ETH Zurich	EPP-SOP-ETH03
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EPP - Standard Operating Procedure

Title: **Analysis of alcohols in culture supernatant by gas chromatography**

Describes the steps required in order to detect and quantify alcohols and other volatile compounds in microbial culture supernatants by gas chromatography with a flame-ionization detector (GC-FID).

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Instruction

Analysis of alcohols in culture supernatant by gas chromatography

1 Introduction/Purpose

This protocol describes the sample preparation and analysis of microbial cultures for the detection of volatile compounds in the culture supernatant. The organic solvent, internal standard, and GC conditions listed below were set for the detection of isobutanol and corresponding side-products.

2 Equipment and chemicals

Please note, this section lists the equipment and chemicals required for sample extraction and GC analysis only, i.e. it does not include equipment for prior microbial cultivation or general laboratory equipment and chemicals such as pipettes, plastic tubes, vortex mixers or distilled water.

2.1 Equipment

Gas chromatograph (Agilent 6890N)
Auto-sampler/injector (Agilent 7683)
DBWAX-UI column, 30 m, 0.25 mm (Agilent)
H_2 generator (for FID; NMH_2 100, Linde)
Glass vials (11 mm crimp/snap, brown, 2 mL; Wicom)
PP-PTFE caps (11 mm crimp/snap; Wicom)

2.2 Chemicals

Methyl tert-butyl ether (MTBE; SupraSolv^R for GC, Merck)
n-pentanol (internal standard; Sigma)
isobutanol (for standard curve; Sigma)
Helium (carrier gas; He 4.6, PanGas/Linde)
N_2 (for FID ; on-site gas port, PanGas/Linde)
Air (for FID; air, PanGas/Linde)

N.B.: All compounds for analysis should have the highest purity grade available.

3 Media and buffers

3.1 Culture samples and standard samples

≥ 1.5 mL of culture broth per sample
≥ 1.5 mL of culture medium incl. defined standard concentration of isobutanol (for each point of the standard curve, e.g. from 0 mM to 20 mM)

4 Procedures

4.1 Sample preparation

Spin down 1.5 mL of each sample in a plastic tube for 5 min at max. speed and 4°C. If required, additionally sterile-filter with a 0.2 µm syringe filter. Next, add 100 µL of internal standard solution (1%(v/v) n-pentanol in pure water) to 1 mL of clear supernatant, mix by vortexing briefly.
Add 500 µL of MTBE, vortex thoroughly for ≥ 30 seconds, subsequently let the two phases separate for a few minutes (if not properly separated, very briefly spin down in bench-top mini centrifuge).
Transfer 400 µL of organic phase into glass vial, close vial with snap-cap and place in the auto sampler unit.

4.2 GC-FID analysis

The following parameters highlight a potential set up for split-less sample injection. Change parameters if the resolution of compound peaks is not sufficient or peaks are not symmetrical, for example use split-injection if column is overloaded.

Carrier gas: He, const. flow mode at 2 mL min^{-1}

FID gases: H_2, 40 mL min^{-1}
air, 450 mL min^{-1}
N_2, 50 mL min^{-1}
Software: GC ChemStation B04.01

Heater/injector at 225°C

Oven temperature profile:

5 min at 40°C
15°C/min to 120°C
50°C/min to 230°C
4 min at 230°C
(total of approx.16.5 min per sample, without oven cooling time)

FID
250°C

First sample as baseline/contamination control: MTBE only, then measure standard curve with standard samples and/or run actual culture samples.

5 Remarks/Troubleshooting

The temperature profile described above is a relatively elaborate ramp program, if you are sure that there is no additional compound(s) in the sample, one could also use an isothermal profile, for example at 70°C for 10 min. Isothermal methods have the advantage that the oven does not have to cool down for the next run, i.e. saves time, but might be limited in the separation of compound peaks.

6 Biosafety

No biosafety issues are associated with this protocol and the microbiological strains

7 Acknowledgements

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