Elimination of bacteria from microalgal culture using antibiotics

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PREAMBLE: Method used to eliminate bacterial contamination of a marine micro-algal culture. Note, this method is not guaranteed to be 100% successful either due to the fact that the antibiotic combination is not lethal to the bacteria present or it is possible that the strain may not be capable of prolonged growth without the presence of bacteria. This method is based on that of Droop (1967), adapted according to Andersen (2005) but using a modern antibiotic mix suggested by S. Slocombe (SAMS).

1. Using appropriate protective clothing, prepare 20ml of an antibiotic solution mixture in pure water as follows:

    - Cefotaxime (500 mg/L)
    - Carbenicillin (500 mg/L)
    - Kanamycin (200 mg/L)
    - Augmentin (200 mg/L)

    Sterilise through a 0.22 micron filter into a sterile bottle. Use aseptically and refrigerate for up to 4 weeks, or store frozen at -20 °C.

2. Prepare a dilution series using 6 tubes of fresh algal culture medium as follows:

Tube	1	2	3	4	5	6
f/2 (+Si)	5 mls	5 mls	5 mls	5mls	5 mls	5 mls
Antibiotic mix	0 (0%)	25µl (0.5%)	50µl (1%)	250 µl (5%)	375 µl (7.5%)	500 µl (10%)
Enriched medium	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop
Algal culture	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml

    The process begins when the algae are added to the tubes. Ensure the contents are well mixed - gently vortex if necessary. Incubate the tubes overnight at the optimum growth temperature in an illuminated incubator.
3. At the following time intervals, take one drop from each tube and inoculate into fresh growth medium:

Algae: Require 20ml of a healthy micro-algal culture in log phase of growth.
Apparatus: Illuminated incubators; a class I biological safety cabinet.
Culture media: 50 tubes containing 5mls sterile f/2 (or f/2+Si for diatoms) medium and 1 tube of an enriched medium such as PPG. 24 small nutrient agar plates made up with ½ strength filtered seawater (SNA) (for recipes see www.ccap.ac.uk/media).
Antibiotics: Cefotaxime, Carbenicillin, Kanamycin, Augmentin (from Sigma Aldrich).
Consummables: 0.22 micron disposable syringe filters, disposable pipettesAll chemicals were purchased from Sigma-Aldrich, unless otherwise stated.

Additional information:
***Droop MR** (1967) A procedure for routine purification of algal cultures with antibiotics. Br. phycol. Bull. 3(2) 295-297.*
***Guillard RRL** (2005) Purification Methods for Microalgae. In Algal Culturing Techniques, Andersen RA (ed). Elsevier Academic Press, pp 117-132.*

culturecollection of algae and protozoa

16 hours, 24 hours, 40 hours, 48 hours, 72 hours, 80 hours.
The above timing intervals are not critical and sample points may be altered plus or minus 6-12 hours for convenience.

4. Incubate the sample tubes and observe for algal growth.
5. In the tubes where algae are seen to be growing, place a drop on an SNA plate, incubate in the dark for up to 1 week and check for bacterial colonies. Always use your initial untreated sample as a positive control.
6. If you have tubes which show algal growth and no demonstrable bacterial growth, you may have produced an axenic culture; however, this should be confirmed by microscopic examination with halogen/tungsten light and in fluorescence after preparing DAPI mounts. If no bacteria are observed you can conclude that*, according to your test methods, your culture is axenic.*
7. To maintain an axenic culture, use very strict aseptic technique and test regularly for bacteria by inoculating in enriched medium as described above.