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AQUIL: A CHEMICALLY DEFINED PHYTOPLANKTON CULTURE MEDIUM FOR TRACE METAL STUDIES[1,2]

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ABSTRACT

The medium Aquil and its variations have been successfully used for trace metal studies of marine phytoplankton (diatoms and dinoflagellates) over the past three years. Here, the recipes, the methods of preparation and the chemical composition of Aquil are presented in detail. To permit complete definition of chemical speciation of the various components as calculated from thermodynamic equilibria, trace element contamination is controlled and the formation of precipitates and adsorbates is avoided. It is established that Aquil is suitable for physiological experiments with a variety of marine phytoplankters representing all major phyla. Modifications of the basic recipe and design of chemically defined media in general are discussed.

Key index words: Aquil; culturing, media; medium, culture; media, defined; metals; nutrients; toxicity; trace metals

The growing body of literature pertaining to the study of trace metals in phytoplankton cultures makes it increasingly desireable that a consistent basic recipe be used to permit meaningful comparison among results. Mounting evidence that chemical speciation of trace metals in the growth medium is critical in determining the toxicity and availability of trace metals to phytoplankton (3,32) as well as to other organisms (4) makes it imperative that the chemistry of the medium be defined as precisely as possible in trace metal studies.

Commonly used recipes for phytoplankton culturing media fall into two general categories—enrichments of natural waters and totally artificial formulations. In each, the concentrations of major nutrients and vitamins have been adjusted over the years to levels favoring the growth of various algal species. This quantitative understanding of the major nutritional requirements of phytoplankton has, historically, outpaced similar understanding of the interactions between trace metals, chelators and phytoplankton. As described by Provasoli et al. (27), additions of "soil extract" and other organic solutions with unknown chemical properties enabled early workers to grow many species of algae. A great deal of empirical work showed that the addition of synthetic chelators such as EDTA (ethylenedinitrilotetraacetic acid) and trace metals in the correct concentration ratios significantly increased the number of species that could be cultured in artifical media (27), though the chemical mechanisms operating were not fully assessable because of the complexity of the metal-ligand interactions which determine the chemical speciation of the trace metals (see 11, 29).

The major debate has been whether chelating agents increase the availability of iron or repress the toxicity of various trace metals, particularly copper (13,18,19,21). It has been proposed that both mechanisms may operate simultaneously (5,6,10,30).

The primary objective in the design of Aquil is to obtain a medium in which the trace metal speciation is known as precisely as possible. Three things are necessary to achieve this. First is the requirement for techniques to control trace contaminants in the salts and the water, the vessels and apparatus and in the procedures. Second is the need for a basic recipe having sufficiently low concentrations of major nutrients and trace metals to make the precipitation of various solids thermodynamically unfavorable, thus avoiding the complications that precipitates and the ensuing adsorption would introduce in the chemistry of the medium. The reduction in major nutrient and trace metal concentrations relative to those of other media achieves the secondary objective of more closely imitating natural conditions. Thirdly, it is necessary to solve the simultaneous chemical equilibrium equations that describe the chemical speciation in the medium. These computations of thermodynamic equilibria can be performed with the computer program MINEQL (33), which is used here. (A useful general description of

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[2] This paper is dedicated to Luigi Provasoli as a modest tribute to the excellence and elegance of his work and in gratitude for his gracious influence on the field of Phycology.

