Maintenance of Cultures

Cultures sent to customers remain alive for at least 14 days provided that the following precautions are observed. Cultures should be unpacked immediately after receipt and stored at 15-18°C under low light intensity (north window, no direct sun light, or weak white fluorescent light). Screw caps or vessels should be loosened but not removed. Further maintenance or multiplication of cultures requires transfer into new culture media. This presupposes experience in simple microbial techniques.

Many species are cultivated and dispatched on agar media for safety reasons but develop their morphological characteristics only in liquid media, e.g. flagellates, colony-forming Volvocales and Chlorococcales. For teaching purposes these species should be transferred into liquid media 2-3 weeks before demonstration, e.g. into Soil Water Media, Basal Medium, or Desmidiacean Medium.

Culture Media

The following media have proved suitable for the maintenance of cultures in test tubes at the SAG for many years. The recipes originate from E. G. Pringsheim and W. Koch, unless stated otherwise. It must be emphasized that the maintenance medium indicated is not always the best medium for the cultivation of a species. There are other media which are just as suitable, e.g. those given in the catalogues of other culture collections of algae (Thompson et al., 1988, Watanabe and Nozaki, 1994, Andersen et al., 1991, Rippka and Herdman, 1992, Starr and Zeikus, 1993). Mass algal culture often requires more concentrated media (for recipes and methods consult Kuhl and Lorenzen, 1964; Starr, 1971; Stein, 1973; Guillard, 1975; Werner, 1982; Castenholz, 1988; Richmond, 2004; Andersen, 2005).

All solutions should be made up with de-ionized water. Media are usually prepared from stock solutions of macronutrients, trace metals, and vitamins which are added to a large proportion of the final volume of water in order to avoid precipitation.

Media may be used as liquid or solidified by 1.0-1.5% agar. Before sterilization the agar has to be dissolved in the medium in a steamer. After this test tubes should be filled with 10 ml of the hot medium, closed with cotton plugs, sterilized (usually by autoclaving at 121°C for 15 min.) and may be stored for several weeks, after cooling, in a refrigerator. Solid media for Cyanobacteria are prepared by mixing, after cooling to 50°C, equal volumes of separately autoclaved double strength solutions of the mineral salts medium and either agar to give a final agar concentration of 0.6-1.0 %.

25. Artificial Seawater Medium with Vitamins (full strength= ASM30 + V; 25. a brackish/ half-strength = ASM15 + V)

(after J. Brand, from Starr & Zeikus 1993, modified)

Preparation: to 944 mL of glass-distilled water, add 30 g NaCl (half-strength = 15 g NaCl) and 1 g TRIS (SERVA). Then add:

ml	stock solution	g/100 mL H_2O
10	$MgSO_4 \cdot 7H_2O$	24.4
10	KCl	6.0
10	$NaNO_3$	10.0
10	$CaCl_2 \cdot 2H_2O$	3.0
10	KH_2PO_4	0.5
6	trace element solution	

Adjust to pH 8.0 with 1 N HCl.

Autoclave, then add (in sterile conditions):

vitamin B_{12} (*)	0,5 ml/l
vitamin B_1 (0,12 g/ 100 ml H_2O)	0,5 ml/l

(*) prepared in two steps: dilute 100 mg B12 in 100 ml H_2O, than dilute 1 ml of this stock solution in 99 ml H_2O.

trace element solution:
add 0,75 g EDTA (Titriplex III) in 1 l aqua bidest., than add salts in the given order(!):

$FeCl_3 \cdot 6\ H_2O$	97 mg
$MnCl_2 \cdot 4\ H_2O$	41 mg
$ZnCl_2$	5 mg
$CoCl_2 \cdot 6\ H_2O$	2 mg
$NaMoO_4 \cdot 2\ H_2O$	4 mg

Optional ingredients: agar at 15 g/L to solidify.

Reference: modified after Starr & Zeikus 1993