DEVICE FOR HYDROGEN SULFIDE REMOVAL IN ANAEROBIC CHAMBERS

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ABSTRACT

We desired to develop, and feasibility test, a column for the removal of hydrogen sulfide (H_2S) from enclosed anaerobic chambers. Build-up of H_2S from the culturing of H_2S-producing bacteria causes undesirable deposits, decreases palladium catalyst life, and harms expensive electronics. Such a column is needed as other methods for H_2S removal, such as bubbling through a silver sulfate solution, lack desired capacity, can be difficult to use, are inefficient at H_2S removal, and/or lack any indication when capacity is reached and are no longer functioning.

The hydrogen sulfide removal column (HSRC) functions by recirculating the anaerobic chamber atmosphere through layers of defined media at a controlled rate. This facilitates chemical interactions that leave the output free of H_2S, based on an H_2S-specific sensitive color-change indicator. Various media were examined. Other important considerations were also addressed in the design and application of the HSRC, including temperature, humidity, use in a contained environment, detection of H_2S, function in an anaerobic atmosphere, byproducts of reaction with H_2S, disposal of spent media, H_2S capacity, chamber atmosphere circulation, and overall HSRC flow characteristics to optimize performance. The HSRC was run continuously without any maintenance under constant exposure to H_2S for a 6-month period and had remaining capacity based on H_2S indicators at the outlet. Inside the chamber, H_2S levels reached as high as ~10ppm during growth of *C. difficile* without the HSRC. Within 10 minutes, the HSRC could lower this to a level undetectable with sensitive lead acetate indicators.

BACKGROUND

- Hydrogen sulfide (H_2S) is produced during normal bacterial growth and metabolism
- This compound can be harmful if found in high enough quantities
- Even very low quantities (0.003 ppm) can cause damage to electronics
- Having a reliable method for H_2S removal from anaerobic chambers would be ideal

Considerations For Column Design:

A.) H_2S Removal Media (20 evaluated)**:**

Two types of media chosen for use the column:

- Balances performance variability of single media resulting from complex set of variables.

1. Activated Carbon
   - works best under in lower humidity
   - enhanced performance due to unique fabrication process
   - functions primarily via adsorption

2. Permanganate Impregnated Media
   - works best under higher humidity
   - color change indication of reaction (not H_2S specific)
   - functions primarily via chemisorption

◆ Shared characteristics of each:
- High capacity for H_2S removal even under anaerobic conditions (with or without CO_2 at elevated temperatures
- Specifically designed/formulated for H_2S removal, but also broad spectrum with potential to remove other unwanted metabolites
- Easily disposable after use

Other media examined and excluded:

Impregnated carbons:
- enhanced removal efficiency potentially offset by narrowing of pores and coverage of adsorption sites
- thermal risks associated with some chemically treated carbons
- require humidity for impregnant reactivity

1. Ferric hydroxide impregnated carbon
   - Limited availability due to manufacturing process
2. Potassium hydroxide impregnated carbon
   - Preliminary testing gave low H_2S removal in anaerobic environment
     - Optimum performance may require O_2 – mechanism-of-action controversial
3. Copper oxide impregnated carbon
   - Concern over bactericidal property of Cu
4. Chromium impregnated carbon
   - Requires hazardous waste disposal

Triazine coated media
- Volatile byproducts of reaction with H_2S

Mixed metal oxides
- Some require high humidity to function
- May require special handling as hazardous material (exothermic reaction when exposed to air and/or heavy metals)

Iron Oxide
- Requires humidity threshold for reaction with H_2S
- Exothermic reaction presents safety/handling concern depending upon substrate and humidity

B.) Flow Optimization

- critical for proper function of media (effective media can be rendered ineffective)
  - important for chemisorption and for adsorption
  - ideal to have single pass H_2S clearance
- avoid design features that yield non-uniform flow
- minimize heat generation from source of air flow

