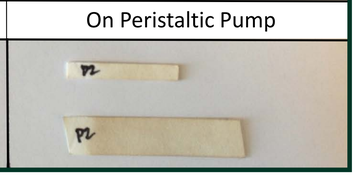
- Two Heated (37°C), dehumidified Coy Vinyl Anaerobic Chambers (size B) with one trilayer and one bilayer HSRC in each
- Average temperature of 36.2 ± 0.7°C, humidity of 37.9 ± 3.7%RH and CO_2 concentration of 3.6 ± 0.2%
- Non-remediated H_2S levels (~25-50 ppb in chamber atmosphere)
- Significant levels of volatile fatty acids produced in addition to H_2S

RESULTS

Mixed culture cultivation volumes over time



- Activation of HSRC:
  - Significantly reduced the level of H_2S in the chamber atmosphere

Lead sulfate accumulation on lead acetate strips after 120 minutes of exposure	
Without HSRC	With HSRC
On Oxygen Analyzer	On Oxygen Analyzer
	
On Peristaltic Pump	On Peristaltic Pump
	

  - Did not affect CO_2 levels
  - Rapidly cleared H_2S (< 5 min) once source of H_2S was removed

LONG-TERM EFFICACY OF HSRC OPERATION

- Reduced levels of H_2S to below the limit of detection for the indicator strips at the outlet of the columns
- HSRC's were capable of long term H_2S removal without saturation (all columns still in operation with initial media)
  - Condition 1 for >8 months
  - Condition 2 for >7 months
- HSRC operation reduced mixed gas consumption under condition 2 by at least 5-fold by eliminating gas purges to remove H_2S
- HSRC allowed continuing function of palladium catalysts
  - >8 months in condition 1 with initial catalyst still in use
  - >1 month without reduction of function in condition 2 (Fig. 1)

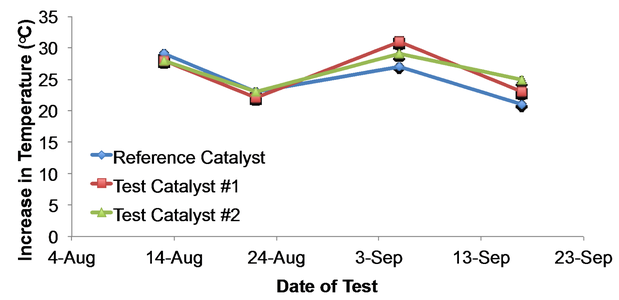


Figure 1: Function of the palladium catalyst was monitored over time by measuring the increase in temperature (°C) that occurred during the catalyzed oxidation of hydrogen to water. Test catalysts #1 and #2 were used during routine anaerobic chamber operation under condition 2. The reference catalyst was not exposed to H_2S or the chamber atmosphere. All catalysts were rejuvenated weekly via heating at 200°C for 2 hrs before testing.

CONCLUSIONS

- HSRC's are convenient, effective tools for removing H_2S from anaerobic chambers operated under a variety of conditions (temperature, humidity, bacterial cultures).
- Lead acetate strips deployed under the column and throughout the anaerobic chamber provide easy-to-read measures of the effectiveness of H_2S removal as well as an indication of when the HSRC media is reaching capacity.

REFERENCES

1. Carlson, PE Jr et al. 2013. The relationship between phenotype, ribotype, and clinical disease in human *Clostridium difficile* isolates. *Anaerobe*. doi: 10.1016/j.anaerobe.2013.04.003.

2. Stern, A.C. 1976. Air Pollution, Volume III: Measuring, Monitoring and Surveillance of Air Pollution. Academic Press, New York USA.

3. More information about this work will be presented during sessions 7.1 and 8.2.

Poster presented at: Anaerobe 2012: The 11th Biennial Congress of the Anaerobe Society of the Americas, June 27-July 1, San Francisco, CA, USA