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### ► **To cite this version:**

Marie Noguier, Sandrine Chifflet, Sylvain Davidson, Florian Haitz, Bernard Angeletti, et al.. A trace metal cleaning protocol for laboratory equipment to enhance the repeatability of methanotroph cultures. *MethodsX*, 2025, 14, pp.103392. <10.1016/j.mex.2025.103392>. <hal-05106687>

**HAL Id: hal-05106687**

**<https://hal.science/hal-05106687v1>**

Submitted on 11 Jun 2025

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## A trace metal cleaning protocol for laboratory equipment to enhance the repeatability of methanotroph cultures



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### ARTICLE INFO

#### Method name:

Trace metal free bioreactor labware preparation

#### Keywords:

Methane oxidation  
Formate production  
Metal contamination  
Labware vessel treatment  
Instrumented bioreactor  
Methanotroph cultivation  
Variability  
*Methylovimicrobium alcaliphilum*

### ABSTRACT

Microbial metabolism relies on metal-dependent enzymes, making trace metal contamination in liquid cultures a critical factor in biotechnological processes. This is particularly relevant for aerobic methanotrophs such as *Methylovimicrobium alcaliphilum* 20Z, a promising bacterium for methane valorization. Despite using conventional cultivation methods (Milli-Q water, analytical-grade reagents, autoclave sterilization), cultures in instrumented bioreactors exhibited poor reproducibility in growth and metabolite production. To address this issue, we developed the “trace metal free” protocol, a specific treatment dedicated to cleaning and removing trace metal contamination from laboratory equipment in order to improve culture stability.

This proposed approach may be beneficial for any microbial culture requiring precise control of trace metal exposure, thereby enhancing the repeatability and productivity of cultures.

- The “trace metal free” protocol treatment combining ultrapure water with an acid wash and sonication steps was applied to remove trace metals from labware and culture vessels.
- Based on a series of batch cultures, the efficiency of the “trace metal free” protocol treatment was demonstrated by the improvement in repeatability of both growth on methane and formate yield (Y formate/methane) parameters known to be closely dependent on the presence of tungsten in the culture medium.
- The 2-hour treatment protocol enabled stable microbial growth from the first batch, whereas untreated cultures required four sequential batches to reach comparable stability.

### Specifications table

|  |   |
|--|---|
| Subject area:                          | Agricultural and Biological Sciences            |
| More specific subject area:            | Microbiology                                    |
| Name of your method:                   | Trace metal free bioreactor labware preparation |
| Name and reference of original method: | None  |

(continued on next page)

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<https://doi.org/10.1016/j.mex.2025.103392>

Received 24 March 2025; Accepted 24 May 2025

Available online 24 May 2025

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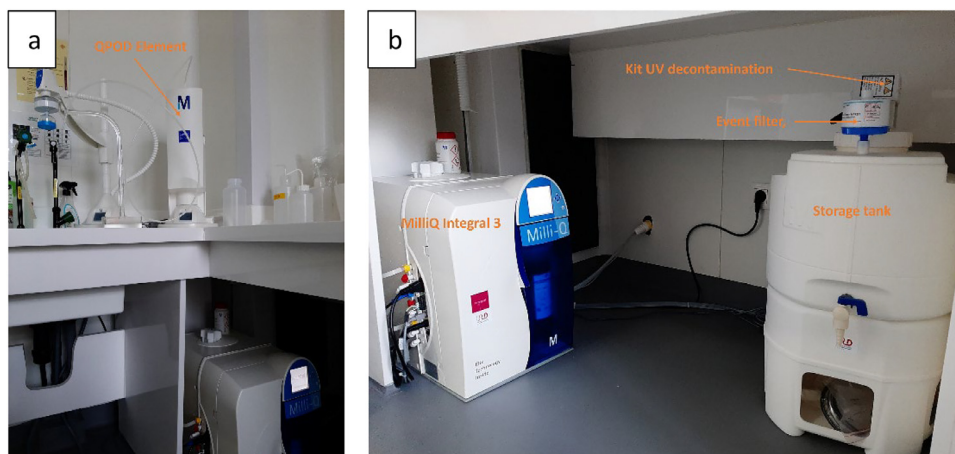
(<http://creativecommons.org/licenses/by/4.0/>)

