BACKGROUND

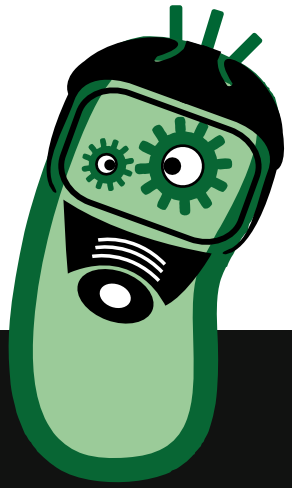
Hydrogen sulfide (H_2S) is a colorless, flammable, and poisonous gas. It has a sweetish taste and a typical smell of rotten eggs. The molecule can be detected by 50% of the population at 0.00047 ppm. However, overtime people become desensitized to the smell. Being heavier than air, H_2S tends to accumulate at the bottom of poorly ventilated spaces, thus making it difficult to detect.

HEALTH RISKS

H_2S has a broad spectrum of toxicity and affects many systems. It is primarily absorbed through the lungs, although it can be absorbed through the gastrointestinal tract and intact skin.

ppm	Effect
10-20	Eyes, nose, throat irritation
50-300	Headache, nausea, cough, vomiting, eyes damage, breathing problems
>300	Shock, convulsion, inability to breath, coma, death

H_2S toxicity is comparable with that of hydrogen cyanide. The gas reacts with iron in the mitochondrial cytochrome enzymes and interrupts the electron transport chain, thus preventing cellular respiration. If you are an asthmatic person you may be at higher risk.



WORKING WITH H_2S PRODUCING BACTERIA

Many natural bacteria use sulfate or cysteine as a substrate for the production of H_2S: these are commonly referred to as sulfur reducing bacteria (SRB). Additionally, several laboratories have engineered bacteria to reduce sulfur for a variety of purposes.

When grown in the laboratory, H_2S producing bacteria can release significant quantities of H_2S, which can potentially pose risks for the researcher. For example, our gas chromatography analyses showed that a sealed 50 ml culture of *E. coli* carrying a synthetic cysteine desulfhydrase gene accumulated between 18 and 30 ppm of H_2S inside of a 250 ml bottle after 4 h of induction. Note that if the culture flask were open to the environment, such a high value would not have been reached.

SAFETY

Here are listed the exposure limits for hydrogen sulfide set in Europe and in the US. Make sure to check the limits set by your country and at your Institution.

EC	Exposure	(ppm)
TWA	Average 8 h/40 h/week	5
STEL	15 min within 8 h	10
USA (OSHA)	**Exposure**	**(ppm)**
TWA	Average 8 h/40 h/week	10
STEL	15 min within 8 h	20
Peak	10 min	50

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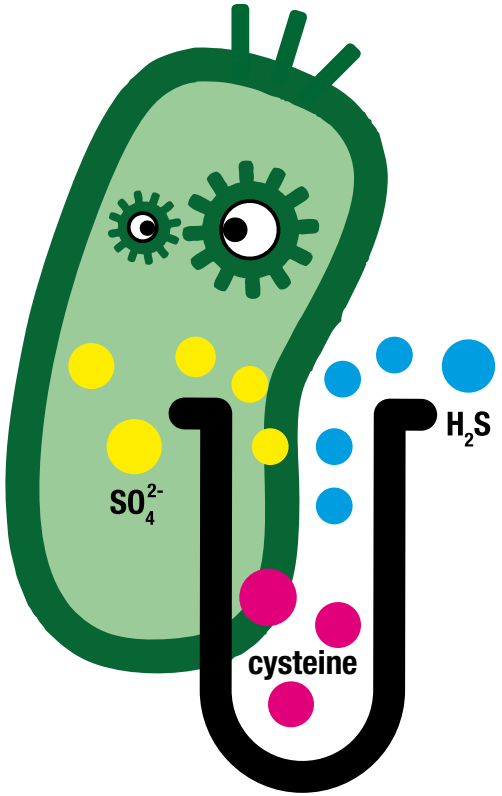
THE SRB HANDBOOK

SAFETY GUIDELINES FOR H_2S PRODUCING BACTERIA



iGEM Trento 2012

ROTTEN EGGS?

