## Culturing of Dethiobacter alkaliphilus

Strain AHT1T was grown anaerobically at 30 °C in Na carbonate buffered mineral medium (22 g/L Na2CO3, 8 g/L NaHCO3, 6 g/L NaCl, 1 g/L K2HPO4) with a pH of 10 and 0.6 M total Na+. Additionally, 4 mM NH4Cl, 1 mM MgCl2 x 6H2O and 1 mlL−1 trace element solution were added [18]. After sterilization, acetate serving as a carbon source (2 mM) and thiosulfate (20 mM) the electron-acceptor, were also added to the medium. The culture (2 L) was grown in a 10 L bottle mounted on a magnetic stirrer whereby the headspace (8 L) was replaced by 100% (v/v) H2, at 0.5 Bar overpressure, acting as the electron-donor.

Preparation of Na carbonated buffer mineral medium

|  |  |
| --- | --- |
| Compound | Weight (g/L) |
| Na2CO3 | 22 |
| NaHCO3 | 8 |
| NaCl | 6 |
| K2HPO4 | 1 |

Additional trace element solution

|  |  |
| --- | --- |
| Compound | Converted measurement |
| 4 mM NH4Cl | 0.004m/L, if dissolving pure NH4Cl, **0.213964g** is needed for 1l of solution.  Molecular weight of NH4Cl x 0.004 = 0.213964g  53.491 g/mol x 0.004 m/L = 0.213964g |
| 1 mM MgCl2 x 6H2O | 0.001m/L, if dissolving premade MgCl2 x 6H2O, **0.2033g** is needed for 1l of solution.  Molecular weight of MgCl2 x 6H2O x 0.001= 0.2033g  203.30 g/mol x 0.001 m/L = 0.2033g |
| 1 mlL−1 trace element solution | 1ml is needed for 1l of solution |

\*note about MgCl2, it is very difficult to accurately measure magnesium chloride as it tends to absorb moisture from the air, hence it is recommended to use MgCl2 x 6H2O

Carbon source

|  |  |
| --- | --- |
| Compound | Converted measurement |
| 1mM hypotaurine | 0.001m/L, if dissolving hypotaurine, **0.109g** is needed for 1l of solution.  Molecular weight of hypotaurine x 0.001= 0.109g  109.147 g/mol x 0.001 m/L = 0.109g |
| 20 mM Thiosulfate | \*This depends highly on the material used, there is no “pure” thiosulfate and they are often compounds with sodium, we need to check what the lab has. |

**Possible protocol**

* Weigh out 22g of Na2CO3, 8g of NaHCO3, 6g of NaCl and 1g of K2HPO4 to one litre of milli-q water.
* Add 4 mM of NH4Cl, 1 mM of MgCl2 x 6H2O and 1 mlL−1 trace element solution according to the table above.
* Filter the buffer solution through a 0.22 μm filter into a sterile flask/bottle (preferably autoclaved prior) or autoclave for 15 to 20 minutes. \*Seems like one cycle would be more logical and safer
* Let the autoclaved solution cool down.
* Add 2 mM Acetate and 20 mM Thiosulfate according to the calculation in the table above (tbc).
* Filter the final solution through a 0.22 μm filter into a sterile bottle for storage (optional but would be good)