| Resource availability: | Required reagents (analytical grade)   |
|------------------------|--|
|                        | <ul style="list-style-type: none"> <li>○ NaCl</li> <li>○ MgSO<sub>4</sub></li> <li>○ CaCl<sub>2</sub> · 2 H<sub>2</sub>O</li> <li>○ KNO<sub>3</sub></li> <li>○ KH<sub>2</sub>PO<sub>4</sub></li> <li>○ K<sub>2</sub>HPO<sub>4</sub></li> <li>○ ZnSO<sub>4</sub> · 7H<sub>2</sub>O</li> <li>○ MnCl<sub>2</sub> · 4H<sub>2</sub>O</li> <li>○ H<sub>3</sub>BO<sub>3</sub></li> <li>○ CoCl<sub>2</sub> · 6H<sub>2</sub>O</li> <li>○ NiCl<sub>2</sub> · 6H<sub>2</sub>O</li> <li>○ Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O</li> <li>○ FeSO<sub>4</sub> · 7 H<sub>2</sub>O</li> <li>○ KOH</li> <li>○ EDTA tetrasodium salt</li> <li>○ CuSO<sub>4</sub> · 5 H<sub>2</sub>O</li> <li>○ NaOH</li> <li>○ Methane (N55, purity&gt; 99.95 %, Air Liquide S.A., France)</li> <li>○ Oxygen (U, purity &gt;99.5 %, Air Liquide S.A., France)</li> <li>○ Nitrogen (U, purity&gt;99.995 %, Air Liquide S.A., France)</li> </ul>   |
|                        | <p>Required materials and equipment:</p> <ul style="list-style-type: none"> <li>○ Ultra-pure water production in ISO5 clean laboratory: <ul style="list-style-type: none"> <li>• Tap water purification: MilliQ Integral3 system (Millipore) including two polishing cartridges (Progard TS2, PROGOTOS2 and Quantum TIX, QTUMOTIX1) to remove trace levels of organics and ions after reverse osmosis purification</li> <li>• Ultra-purification: Q-Pod® Element column (Millipore) including a polishing cartridge (Quantum ICP, QTUMM00ICP) and a 0.1 µm final filter (MPPVICPK1) to lower trace metal concentrations below ng/L</li> <li>• UV decontamination for storage tank (ASM kit, Millipore) to prevent biofilm and control TOC at 2 µg/L</li> </ul> </li> <li>○ Ultraclean labware pre-treatment in ISO5 clean laboratory: <ul style="list-style-type: none"> <li>• Ultrasonic Bath (Branson®, MT 8800-E, 40 KHz, 21 L)</li> <li>• HCl (bi-distillation using Savillex DST-1000, ultra-pure grade)</li> </ul> </li> <li>○ Material for bioreactor setup: <ul style="list-style-type: none"> <li>• Agitated PEEK (PolyEtherEtherKetone) bioreactor (Fairmentech, Germany), 1 L working volume, with double-jacketed glass tank. Agitated stainless-steel bioreactor (Pierre Guerrin, France), 2 L working volume, with double-jacketed glass tank</li> <li>• 500 mL – 5000 mL Culture medium supply bottles in borosilicate glass 3.3 (Duran, France)</li> <li>• Temperature probe (Pt 100, Prosensor, France)</li> <li>• Combined pH/RedOx probe (InPro 3253, Mettler Toledo, Switzerland)</li> <li>• Optical dissolved oxygen probe (Visferm, Hamilton, Switzerland)</li> <li>• Four mass-flow meter 0–100 ml/mn (InFlow, Bronkhorst, Netherland)</li> <li>• Tubing: 4 mm polyurethane tube for gas flux (Legris, France) and polypropylene-based thermoplastic elastomer Pharmed-BPT (St Gobain, France) for peristaltic pump with polypropylene connectors.</li> <li>• Two scales for NaOH supply bottle, and bioreactor weight tracking (Sartorius Combiics 1 and BP400, Germany)</li> <li>• Cooling circulation Bath for condenser (Julabo dyneo, Germany)</li> <li>• Heating circulation bath (Julabo SE 6, Germany)</li> <li>• Peristaltic Pumps (Watson-Marlow, France)</li> <li>• Online micro-GC with Thermal Conductivity Detector, (R3000, SRA Instrument, France)</li> <li>• 0.20 µm filter (Filtropur S PES, Sarstedt, Germany and Midisart® 2000 0.2 µm, Sartorius, France)</li> <li>• CO<sub>2</sub> probe (CARBOCAP GMT 221, Vaisala, Finland )</li> <li>• Supervision system (BatchPro software, Decobecq Automatismes, France)</li> <li>• Automate (Wago PLC, France)</li> <li>• Cartridge filled with carded cotton for gases feeding the bioreactor</li> <li>• Centrifuge (miniSpin, Eppendorf, France)</li> <li>• Spectrophotometer (GeneQuant 1300, GE Healthcare, USA)</li> <li>• pH Buffer solutions 9.21, 7.00, 4.01, (Mettler Toledo, Switzerland)</li> <li>• Autoclave (Lequeux, 205 litres)</li> </ul> </li> </ul> |