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RESULTS

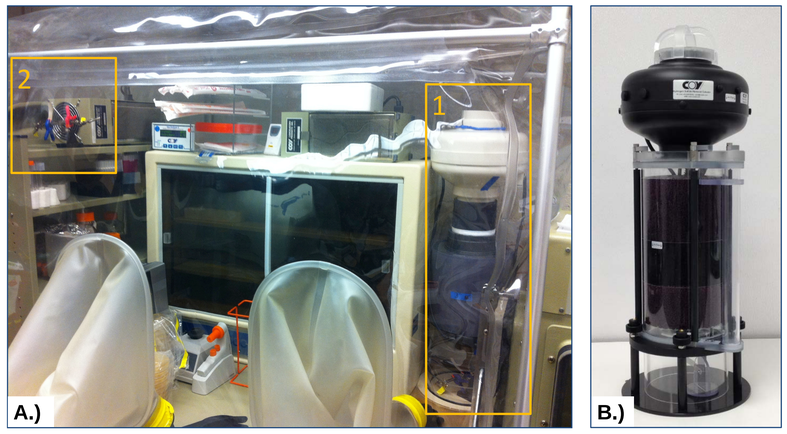

Figure 1: Testing procedure for H_2S removal column.
(A) Chamber setup during testing procedure. Detection strips (box #2) placed on opposite end of chamber from column (box #1). Detection strips also placed under column to monitor life-span. Chamber routinely run with ~4.0% hydrogen using gas mix containing H_2:CO_2:N_2 (10:5:85%). CO_2 levels were approximately 3.0% after 8 months of column use. Chamber was used to consistently grow relatively high levels of *Clostridium difficile*, a bacterium known to produce H_2S. Cultures were primarily growth in Brain Heart Infusion broth/plates supplemented with yeast extract and 0.1% cysteine (BHIS). Calculated H_2S capacity of column >700g or 1.5 lbs. NOTE: For proper function of any H_2S removal system, the chamber environment needs to have good mixing to avoid "dead spots" for H_2S to collect.
(B) Beta version of H_2S removal column for subsequent testing has disposable media-filled cartridge & fits through the chamber airlock.