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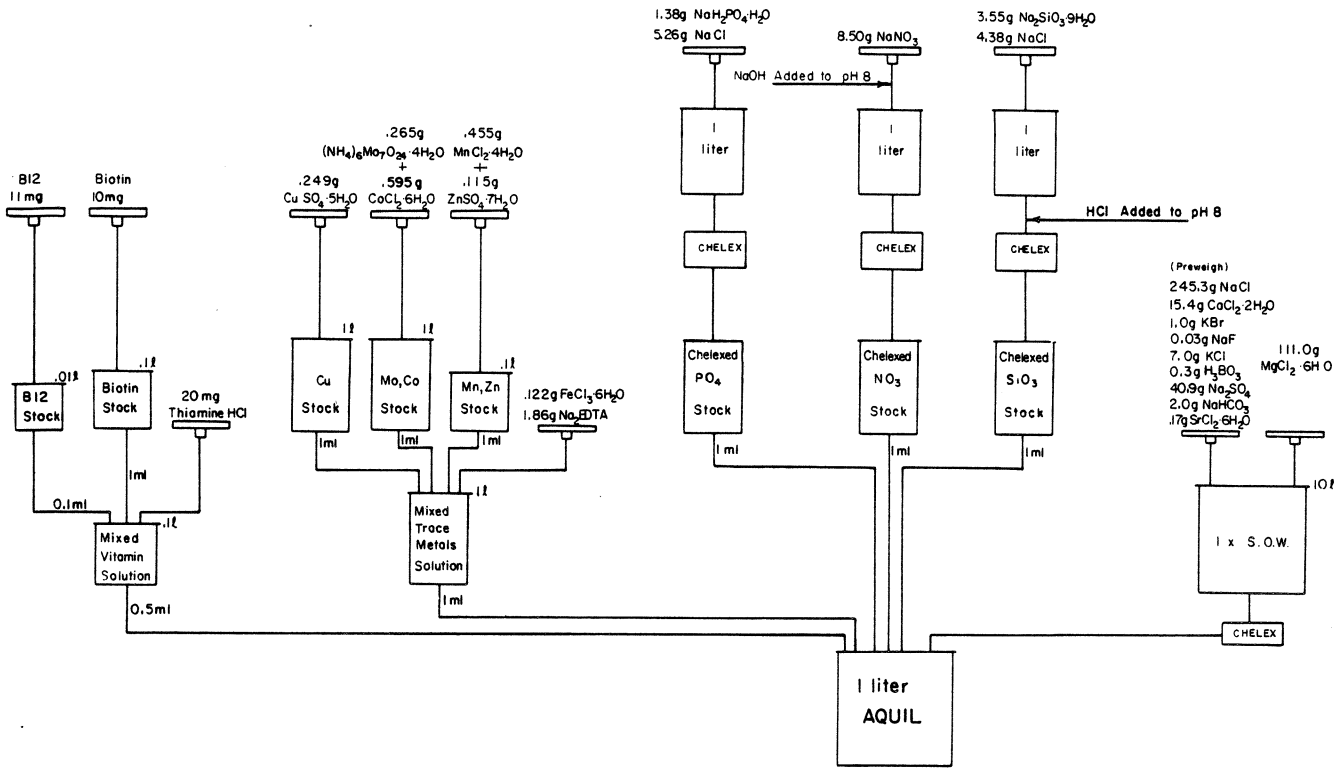

FIG. 1. Scheme for preparation of Aquil medium: see text for details.

metal-ligand interactions in aqueous media is given by Stumm and Morgan, see especially sections 6.7–6.9 in reference 31.)

The origin of Aquil is easily traced to well-known standard recipes: the major seawater salts are those of the Standard Ocean Water (SOW) recipe (12), the vitamins are identical to those of the medium f (15), and the nutrients and trace metal concentrations correspond approximately to a 50-fold dilution of the medium f.

PREPARATION AND COMPOSITION

The background salts (major nutrients), vitamins, and trace metals which compose the medium Aquil are prepared and mixed according to the diagram of Fig. 1. Table 1 gives the final concentrations of all components.

Seawater salts (SOW). The seawater salts of Aquil, except $MgCl_2$, are weighed in advance and stored as a mixture until needed. Magnesium chloride is weighed and added after the "preweigh" has been dissolved in distilled, deionized water. As all other hygroscopic salts, it should be dried before weighing to obtain good precision. (Due to problems with salt dissolution, the original method of preparation (23) which used 2 × SOW has been modified.) Before mixing with the nutrients, vitamins, and trace metals, the SOW solution is passed through an ion exchange column to remove trace metal impurities, as described later.

Nutrients. The major nutrients (phosphate, nitrate, silicate) are prepared as individual stock solutions at 1000× the final concentrations. Each solution is passed individually through an ion exchange column and added at 1 $ml \cdot l^{-1}$ to SOW. To use only one ion exchange column for this purpose, each nutrient stock solution has to be adjusted to the same Na concentration of 10^{-1}M (hence the addition of NaCl in the phosphate and the silicate stocks) and equilibrated to the same pH (=8.0) by addition of 1N HCl or 1N NaOH.

