## 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 %.

# 23. Enriched Seawater Medium "f/2" (= f/2)

| | stock solution [g/100 ml] | nutrient solution [ml] |
|---|---|---|
| $NaNO_3$ | 7.5 | 1 |
| $NaH_2PO_4 . H_2O$ | 0.5 | 1 |
| $Na_2SiO_3 . 9H_2O$* | 3 | 1 |
| micronutrient working stock solution ** | | 1 |
| vitamin working stock solution *** | | 1 |
| filtered seawater | | 1000 |

* Recommended for diatom culture.

** Preparation of micronutrient solution

| | primary stock solution [g/100 ml dist.$H_2O$] | working stock solution |
|---|---|---|
| $CuSO_4 . 5H_2O$ | 0.98 | 1 ml |
| $ZnSO_4 . 7H_2O$ | 2.2 | 1 ml |
| $CoCl_2 . 6H_2O$ | 1 | 1 ml |
| $MnCl_2 . 4H_2O$ | 18 | 1 ml |
| $Na_2MoO_4 . 2H_2O$ | 0.63 | 1 ml |
| $Na_2EDTA$ | | 4.36 g |
| $FeCl_3 . 6H_2O$ | | 3.15 g |
| de-ionized or distilled water | | 1000 ml |

*** Preparation of the vitamin solution

| | primary stock solution [mg/ml dist. $H_2O$] | working stock solution [in 1000 ml dist. $H_2O$] |
|---|---|---|
| vitamin $B_{12}$ | 1 | 1 ml |
| Biotin (hygroscopic, weigh approx. 10 mg and add 9.6 ml dist. $H_2O$/mg biotin) | 0.1 | 10 ml |
| thiamin HCl | | 200 mg |

Sterilize by filtration or - slightly acidified with HCl - by autoclaving. Add vitamin working stock to nutrient solution after autoclaving.

**Ref.:** Guillard, R.R.L. – Culture of phytoplankton for feeding marine invertebrates. In: W. L. Smith and M. H. Chanley, eds., Culture of marine invertebrate animals. P. 29-60, Plenum Book Publ. Corp., New York, 1975.

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Werner, D. – Biologische Versuchobjekte. Kultivierung und Wachstum ausgewählter Versuchsorganismen in definierten Medien. 432pp. Fischer Verlag, Stuttgart, New York, 1982.

## Further recommended literature about culturing algae:

**Andersen, R.A. (ed.) (2005) Algal Culturing Techniques. Elsevier Academic Press, Burlington. ISBN 0-12-088426-7.**

Belcher & Swale (1982) Culturing Algae - a guide for schools and colleges. ISBN 1-871105-04-8 (ask for at ccap@sams.ac.uk). (Currently unavailable).

**Isaac & Jennings (1995) Microbial Culture. Bios Scientific Publ., Oxford. ISBN 1-872748-92-9.**

**Richmond (ed.) (2004) Handbook of Microalgal Culture. Biotechnology and Applied Phycology. Blackwell Publ., London. ISBN 0-632-05953-2.**

Streble & Krauter (2006) Das Leben im Wassertropfen. Kosmos (Franckh-Kosmos), Stuttgart. ISBN 3-440-10807-4.

Von Berg, Linne & Melkonian (2004) Der Kosmos-Algenführer. Kosmos (Franckh-Kosmos), Stuttgart. ISBN 3-440-09719-6.