Chlorophyll (Chl) a analysis.

1. Remove a random sample of 1 or 2 (or more) ml of cyanobacterial culture and add to microfuge tube (1.0 ml sample) or a 15 ml polystyrene conical centrifuge tube (2.0 ml or more sample). Centrifuge at maximum rpm at room temperature in a microfuge (1.0 ml samples) for 1 min or in a clinical centrifuge (2.0 ml sample) for 5 min.

2. Remove 90% of the liquid with a pipetman or pipet and then add an equivalent amount of 100% methanol to the liquid pellet; yields 90% methanol final concentration. Any variation of volumes to concentrate or dilute sample is fine, as long as one extracts with 90% methanol. Mix vigorously on a micromixer (vortex) to fully suspend the pellet. Allow to sit for at least 5 min in the dark to extract the chlorophyll. Vortex again.

3. Centrifuge methanolic extract as in harvesting cells (step 2). Pellet should be purplish-blue. If green, and the methanol is light green, vortex and extract for a longer time. If the methanol is dark green, remove and save the supernatant, and re-extract the pellet as in 2 above. Recombine the methanolic extract from the two extractions and measure as below.

4. Add 100 ml of methanolic extract to a micro cuvette (1.0 ml sample) or 1.8 ml of methanolic extract to a standard cuvette (2.0 ml sample). Read Absorbency at 665 nm against a 90% methanol blank.

5. Based on an extinction coefficient of 78.74 liter/gram/cm for Chl a in 90% methanol, multiply the A665 by 12.7 to obtain μg Chl a per ml (the value 12.7 is derived from 1/78.74 x 103). Adjust for any sample dilution or concentration.

Reference on extinction coefficient, Meeks and Castenholz. 1974. Arch. Mikrobiol. 78:25-41.