Major nutrient ions present a contamination problem in the background salt solution which is similar to, though less severe than, that of the trace metals. For example, concentrations of phosphate in excess of 0.5 μM have been measured in artificial seawater prepared from reagent grade chemicals in double distilled water (7). In this particular instance, the contamination was due to $MgCl_2$. SOW has been measured to contain 1 μM silicate. In general, it can be expected that some lots of reagent grade chemicals will contain up to the guaranteed limit of P, N and Si impurities. This difficulty can be resolved in special experiments by selecting stock chemicals that are relatively clean.

The phosphate stock solution should be stored in pyrex bottles at 4 C since phosphate ions are strongly adsorbed onto polyethylene but only slightly onto glass surfaces (17). The great solubility of NH_3 makes exacting demands on the storage of solutions, which can easily pick up micromolar concentrations in a few days if the flasks are not properly stoppered. If necessary, NH_4 can be removed by use of an Amberlite IR-120 resin (Rohm and Haas Co., Philadelphia, Pennsylvania).

Silicic acid from the dissolution of glass vessels is available for diatom growth, making it imperative to select alternative materials if silicon limitation is desired. In this situation, clean nonvitreous material (e.g. teflon) is recommended for storage of all solutions except SiO_3, which can be stored in glass. Polycarbonate is convenient for the culture vessels. The silicate stock solution at pH 8 is unstable for periods longer than 2 wk so that the original stock (normal pH ≈ 12.5) should be acidified and passed through the ion exchange column only as needed.

Vitamins. The vitamin preparation is that of f/2 medium (15) and follows the procedure of Guillard (14). The biotin and vitamin B_{12} primary stock solutions are made from crystalline compounds, made slightly acidic (ca. pH 5), sterilized and stored

TABLE 1. *Composition of Aquil medium.*

	Substance	Initial weight g	Initial stock volume l	Initial stock concentration M	Final concentration M
AQUIL salts (SOW)	NaCl	245.3	10	8.39×10^{-1}	4.20×10^{-1}
	$CaCl_2 \cdot 2H_2O$	15.4	10	2.09×10^{-2}	1.05×10^{-2}
	KBr	1.0	10	1.68×10^{-3}	8.40×10^{-4}
	NaF	0.03	10	1.43×10^{-4}	7.14×10^{-5}
	KCl	7.0	10	1.88×10^{-2}	9.39×10^{-3}
	H_3BO_3	0.3	10	9.70×10^{-4}	4.85×10^{-4}
	Na_2SO_4	40.9	10	5.76×10^{-2}	2.88×10^{-2}
	$NaHCO_3$	2.0	10	4.76×10^{-3}	2.38×10^{-3}
	$SrCl_2 \cdot 6H_2O$	0.17	10	1.28×10^{-4}	6.38×10^{-5}
	$MgCl_2 \cdot 6H_2O$	111.0	10	1.09×10^{-1}	5.46×10^{-2}
Nutrients	$NaH_2PO_4 \cdot H_2O$	1.38	1	1.00×10^{-2}	1.00×10^{-5}
	$NaNO_3$	8.50	1	1.00×10^{-1}	1.00×10^{-4}
	$Na_2SiO_3 \cdot 9H_2O$	3.55	1	1.25×10^{-2}	1.25×10^{-5}
Trace metals	$CuSO_4 \cdot 5H_2O$	.249	1	9.97×10^{-4}	9.97×10^{-10}
	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	.265	1	1.50×10^{-3}	1.50×10^{-9}
	$CoCl_2 \cdot 6H_2O$	.595	1	2.50×10^{-3}	2.50×10^{-9}
	$MnCl_2 \cdot 4H_2O$	.455	0.1	2.30×10^{-2}	2.30×10^{-8}
	$ZnSO_4 \cdot 7H_2O$	.115	0.1	4.00×10^{-3}	4.00×10^{-9}
	$FeCl_3 \cdot 6H_2O$	.122	1	4.51×10^{-4}	4.51×10^{-7}
	Na_2EDTA	1.86	1	5.00×10^{-3}	5.00×10^{-6}
Vitamins	B_{12}	.011	0.01	1.1×10^{0} g/l	5.5×10^{-7} g/l
	Biotin	.010	0.1	1.0×10^{-1} g/l	5.0×10^{-7} g/l
	Thiamine HCl	.020	0.1	2.0×10^{-1} g/l	1.0×10^{-4} g/l

frozen. The mixed vitamin solution is prepared by diluting the biotin and B_{12} primary stocks to 1 liter and adding thiamine-HCl according to Fig. 1. Both the vitamin primary stocks and the mixed solution can be dispensed into ampoules or screw-capped containers, sterilized and stored refrigerated.