## Background

Culture media contamination by trace metals from environmental sources including labware and water has been reported to affect bacterial metabolic activity [1,2]. These contaminations result in decreasing the performance and productivity of cultures in biotechnological processes, thus making their control a critical factor.

Aerobic methanotrophs, in particular, are well known to exhibit high sensitivity to trace metal, including copper and rare-earth elements, which play an essential role in their metabolism [3]. Among them, *Methylovimicrobium alcaliphilum* [4] and *Methylovimicrobium buryatense* [5] are particularly studied for their potential to convert methane into value-added molecules. However, due



**Fig. 1.** Equipment dedicated to water treatment. (a) Q-Pod Element containing a polishing resin (Quantum ICP) and a 0.1  $\mu\text{m}$  final filter). (b) MilliQ Integral 3 containing two polishing resins (Progard TS2 and Quantum TIX) connected to the water storage tank.

to their halophilic and alkaliphilic nature, these species are prone to trace metal precipitation in liquid cultures, leading to variability in metal availability and, consequently, metabolic fluctuations.

Formate accumulation by *M. alcaliphilum* has been shown to be suppressed by the addition of tungsten [6] which activates a tungsten-dependent formate dehydrogenase. Bordel et al. [7] reported a high methane-to-formate conversion yield (0.45 mol/mol), in contrast to Kalyuzhnaya et al. [8] who measured a yield of 0.12 mol/mol under similar culture conditions without tungsten supplementation. In our study on the ecophysiology of *M. alcaliphilum*, we encountered significant reproducibility issues when culturing this organism in sequential batch experiments using an instrumented bioreactor, despite adhering to conventional microbiology practices, including the use of analytical-grade reagents and Milli-Q water. We therefore hypothesized that the variability of results reported in the literature and our own experimental inconsistencies were linked to trace metal contamination in the culture environment, potentially influencing the activity of the tungsten-dependent formate dehydrogenase.

To address this issue, we developed a specific protocol to optimize equipment cleaning procedures and minimize trace metal contamination from environmental sources. This approach is inspired by methods established by geochemists in the GEOTRACES community (<https://www.geotraces.org/>) for studying trace metal in marine environments. From controlled cultures of *M. alcaliphilum* 20Z, formate and cell yields on methane were used as proxies to determine the presence or absence of trace metal contamination. More broadly, this methodology may benefit any microbial culture requiring precise control of trace metal exposure, ultimately enhancing reproducibility in biotechnological applications.

## Method details

### Ultra-pure water

All stock solutions and culture media were prepared in a clean laboratory (ISO 5) with ultra-pure water. Ultra-pure water was generated through the purification of Milli-Q® water in a Q-Pod® Element column (Millipore, France; Fig. 1). The quality of the produced ultra-pure water was carefully controlled every three months for the following indicators: R ultra-pure = 18.2  $\text{M}\Omega\cdot\text{cm}^{-1}$ , TOC < 2  $\mu\text{g}/\text{L}$ , rejection > 92 %, R reverse osmosis = 15  $\text{M}\Omega\cdot\text{cm}^{-1}$ . In addition, the reverse osmosis filter is chlorinated every 3 months, and the storage tank is irradiated every night to prevent biofilm proliferation in the MilliQ Integral3 system and storage tank, respectively. Trace metal analyses were performed using an inductively coupled plasma mass spectrometer (ICP-MS NexION 300; Perkin Elmer). This procedure removed metallic ions from the water down to a level below the ICP-MS detection limits (< 10 ng/L).