This brief document is meant to help other iGEM teams that will work in the future with H_2S producing bacteria. We hope that our experience will be helpful to others.

If you decide to work with a H_2S producing bacteria, we suggest that you brush up on your H_2S chemistry and that you understand the potential risks involved. Awareness always helps to avoid problems and mistakes. This handout is a good starting point, but you should not limit your knowledge to this document alone.

TIPS & TRICKS IN THE LAB

- Before starting an experiment, always plan well in advance all of the details of the assay and material needed. In this way you will not be found unprepared.
- Be sure to have emergency numbers always within reach. Many European Universities have emergency units for first aid.
- Consult your safety office and supporting laboratory staff before starting your project. Discuss together a strategy to organize your work. For example, you could designate a chemical hood only for H_2S producing bacteria.
- Do not work alone in the lab if you are using H_2S bacteria. Make sure that there are other labmates around in case you need help.
- Ask yourself if it is really necessary to grow this culture? If possible, minimize the volume and the amount of cultures grown.
- Always wear goggles, lab coat, and gloves.
- H_2S is highly flammable. Keep potential triggers (flames, sparks, electric discharges etc.) away from cultures.
- Always work under a chemical hood. Flasks or tubes containing bacteria producing H_2S should be sealed tightly and only opened under a chemical hood. If you need to use the culture for a measurement, e.g. optical density, it is preferable that small portable instruments are used under the hood.
- Dedicate a separate waste container for H_2S containing media and solutions. Keep the waste under the hood. Inform the appropriate staff at your university so that waste is safely disposed of.
- H_2S has a strong smell of rotten eggs and most people can easily detect it at low concentrations. However, people quickly become desensitized to the smell, so do not overly rely on smell.
- Although smelling your samples is an easy and immediate way to qualitatively assess if your system is working, we strongly recommend that you do not do so. An easy and quick way to determine if H_2S is present is to use lead acetate-soaked paper strips, which turn black in H_2S presence. Note that lead containing waste must be disposed of properly.
- When using a part that produces H_2S, it is wise to place it downstream of a well-controlled inducible system. Avoid placing the biological part behind a constitutive promoter, as you won't be able to control H_2S production. It is preferable to use a well characterized system with low levels of basal expression.
- Label properly cell stocks and plasmids. Store them in boxes that are easily recognizable as containing material that produces H_2S.
- If you are not feeling well, leave the laboratory immediately and call for medical help.

MORE READING ON THE TOPIC

http://www.osha.gov/OshDoc/data_Hurricane_Facts/hydrogen_sulfide_fact.pdf

www.atsdr.cdc.gov/toxprofiles/tp114.pdf

www.npi.gov.au/substances/hydrogen-sulfide/index.html

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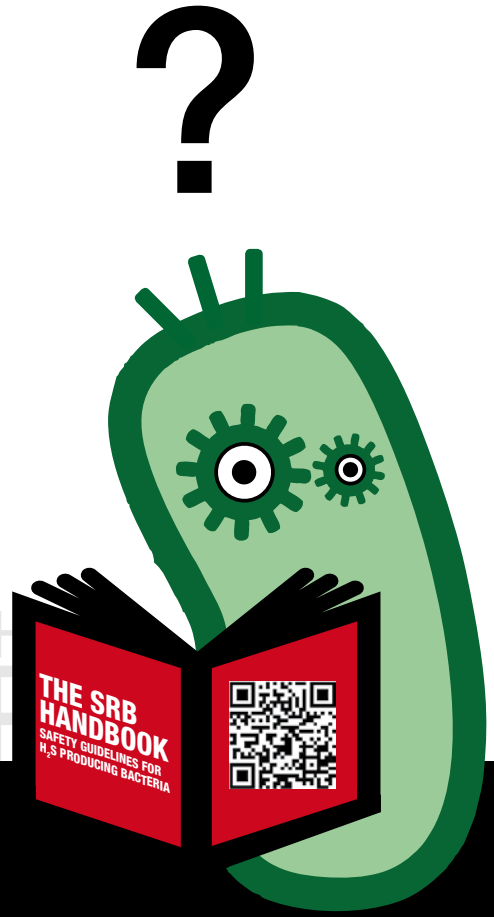

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