Trace metals. Trace metal stock solutions are prepared from reagent grade chemicals. Three separate stocks (copper, molybdenum and cobalt, manganese and zinc) are shown in Fig. 1 but other combinations are possible depending on the purpose. Sodium EDTA and $FeCl_3$ are added directly to the combined trace metal solution which is made up of a 1000-fold dilution of the individual stocks. It is important to add Na_2EDTA before $FeCl_3$ in order to prevent precipitation of ferric hydroxide.

Trace metal impurities in salt solutions prepared from reagent grade chemicals can easily exceed the nominal metal concentrations of Aquil. These impurities are reduced to acceptable levels by the use of ion exchange columns following the procedure of Davey et al. (9) as described in the next section. Impurities introduced from the materials (flasks, stoppers, tubing, etc.) are minimized by using "clean" materials whenever possible (e.g., silicone stoppers rather than rubber stoppers; polycarbonate erlenmeyer flasks rather than glass culture containers) and by soaking all vessels in 1N HCl. Distilled-deionized-distilled water (DDDW) is used for preparation of solutions and final rinse of materials. Stock solutions of individual trace metals and the combined trace metal solution should be stored in polyethylene bottles. These solutions are sufficiently concentrated that little change takes place with time.

The possibility of steam-carried contaminants during autoclaving is unavoidable inasmuch as either the flasks and sterilization apparatus or the flasks with medium must be autoclaved. The use of distilled water in the autoclave is a worthwhile precaution when possible. Steam flow into culture flasks can be reduced to a minimum by filling them with DDW which is aseptically emptied after autoclaving. (Another source of metal contamination that should be considered in phytoplankton experiments is simply the carryover from the inocula. Stock cultures themselves need to have controlled metal concentrations low enough to yield negligible concentrations when the inocula are diluted by the fresh media.)

Ion exchange columns. The target concentrations of trace metals in Aquil are so low that it is necessary to remove impurities from the chemical reagents used in preparing the medium. This involves passing the major salt and nutrient solutions through ion exchange columns. The procedures for column preparation are sometimes difficult, so they are described in some detail. The selectivity of the resin Chelex 100 (Bio-Rad Laboratories, Richmond, California) is based on chelate formation rather than on cation charge, size or physical characteristics, so it shows a high preference for transition metal ions over alkali or alkaline earth metal ions, and is especially suited for the removal of trace metals from nutrient and salt solutions (9). The chelating strength and ionic form of Chelex 100 vary significantly with pH. In order to avoid alkalinity changes in the medium as the resin gains or loses protons, it is necessary to equilibrate each column to the pH and major cation concentrations (ionic strength) of the particular solution being purified. The procedures for preparing the resin and packing one column are as follows for each of the solutions to be cleaned of trace metals (SOW, stock nutrients—NO_3, PO_4 or SiO_4 which use the same column):

i) In a 400 ml beaker, rinse 15 g Chelex 100 (Drymesh 100–200, Na form) in methanol to remove residual organics; then rinse 3× with DDDW (to rinse, allow the resin to settle, decant excess water; resuspend in 200 ml fresh DDDW).
ii) Slurry the resin with 300 ml of the solution to be cleaned and, while stirring, titrate (patiently!) to pH 8. This requires ca. 2.2 ml of 1N NaOH for SOW and <0.5 ml 1N HCl for the individual nutrient stocks with adjusted pH and ionic strength. Equilibration of the slurry for each addition of acid or base may take 10–30 min.
iii) Fill a 10 × 250 mm glass (or polycarbonate) fritted chromatography column with the solution to be cleaned. While the column is dripping at ca. 40 ml/min, constantly pour in resin slurry to insure a continuous "snowfall" appearance. When all the slurry has been added, add the appropriate

TABLE 2. *Chemical speciation of Aquil (computations performed with chemical equilibrium program MINEQL (33): this involved 99 complexes, 27 possible solids, fixed pH of 8.10, ionic strength of 0.5 M).*