### Inoculum and bioreactor culture medium recipe

*M. alcaliphilum* 20Z strain was purchased from DSMZ cell bank (DSM-19,304) and routinely stored at 4 °C. Table 1 reports the culture medium used for growth of this bacterium (inspired by Ojala, Beck, et Kalyuzhnaya [9]).

### Stock solutions

The following stock solutions were prepared as follows:

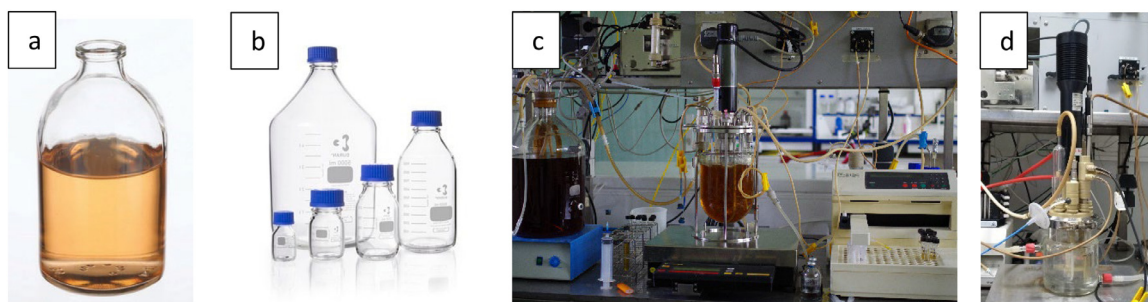
- Phosphate stock solution: dissolve 5.4 g/L  $\text{KH}_2\text{PO}_4$  and 10.7 g/L  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  in ultra-pure water and adjust pH to 7.2. Autoclave the solution.

**Table 1**  
*M. alcaliphilum* medium preparation steps and composition.

| Component   | Content |
|---|---------|
| <b>Addition before autoclaving:</b>   |         |
| CaCl <sub>2</sub> · 2 H <sub>2</sub> O  | g/L     |
| NaCl  | 0.013   |
| MgSO <sub>4</sub>   | 15      |
| KNO <sub>3</sub>  | 0.5     |
|   | 1.0     |
| <b>Addition from stock solutions after autoclaving, in a medium cooled to room temperature and flushed under N<sub>2</sub> flow to prevent oxidation of metal components:</b> |         |
| Phosphate stock solution  | mL/L    |
| Trace Element stock solution (TES)  | 20      |
| Copper stock solution   | 2       |
| Chelated iron stock solution  | 1.8     |
|   | 0.12    |

**Table 2**  
Concentrations of EDTA chelating agent and metals in the medium of *M. alcaliphilum*.

| Component   | concentration (mg/L) | concentration (μM) |
|---|----------------------|--------------------|
| Na-EDTA, 2 H <sub>2</sub> O                           | 1.0                  | 2.7                |
| CoCl <sub>2</sub> , 6 H <sub>2</sub> O                | 0.1                  | 0.4                |
| MnCl <sub>2</sub> , 4 H <sub>2</sub> O                | 0.01                 | 0.1                |
| ZnSO <sub>4</sub> , 7 H <sub>2</sub> O                | 1.6                  | 5.6                |
| NiCl <sub>2</sub> , 6 H <sub>2</sub> O                | 0.004                | 0.02               |
| H <sub>3</sub> BO <sub>3</sub>                        | 0.06                 | 1.0                |
| Na <sub>2</sub> MoO <sub>4</sub> , 2 H <sub>2</sub> O | 0.1                  | 0.4                |
| FeSO <sub>4</sub> , 7 H <sub>2</sub> O                | 2.1                  | 7.6                |
| CuSO <sub>4</sub> , 5 H <sub>2</sub> O                | 0.15                 | 9.0                |

**Fig. 2.** Vessels used to store stock solutions, media and conduct cultivations. (a) Serum bottle; (b) Medium supply bottles; (c) stainless-steel bioreactor; (d) PEEK bioreactor.

