PREPARATION OF FE-FREE TAP MEDIUM FOR ICP-MS MEASUREMENTS

Iron-deficiency is easily obtained in *Chlamydomonas* and is visually recognized by chlorosis in iron supplements below 1 μM, depending upon the strain used. In experiments where it is essential to know the absolute metal content of the medium, more care must be taken. I have prepared samples comparing the effects of autoclaving vs. filtering (0.22 μM) and the iron liberated from the glass during aging.

METHODS

For autoclaved medium: Fe-free TAP medium was prepared in acid washed flasks. A separate water control was also performed. A 9.65 mL aliquot was removed prior to autoclaving. Flasks were capped with aluminum foil and autoclaved for 45 min (120°C, 22 psi). A 9.65 mL aliquot of post-autoclaved medium was taken when it had reached room temperature. Flasks were aged on the bench top with occasional shaking for 5 d and a 9.65 mL aliquot was taken. A second aliquot was filtered through a 0.22 μm PVDF filter (Millipore MillexHV) and 9.65 mL was saved.

For filtered medium: Fe-free TAP medium was prepared in acid washed graduated cylinder and filtered into acid washed and baked (12 h 250°C) flasks (50 mL in 125 mL Erlenmeyer flasks) in duplicate. A separate water control was also performed. A 9.65 mL aliquot was taken after filtering. Flasks were capped with aluminum foil, aged on the bench top with occasional shaking for 5 days and a 9.65 mL aliquot was taken. A second aliquot was filtered through a 0.22 μm PVDF filter (Millipore Millex HV) and 9.65 mL was saved.

Sample handling: 14 mL blue cap tubes were washed in Alconox, rinsed with MilliQ water and soaked in 10% nitric acid for 3 days prior to use. All samples were collected in washed blue cap tubes. Samples (9.65 mL) were mixed with 0.35 mL concentrated nitric acid (Fisher ultra pure, 67-70% HNO_3) and stored at room temperature until measurement.

ICP-MS analysis: Samples were measured at UCLA using an Agilent 7500CE ICP-MS with yttrium as an internal standard. Iron was measured as ^{56}Fe with hydrogen as a carrier gas. The detection limit was calculated to be 1.48 nM.

RESULTS

Overall, all Fe-free TAP medium samples had low levels of iron contamination (Table I). In water control samples, the level of iron detected never increased above the detection limit. It appears that the source of iron contamination appears to be due to leaching from the glassware. It is likely that the EDTA in the medium can solubilize iron in the medium and that there is a basal level of iron that can be solubilized (approx. 10 nM) because no iron was detected in water alone samples. Autoclaving has a clear effect on the amount of iron liberated from the glassware, as levels increase approx. 5 fold due to autoclaving. In filtered medium this release of iron is slower but reached approx. the same ending value. Filtering prior to sampling did not have an effect on the amount of iron detected; indicating that the released iron is indeed soluble.

CONCLUSIONS

In order to achieve the lowest possible levels of iron contamination, either method of media preparation can be acceptable. In experiments where growth in low Fe is desired for a short period, filter sterilizing is preferred whereas in longer duration experiments either method is acceptable. Filtering samples after collection, before digestion is unnecessary.

Sample Treatment	Fe content (nM)
Pre-autoclave	1.84 ± 0.31
Post-autoclave (day 0)	10.61 ± 1.24
Post-autoclave (day 5)	9.53 ± 1.46
Post-autoclave (day 5), filtered	11.11 ± 1.49
Filter sterilized (day 0)	3.38 ± 2.15
Filter sterilized (day 5)	10.40 ± 7.02
Filter sterilized (day 5), filtered	9.71 ± 2.14

Table I. Iron content of medium. Fe content measured by ICP-MS in expressed nM with standard deviation of duplicate flasks.

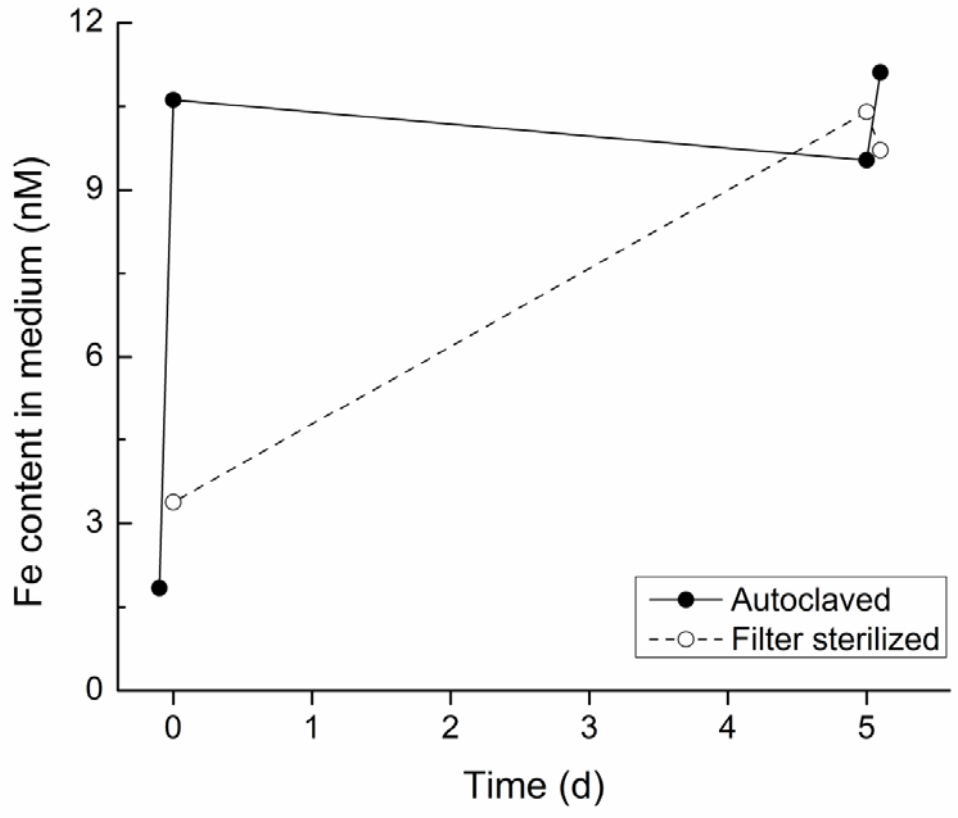

Figure 1. Iron content of medium. Data from Table I are shown graphically as a time course.