	Analytical concentration[a] M	Computed −log activity[b]	Computed major species (%)
Bromide	8.40×10^{-4}	3.24	Br^-(100)
Borate	4.85×10^{-4}	4.30	H_3BO_3(85); $B(OH)_4^-$(15)
Calcium	1.05×10^{-2}	2.67	Ca^{2+}(87); $CaSO_4$(12)
Carbonate	2.38×10^{-3}	4.97	HCO_3^-(65); $MgHCO_3^+$(17); $MgCO_3$(7); $NaCO_3^-$(3); $CaHCO_3^+$(3); CO_3^{2-}(2)
Chloride	5.59×10^{-1}	0.41	Cl^-(100)
Cobalt	2.50×10^{-9}	11.50	CoY^{2-}(99)
Copper	9.97×10^{-10}	14.41	CuY^{2-}(100)
EDTA(Y)	5.00×10^{-6}	14.68	CaY^{2-}(87); $FeYOH^{2-}$(9); MgY^{2-}(4)
Fluoride	7.14×10^{-5}	4.54	F^-(59); MgF^+(39); CaF^+(2)
Iron	4.51×10^{-7}	20.23	$FeYOH^{2-}$(98); FeY^-(1); $Fe(OH)_2^+$(1)
Magnesium	5.46×10^{-2}	1.96	Mg^{2+}(85); $MgSO_4$(14); $MgCO_3$(1)
Manganese	2.30×10^{-8}	8.89	$MnCl^+$(38); $MnCl_2$(10); $MnCl_3^-$(2); Mn^{2+}(24); MnY^{2-}(22); $MnSO_4$(3)
Molybdate	1.50×10^{-9}	9.45	MoO_4^{2-}(100)
Nitrate	1.00×10^{-4}	4.16	NO_3^-(100)
Phosphate	1.00×10^{-5}	10.25	HPO_4^{2-}(48); $MgHPO_4$(46); $CaHPO_4$(3); $H_2PO_4^-$(3)
Potassium	1.03×10^{-2}	2.16	K^+(97); KSO_4^-(3)
Silicate	1.25×10^{-5}	11.26	$HSiO_3^-$(6); H_2SiO_3(94)
Sodium	4.80×10^{-1}	0.49	Na^+(99); $NaSO_4^-$(1)
Strontium	6.38×10^{-5}	4.83	Sr^{2+}(100)
Sulfate	2.88×10^{-2}	2.53	SO_4^{2-}(44); $MgSO_4$(27); $NaSO_4^-$(24); $CaSO_4$(4); KSO_4^-(1)
Zinc	4.00×10^{-9}	11.50	ZnY^{2-}(100)

[a] Contains also vitamins as in f/2 medium; analytical concentrations computed as in TABLE 1 and given to three significant places for consistency: in many cases actual concentrations in solution are not known with such accuracy.
[b] Mantissas of these logarithms given to two significant places for internal consistency: accuracy not generally applicable to actual medium. The activity coefficients are computed according to Davies formula (31).

solution until the Chelex resin has been completely packed. (Always maintain a fluid head of at least 10 cm over the packed surface.)

iv) Continue to run the solution to be cleaned through the column until an effluent pH sample bubbled *vigorously* with air equals the influent pH (ca. 8). Due to CO_2 exchange effects, the effluent may not be immediately in equilibrium with the air, which will result in a difference between the pH of the influent and pH of an unequilibrated sample of the effluent.

It is advisable to equilibrate 30 g or more of resin at one time and to save the unused portion for future use. The resin for the salt and nutrient solutions, respectively, should be replaced or regenerated after 50 and 100 liter have been eluted, or, at such times that the column becomes discolored or clogged.

The optimum drip-rate is 5 ml/min for all columns. If the columns run dry accidentally, the resin should be totally resuspended and settled again. Operationally, it is advisable to make an airtight connection between the feed bottle and the column so the quantity of solution entering the column is the same as that dripping out. Alternatively, a siphon tube can be installed to insure a constant head above the resin bed.

Chemical speciation. The precautions taken to remove chemical impurities from the chemicals and vessels make it possible to compute the equilibrium speciation of Aquil with some confidence. Table 2 lists major salts, nutrients and trace metals with their final total concentrations in Aquil, their free ion activities (in log form), and the species which account for >1% of their total concentrations in the medium. The free ion activities and the speciation of the components are calculated on the basis of thermodynamic equilibrium in the system using the computer program MINEQL (33). Since there is no satisfactory method to measure the concentrations or activities of any of the trace species in systems as complex as culturing media, the results of the theoretical calculations remain basically unverified. This situation is mitigated by two factors: i) speciation of most trace metals is dominated by chelation with EDTA and the constants for such chelation are relatively well known; ii) systematic results obtained in relating trace metal activities to their effects on phytoplankton growth in Aquil imply a good degree of self-consistency in the computed activities. Still, it should be remembered that equilibrium calculations are only as good as the thermodynamic constants on which they are based and that these should be verified whenever possible.