- The Trace Element stock Solution (TES, 500X) contains in g/l: 0.8 ZnSO<sub>4</sub> 7H<sub>2</sub>O, 0.005 MnCl<sub>2</sub> 4H<sub>2</sub>O, 0.03 H<sub>3</sub>BO<sub>3</sub>, 0.05 CoCl<sub>2</sub> 6H<sub>2</sub>O, 0.002 NiCl<sub>2</sub> 6H<sub>2</sub>O, and 0.05 Na<sub>2</sub>MoO<sub>4</sub> 2H<sub>2</sub>O. To prepare the TES, ultra-pure water was previously boiled under a stream of O<sub>2</sub>-free N<sub>2</sub> gas, then 0.5 g/l of the chelating agent (Na<sub>2</sub>-EDTA) was added, and the pH was adjusted at 7.0 with KOH. After dissolving each trace element of TES in an aliquot of the latter solution, the aliquots were finally pooled and mixed. The TES solution was stored at 4 °C and protected from light.
- Copper stock solution: dissolve 1.25 g/L CuSO<sub>4</sub> 5H<sub>2</sub>O in the ultra-pure water previously boiled under a stream of O<sub>2</sub>-free N<sub>2</sub> gas. Sterilize through 0.2 μm filter and store at 4 °C protected from light.
- Iron stock solution: dissolve 20 g/L KOH in the ultra-pure water previously boiled under a stream of O<sub>2</sub>-free N<sub>2</sub> gas, add 18.5 g/L Na<sub>2</sub>-EDTA, and adjust pH to 9.0 with H<sub>2</sub>SO<sub>4</sub>. Dissolve 17.6 g/L FeSO<sub>4</sub> 7H<sub>2</sub>O. Sterilize through 0.2 μm filter and store at 4 °C protected from light.

**Table 2** reports the concentrations of the chelating agent (EDTA) and metals in the culture medium.

All these solutions were prepared using the ultra-pure water produced in an ISO5 clean laboratory as described above and stored in serum bottles (Clear Type 1B Borosilicate) or media bottles (DURAN® borosilicate glass 3.3) (Fig. 2). The TES solution, an adaptation of the trace element solution made by Ojala et al. [9] without iron and copper, was prepared as separate stock solution to prevent the oxidation of metals. In addition, compared with Ojala et al.'s medium, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> were not introduced in the medium during bioreactor cultures to avoid their precipitation, and the pH was controlled with NaOH for the duration of the culture.



Fig. 3. HCl batch positioned in ultrasonic bath.

### Bioreactor setup

Microbial cultures were carried out in two bioreactors equipped with two axial impellers and with working volumes of 1.0 L and 2.0 L. Temperature, pH, redox potential, and dissolved dioxygen were tracked by specific probes. The inlet  $\text{CH}_4$ ,  $\text{O}_2$ , and  $\text{N}_2$  gas streams were controlled with mass-flow meters, sterilized through a filter made of carded cotton, and injected through a nozzle immersed in the bioreactor. At the bioreactor exit, the steam in the outlet gas stream was condensed into a glass exhaust water cooler temperature-controlled at 4 °C by a cooling bath equipped with a pump to prevent water evaporation. Downstream from this water-cooler glass exhaust, a micro-GC, equipped with a catharometric detector, and a  $\text{CO}_2$  probe, allowed online to assess the  $\text{CH}_4$ ,  $\text{O}_2$ ,  $\text{H}_2$ ,  $\text{N}_2$ , and  $\text{CO}_2$  in the bioreactor outlet gas. An additional mass-flow meter was used to control a  $\text{N}_2$ -stream directed toward the feeding bottle containing the sterile culture medium to maintain anoxic conditions. Bioreactor was heated with water circulated into the double jacket using a heat bath equipped with a pump. Bioreactor was connected to three pumps dedicated to (i) supplying sterile culture medium, (ii) emptying the reactor, and (iii) adjusting pH using the NaOH solution. The weights of the bioreactor, and the NaOH solution were monitored by two scales to track NaOH consumption during batch cultures and to manage the filling and emptying operations of the bioreactor tank. Connections between the bioreactor, the medium feeding bottle, the spent medium collecting bottle (bioreactor exit), and the NaOH solution were made using polypropylene-based thermoplastic elastomer tubing.

