

STANDARD OPERATING PROCEDURE (SOP)

METHANE INCUBATION OF METHANOTROPHIC BACTERIA

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SOP #	Methane incubation of methanotrophic bacteria
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1. INTRODUCTION/PURPOSE

This is a standard procedure for methane incubation of methanotrophic bacteria. Methanotrophic bacteria derive their carbon from methane, CH₄. In order to grow these bacteria, methane must be injected into an anaerobic culture. This is done by releasing methane gas from a tank into a rubber balloon, from which the gas is extracted into a syringe. The syringe is then punched through the rubber stopper in the growth flask and the gas is injected into the headspace. Upon withdrawal of the syringe the rubber stopper will close around the hole made by it. In this way, the methanotrophic bacteria can be grown in the presence of methane in a lab setting.

2. SAFETY

General laboratory safety applies. For more information see IBV's HSE webpages: <http://www.mn.uio.no/ibv/om/hms/>. Key procedures for working in the laboratories are listed in HSE in the laboratories: <http://www.mn.uio.no/ibv/english/about/hss/laboratorier/>

Overall risk for this SOP is

 Risk assessment of this procedure	There is a medium overall risk associated with the use of this procedure, provided that this procedure is followed.
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3. NECESSARY SAFETY EQUIPMENT

The user of the SOP should wear lab coat and gloves. This procedure should be carried out in a fume hood.

4. RESPONSIBILITIES

The head of the department has overall responsibility for systematic HSE management. He/She must ensure that the section leaders who report to them, and their staff, have adequate training in HSE.

Employees shall participate in the implementation of HSE, and in accordance with the orders and instructions of IBV. Employees shall be informed of their duty to actively contribute to systematic HSE management. They shall help prevent and limit injuries and ensure work is stopped when it cannot continue without risk to life and health. Injury or illness which they believe may be due to the working environment shall be reported to the employer/manager.

5. EQUIPMENT, MATERIALS AND SOLUTIONS

- Methane tank w/ valve
- Rubber balloon
- Syringe w/ 2 um filter
- 250/500 mL Erlenmeyer flasks
- Pipette and pipette tips
- Methylococcus capsulatus bacterial culture (or other methanotroph)
- Methylococcus capsulatus growth medium (or for other methanotroph)
- Incubator (anaerobic glove box)
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CAS no	Supplier	Name
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6. PROCEDURES:

We always wear lab coats when working in the lab.

Specific for this procedure use fumehood and gloves.

This is a general procedure for methane incubation of methanotrophic bacteria. The risk associated with growth and expression of cells are described in separate SOPs.

The waste disposal is described under 7. WASTE DISPOSAL

Before starting:

Culture *M. capsulatus* (or other methanotroph) in appropriate medium, keep at 4 C.

1. Depressurization step
2. Attach rubber balloon to methane tank (valve closed).
3. Open valve so that balloon is filled.
4. Close valve so a set amount of methane gas is trapped in the balloon.
5. Using syringe w/ 2uM filter, suck up methane gas from the balloon.
6. Drive needle through rubber stopper in the growth flask and inject desired amount of methane, 25 % vol in the headspace. In a 500 mL Erlenmeyer flask, with 100 mL medium with bacteria, this means 123 mL methane in a headspace of 500 mL.
7. Withdraw needle.
8. Place culture in an incubator.

After injection:

Monitor growth of bacterial culture by OD₆₀₀ measurement. Once this reaches an appropriate level, extract DNA (described in a separate SOP).

7. RISK ASSESSMENT

The general risk factor of a SOP can be calculated using the part of the procedure with the assumed highest risk factor. The risk assessment associated with this SOP is based on the user following the precautions stated in the step by step risk assessment below.

List of chemicals and their H and P phrases

Chemicals	Hazard symbol	H phrases	P phrases Precautions
Methane gas		H220 - Extremely flammable gas. H280 - Contains gas under pressure; may explode if heated. Asphyxiant in high concentrations.	P210 - Keep away from heat, sparks, open flames or hot surfaces. – No smoking. P377 - Leaking gas fire: Do not extinguish, unless leak can be stopped safely. P381 - Eliminate all ignition sources if safe to do so. P403 - Store in a well-ventilated place.

Risk assessment; step by step

Part of procedure	Unwanted scenarios	Precautions	Emergency planning	S*K	
1	Depressurization	Release of methane gas; fire/explosion hazard	Fume hood; valve; no open flames	See P-statements above	1*1(green)
3	Filling balloon w/ methane gas	Release of methane gas; fire/explosion hazard	Fume hood; valve; no open flames	See P-statements above	1*1(green)
5	Sucking up methane gas w/ syringe	Needle puncturing skin/eye	Wear gloves/goggles	-	1*1(green)
6	Injecting methane gas into growth flask	Needle puncturing skin/eye; Release of methane gas; fire/explosion hazard	Wear gloves/goggles Fume hood; no open flames	-	1*1(green)
7	Withdrawing needle	Release of methane gas; fire/explosion hazard; turning over the flask, spillage	Wear gloves/goggles/lab coat, fume hood	See P-statements above -	1*3(green)

Overall risk assessment for this SOP

If procedure specified above is followed there is MINIMAL RISK associated with the use of this SOP. If you get a yellow part of the procedure, new precautions must be assessed. If you get a red part of the procedure it will have to be changed.

8. WASTE DISPOSAL

According to waste procedures:

- Chemical waste
- Methane
- Cells and cell-media with antibiotics must also be disposed as risk waste.

- Other waste used in this SOP can be disposed in a waste bin right away.

9. REFERENCES

MSDS
ECOonline