Precipitation; adsorption. Minimization of precipitation and adsorption is an important part of the preparation of chemically defined media. Adsorption onto container surfaces leads to unknown decreases in constituent concentrations. Precipitation of solids in the medium results in kinetic and aging processes difficult to quantify; adsorption phenomena on the surface of the new particulates add further complications.

Adsorption onto the walls of the culture vessels is decreased by the choice of material. If glass is used, it should be coated with a silicone film such as S.C. 87 dry film (Pierce Chemical Company, Rockford, Illinois; 9). Adsorption can be minimized further by a preconditioning treatment of 24 h soaking with a medium of exactly the same composition as that to be used in the experiment.

Precipitate formation (and ensuing adsorption onto the particulate phase) is avoided by making solid precipitation thermodynamically unfavorable or, when this is not possible, kinetically slow. The composition of the medium is chosen to achieve the first goal and only five solid species are calculated to be saturated in Aquil: $CaCO_3$; $Ca_5(PO_4)_3OH$; $Mg_3(PO_4)_2$; MnO_2; $Fe(OH)_3$. The first four remain supersaturated for periods of weeks if care is taken not to precipitate them during the sterilization process. Filter sterilization, using "clean" filter apparatus (e.g., acid-washed polycarbonate apparatus and acid-washed 0.4 μm Nuclepore filters) is normally preferred. If the medium is autoclaved, immediate bubbling with sterile 100% CO_2 upon removal from the autoclave will avoid precipitation by lowering the pH which will then rise again to its equilibrium value. (Note that in Aquil the concentrations of major constituents of seawater, especially calcium, have *not* been lowered to avoid precipitation.)

There is some uncertainty about ferric hydroxide precipitation

in Aquil reflecting the uncertainty regarding the applicable solubility constant. The colloidal nature of the possible precipitate and its low concentration make it difficult to verify if precipitation has actually occurred. In situations where it is thought to be critical, a reduction of the Fe concentration to 2×10^{-8} M will insure that the amorphous precipitate will not form while satisfying the Fe requirements of most algae. The most stable crystalline oxides of Fe (e.g., goethite) will still be saturated but these are notably slow to form and do not create difficulties in the time frame of phytoplankton growth experiments.

PHYTOPLANKTON GROWTH AND MODIFICATIONS OF BASIC RECIPE

Culturing tests. Given its complicated preparation and its low nutrient concentrations which result in low cell yields, Aquil is obviously not a good maintenance medium to be used routinely. However, to be useful for physiological studies, a medium has to support the growth of most typical phytoplankters in culture. Due to difficulties often encountered in completely artificial seawater media, Aquil was tested in two ways: i) as a medium; ii) as a basic synthetic seawater which is further enriched as f/2 (referred to as Aquil+).

Table 3 summarizes the results of a systematic test on 21 clones of marine planktonic species representing seven algal classes. The culturing techniques were those routinely employed to maintain the culture collection at Woods Hole Oceanographic Institution (14). The significance of the number of transfers relates to the dilution (ca. 1:100 for each transfer) of critical growth factors that may be introduced with the original inoculum and be absent from the medium. Cells which grow after six transfers are considered to be maintainable indefinitely in Aquil.

In a qualitative way, Aquil resembles a dilution of f (ca. f/50) made with open ocean (Sargasso Sea) water diluted to salinity ca. 32‰ and autoclaved. Cultures are nutrient-limited compared to f/2, and maintenance of sensitive species is somewhat difficult because stationary phase populations do not survive nearly as long in Aquil (or f/50) as they do in Aquil+ (or f/2), hence there is little leeway in timing of transfer.

Of the 21 cultures, 13 were carried through six transfers in Aquil on approximately the same transfer schedule as used for the culture collections of algae kept in filtered seawater with f/2 at the Woods Hole Oceanographic Institution (Table 3). Seven species were difficult to keep in Aquil on the regular schedule and were, at one transfer or another, changed into Aquil+, upon which all went through at least six transfers without difficulty (Table 3, second column). One culture, (*Oscillatoria*), was only tried on Aquil+.