Temperature, pH, gas flow rates, dissolved dioxygen, and stirrer speed were regulated through control units (local loops). All this equipment including mass-flow meters, heat, and cooling baths, and pumps were connected to an automat via either a serial link (RS232/RS485), a 4–20 mA analog loop, or a digital signal. The automat was connected to a computer for process monitoring and data capture. BatchPro software was used to monitor and manage the process with good flexibility and accuracy.

Before each series of cultivation cycles (see details in “Experimental conditions” section), the bioreactor (vessel and internal components) was dismantled, washed with detergent and tap water, and either (i) rinsed once with Milli-Q water for the condition “without treatment” (corresponding to conventional microbial protocols) or (ii) treated against trace metals contamination, using the “trace metal free” protocol treatment, for the condition “with treatment” as described in “Experimental conditions” section. Clean bottles, subsequently used for fresh medium and acidic or basic solution, were rinsed three times with ultra-pure water before use. The clean bioreactor connected to the medium feeding bottle, the NaOH bottle, and the bottle dedicated to collecting the spent medium were autoclaved together at 120 °C for 20 min. The autoclave was loaded with filtered (through 20 and 10  $\mu\text{m}$  filters) and deionized (ion exchange resins) water.

### The “trace metal free” protocole treatment for trace metals contamination removal

The “trace metal free” protocole treatment designed to eliminate trace metal contamination was performed as follows:

- Rinse 3x dismantled bioreactor with ultra-pure water
- Submerge dismantled bioreactor in HCl (10 % v/v, bi-distilled) bath
- Sonicate for 1 h (Fig. 3)
- Again, Rinse 3x with ultra-pure water

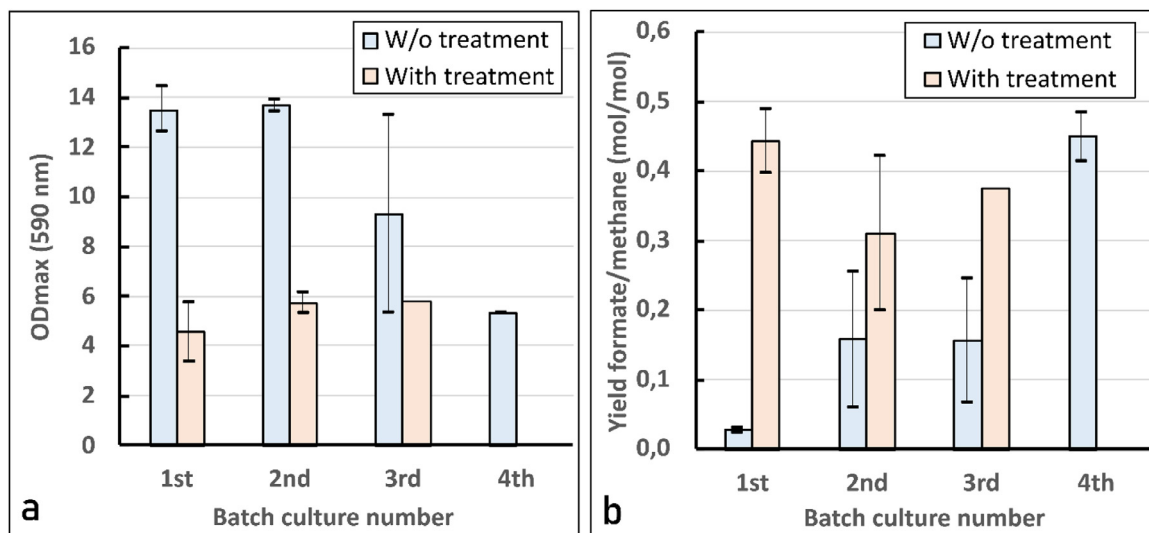


Fig. 4. Effect of the “trace metal free” labware protocol treatment on the metabolic activity of *M. alcaliphilum* depending on batch number in sequential cultures at  $3.0 \pm 0.4$  days; (a) Maximum optical density measured at 590 nm and (b) formate yield from methane. Bars represent the mean of two values and error bars represent min and max values.