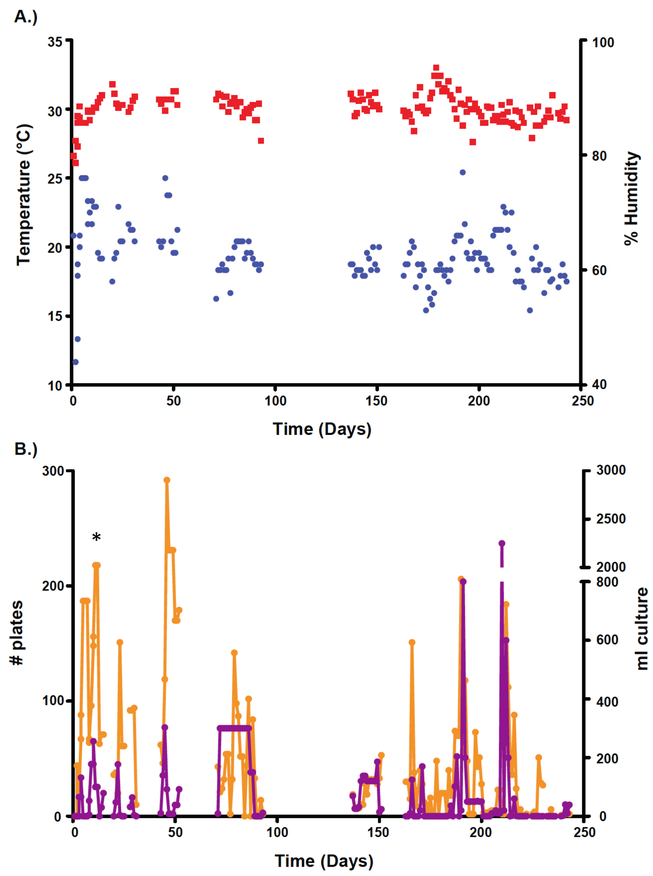

Figure 2: Chamber conditions and usage during column testing.
(A) Chamber conditions. Temperature (red symbols) remained relatively constant throughout the test period while humidity (blue symbols) varied somewhat. Peaks in humidity correlate to peaks in chamber usage as high numbers of plates can lead to a significant increase in humidity. A dehumidifier was run constantly throughout this testing to keep humidity levels from reaching the condensation point.
(B) Chamber usage. As a measure of usage, and therefore potential H_2S production, the total number of 100mm plates (orange symbols) and ml of culture (purple symbols) was recorded daily. While usage varied significantly, levels of H_2S produced throughout the testing period were relatively high.
* HSRC activated on day 11.

RESULTS

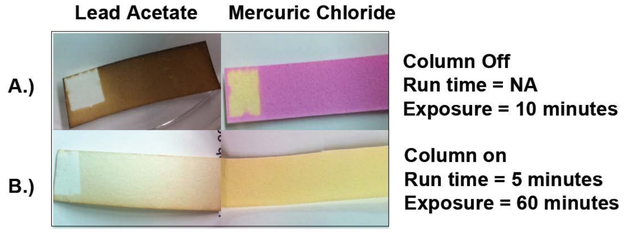

Figure 3: Detection and clearance of hydrogen sulfide before and after column activation.
Both lead acetate tape and mercuric chloride strips were used to detect H2S levels. Lead acetate changes from white to dark brown in the presence of H_2S and is H_2S specific and cumulative. In contrast, while mercuric chloride turns yellow to pink in the presence of H_2S, these strips are time sensitive and do allow for quantification of H_2S levels. Light colored rectangular areas on strips are initial non-reacted color.
(A) Initial levels of hydrogen sulfide were measured before activation of the column (day 11). Test strips shown were exposed to chamber environment for 10 minutes. The level of change observed on mercuric chloride strips over this time is equivalent to 10ppm H_2S.
(B) Following activation of the column, the device was allowed to run for only 5 minutes prior to measuring levels of H_2S again. Test strips were exposed to the chamber environment for over 1 hour following this initial run time (column remained running during exposure). Little/no change is evident, indicating rapid clearance of H_2S from the environment.

Figure 4: Routine detection and clearance of hydrogen sulfide.
Lead acetate strips showing cumulative levels of H_2S detection during chamber usage (see Figure 1 for locations). Test strips in the chamber change to a dark color in 2 days during heavy usage (A) as opposed to minutes in Figure 3A; however, upon removal of active cultures, the chamber is still rapidly cleared of H_2S (B). Under normal usage, test strips should be changed at least weekly (C). Test strips placed under the column show no signs of H_2S exposure, even after 8 months of use, indicating that the column has a long life-span (D).

NOTES:
- Test strips cycled weekly from under column to the chamber to ensure that they were still functional.
- The strips will transition from lighter shades of brown to the darker colors seen above. During heavy chamber usage, the initial color change occurred within hours of placing the strip in the chamber.
- Catalyst rejuvenated every 2 weeks without HSRC and < 1 time per month with active H_2S removal.

SUMMARY

- **We have developed and tested a device for successful removal of hydrogen sulfide from anaerobic environments.**
- **HSRC is designed to function under a variety of anaerobic chamber conditions.**
  - **Humidity – non-condensing and <65% RH**
  - **Temperature – ideally 25-37C**
  - **Gases – H_2, CO_2, N_2**
- **HSRC removed hydrogen sulfide produced by high levels of *Clostridium difficile* culture over a period of 8 months without changing media and with additional capacity remaining.**
- **No maintenance of the HSRC was required.**
- **Airflow from the HSRC did not disrupt work in the chamber.**