**McIlvaine’s minimal media**

The appropriate volume of dH2O was added to a glass bottle and autoclaved at 121°C for 15 min. The following components were added to the sterile water aseptically, according to table 2.3: 18 mM C6H8O7, 56.6 mM Na2HPO4, 5 mM K2HPO4, 0.4 mM MgSO4.7H2O, 7.6 mM (NH4)2SO4, 3 μM thiamine, 6 μM (NH4)2SO4.FeSO4.6H2O. The final solution was mixed well. Stocks of each media component were prepared, autoclaved and stored at 4°C until needed. The thiamine solution was not autoclaved, as thiamine is heat sensitive, and was instead filter-sterilised. A supplement stock was prepared separately (Table 2.4) and stored at 4°C. 15

**Table 2.3:** Media components and volumes required for 1000 ml McIlvaine’s medium/Soil extract medium (0.4% glucose). **Component**

|  |  |
| --- | --- |
|  | **Amount (ml)** |
| 0.4 M Na2HPO4 | 141.5 |
| 0.2 M Citric acid | 90 |
| 0.4 M K2HPO4 | 12.5 |
| Supplement stock | 31 |
| dH2O/soil extract | 705 |
| 20% glucose stock | 20 |

**Table 2.4:** Supplement stock volumes for 31 ml.

|  |  |
| --- | --- |
| **Supplement stock component** | **Volume (ml)** |
| 40 mM MgSO4.7H2O | 10 |
| 10 g/100 ml (NH4)2SO4 | 10 |
| 0.1 mg ml-1 thiamine | 10 |
| 6 mM (NH4)2SO4.FeSO4.6H2O in 0.1 M HCl | 1 |