### Experimental conditions

One cultivation cycle included three steps: the reactor feeding, the culture phase, and the reactor emptying. Description of the three steps of a culture cycle:

- **Step 1:** Reactor feeding. The reactor was filled with 0.4L or 0.8 L of fresh medium. During the filling step, incoming gas streams of  $N_2$  adjusted at 100 mL/min and 50 mL/min were used to maintain the anoxia in the bioreactor and the feeding bottle, respectively.
- **Step 2:** Culture phase. For the first culture cycle of a series of cycles, the bioreactor inoculation was performed with 25 mL of a recent *M. alcaliphilum* 20Z culture (less than 8 days old) prepared in serum bottles. For the next culture of the following cycles, 10 mL of the previous culture ( $n - 1$ ) was kept in the bioreactor to inoculate the current culture cycle ( $n$ ). The temperature and stirrer speed were regulated at  $27.9 \pm 0.5$  °C and 500 rpm, respectively. The pH was regulated at  $8.8 \pm 0.2$  by adding NaOH (2 M). The pH probe was calibrated with pH 7.0 and 9.2. The incoming gas stream of  $CH_4$  was set to represent 50 % of the input gas ( $CH_4:O_2:N_2$ ) in the bioreactor. The dissolved  $O_2$  was regulated at 10 % of saturation, i.e. 0.4 mg/L, through adjustment of the inlet gas stream of  $O_2$ . The Inlet gas stream of  $N_2$  was proportionally adjusted so that the sum of  $O_2$  and  $N_2$  flows would remain constant and thus liquid-gas transfer efficiency would not be affected during cultivation.
- **Step 3:** Reactor emptying. At the end of the cultivation phase (step 2), the bioreactor was emptied, leaving 10 mL to be used as inoculum for the next cultivation cycle. The process was therefore ready for a new cycle ( $n + 1$ ).

### Analytics

From bioreactor samples, the cell density (OD) was assessed by spectrophotometry at 590 nm (suitably diluted so that the OD was  $<1.0$ ). These samples were centrifugated at 13,400 rpm for 15 min, and the formate concentration in the supernatant was determined by HPLC (Dionex ICS5000, Thermofisher) using a UV detector at 210 nm (Dionex Ultimate 3000) and the Chromeleon software (Thermofisher). 900  $\mu$ L of sample supernatant were loaded with a Dionex AS/DV autosampler onto an Aminex HPX-87H column (Biorad) set at 45 °C and eluted at 0.6 ml/min with  $H_2SO_4$  solution (0.5 mM). Gas chromatograph (micro-GC) was used to measure  $CH_4$ ,  $O_2$ ,  $H_2$ , and  $N_2$  content in the outlet gas stream. The temperatures of the injector, the column, and the detector were adjusted to 90, 100, and 100 °C, respectively. The pressure of argon, used as the carrier gas, was 30 kPa. The gas analysis was repeated every 2 min. The chromatogram treatments were performed by SOPRANE software (SRA Instrument, France).

### Determination of $CH_4$ consumption rate

Inflow gas debits and outflow gas analyses ( $N_2$ ,  $O_2$ ,  $CH_4$ ,  $H_2$ ,  $CO_2$ ) were used to calculate  $CH_4$  consumption  $Q_{CH_4^{consumed}}$  (Eq. (1)).

$$Q_{CH_4^{consumed}} = \left( Q_{CH_4^{in}} - Q_{out} \times p_{CH_4} - \frac{dp_{CH_4}}{dt} \times \frac{V_{headspace}}{V_{liq}} \right) \times V_m \quad (1)$$

With  $Q_{CH_4^{out}}$  and  $Q_{CH_4^{in}}$  the outflow and inflow of  $CH_4$  into the bioreactor, respectively, and  $Q_{out}$  the total gas outflow ( $N_2$ , residuals  $O_2$  and  $CH_4$ ,  $CO_2$ , and  $H_2$  produced). The partial pressure of  $CH_4$  in the outflow  $p_{CH_4}$ , volumes of liquid ( $V_{liq}$ ) and headspace phase ( $V_{headspace}$ ), and molar volume  $V_m$  were considered at the reactor's temperature.

### Method validation

The present method was validated by comparing growth of *M. alcaliphilum* 20Z on methane and formate production in sequential batch cultures in a bioreactor with and without treatment for trace metal contamination removal as described above. Here, the formate yield was used as an indicator of the absence of trace of tungsten. As a matter of fact, it was shown that formate accumulation was suppressed by the addition of 0.05 - 0.24  $\mu M$  of tungsten [6,10]. The Fig. 4 presents (a) the maximum optical density reached after  $3.0 \pm 0.4$  days and (b) the formate yield when *M. alcaliphilum* was successively cultured with methane as substrate with or without treatment to limit trace metal contamination. Without treatment, maximum optical density and formate yield highly varied from 5.3 to 13.5 OD<sub>590nm</sub> units (Fig. 4a) and from 0.03 to 0.45 mol formate/mol methane (Fig. 4b) from the first to the fourth successive batch, respectively. It was noteworthy that, without treatment, the maximum optical density decreased and formate yield increased, with the succession of batches, suggesting a progressive wash-out of the trace metal influencing metabolic activities. With treatment, formate yield was high (0.40–0.49 mol/mol) from the first batch and much higher than without treatment (0.02–0.03 mol/mol) during the first batch.

In addition, without treatment, four successive cultures were required to reach the formate yield observed at the first batch with the treatment (Fig. 4b). For all cultures ( $n = 8$ , without treatment, and  $n = 5$  with treatment), the treatment reduced substantially the coefficient of variation of the measures of maximum optical density and formate yield from 92 % to 29 % and from 41 to 21 %, respectively.

### Limitations

Here, we report on the “trace metal free” protocole treatment, a cleaning protocol designed to eliminate trace metal in water, vessels, and parts of the submerged bioreactor that allowed us to increase the repeatability of growth and formate production by *M. alcaliphilum*. However, these precautionary measures may not be sufficient under all conditions of bacterium culture. Trace metal contamination may be encountered through (i) sterilization processes, particularly when using an autoclave [11], (ii) the release of elements from tubing, or (iii), the use of reagents and commercial solutions [2], sodium bicarbonate [12], cell culture media [13,14], yeast extract [15]. If trace metals contamination is suspected, scientists are encouraged to take additional precautionary measures including substituting the sterilization method, regularly changing the tubing or changing its material, and increasing the quality of the reagent to be used.

### Related research article

None.

### Ethics statements

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### CRedit author statement

Marie Noguér: conceptualization, methodology, validation, investigation, writing-original draft; funding acquisition; Sandrine Chifflet: methodology, investigation, writing – review and editing; Sylvain Davidson: conceptualization, investigation, methodology, data curation, resources; Florian Haitz: investigation; Bernard Angeletti: resources, investigation; Jean Borgomano: project administration; supervision; writing – review and editing, funding acquisition; Bernard Ollivier: supervision; writing – review and editing; Alain Dolla: project administration; supervision; writing – review and editing, funding acquisition; Combet-Blanc Yannick: conceptualization, supervision, resources, validation, writing – review and editing.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

The data that has been used is confidential.

## Acknowledgments

This work was performed in the frame of the MicroBioH2Prod project funded jointly by Akkodis; the AMIDEX industrial chair Carbonate geosciences: energy, environment, education (Carb3e); and the Mediterranean Institute of Oceanology (MIO). The authors are particularly grateful to Sandrine Chifflet for enabling the collaborative work carried out between the MIO biotechnology platform and the MIO 2METO platform for clean rooms facilities and the CEREGE LA-ICP-MS platform for ICP-MS analyses. We would also like to thank Camille Faure and Michael Chepy from Akkodis for their contribution to bibliographical review and project administration, respectively.

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