The diatoms, notably *Rhizosolenia setigera* and *Ditylum brightwellii,* formed auxospores in Aquil or Aquil+. *Skeletonema menzellii* (Men 5), a tropical oceanic isolate, grew more rapidly and luxuriantly in Aquil+ than in other media in which it has been grown, but this was not studied quantitatively. The oceanic *Emiliania (Coccolithus) huxleyi* (Clone BT-6), which grew very well in Aquil—even producing motile cells on one occasion—has not grown at all on other synthetic seawaters we have tried, though clones isolated from coastal regions were relatively inexacting. (It has been possible to maintain clone BT-6 on a specially designed rich synthetic seawater; L. Provasoli and I. J. Pintner, pers. comm.)

In general, it appears that physiological experiments can be conducted with all species tested in Aquil. In addition to the species of Table 3, several others (*Thalassiosira pseudonana* clone 3H, *Skeletonema costatum* clone Skel; *Gonyaulax tamarensis* clone Gony; *Thalassiosira fluviatilis,* clone Actin) have been used extensively for trace metal experiments with

TABLE 3. *Phytoplankton species carried through axenic transfers in Aquil or Aquil+: nomenclature according to Parke and Dixon (26).*

Species	Clone[a]	Number of transfers[d] Aquil	Aquil+
BACILLARIOPHYCEAE			
Bacteriastrum sp.	A103	6	—
Corethron sp.	A99	6	—
Ditylum brightwelli West	DB	6	—
Rhizosolenia setigera Brightwell	Rhizo	3	6
Skeletonema menzellii Guillard, Carp. & Reim.	Men5	6	—
Thalassiosira pseudonana (Hust.) Hasle & Heim.	13-1	6	—
DINOPHYCEAE			
Gonyaulax polyedra Stein	GP60e	4	6
Gymnodinium nelsoni Hulburt	GSBL	3	6
Scrippsiella trochoidea (Stein) Loeblich III (ex *Peridinium trochoideum*)	Peri	6	—
Pyrocystis lunula Schütt	Plun	4	6
HAPTOPHYCEAE			
Emiliania huxleyi (Lohm.) Hay & Mohler (ex *Coccolithus huxleyi*)	BT-6	6	—
Unidentified coccolithophore	A-112	6	—
Pavlova sp.	Nep	6	—
Phaeocystis pouchetii (Hariot) Lager.	677-3	6	—
Undetermined flagellate	Θ	6[c]	—
CHRYSOPHYCEAE			
Olisthodiscus luteus Carter[b]	Olisth	4	6
CRYPTOPHYCEAE			
Chroomonas salina (Wislouch) Butcher	3C	6	—
Unidentified cryptomonad	ϕ	3[c]	6[c]
PRASINOPHYCEAE			
Pyramimonas sp.	13-10 Pyr	6	—
CYANOPHYCEAE			
Synechococcus bacillaris Butcher	Syn	5	6
Oscillatoria sp.	Sm24	—	6

[a] Woods Hole Oceanographic Institution designation.
[b] This species has been moved from the Chrysophyceae to the Chloromonadophyceae and the name changed to *Chattonella luteus* by Loeblich and Fine (20).
[c] Cannot use nitrate, so 500μM NH_4Cl supplied.
[d] — = not tested.

Aquil in which they exhibit as high a maximum growth rate as in enriched seawater (3,24,25). From visual inspection, other species also appear to grow fast in Aquil. *Gonyaulax polyedra, Gymnodinium nelsoni* and *E. huxleyi* seem to be exceptions although they were not tested systematically.

Changes of nutrient levels, salinity. For the diatoms, we found that silicic acid limits cell yield in Aquil. A 9-fold increase of the silicic acid concentration (from 1.25×10^{-5}M to 1.13×10^{-4}M) removes this limitation and nitrate then becomes the limiting nutrient. In cultures where a high cell yield is desirable, nitrate can be increased 3-fold (from 1×10^{-4}M to 3×10^{-4}M). These two enrichments of the basic Aquil recipe have negligible effects on the chemistry of the medium, leaving metal speciation and activities virtually unchanged.

For optimum growth, many estuarine phytoplankton species require lower salinities than the 32‰ of SOW. For example, a medium of 26‰ salinity is typically prepared for experiments with *Gonyaulax tamarensis.* Although the resulting change in ionic strength per se has no measurable effect on the trace metal activities, the decrease in Ca^{2+} and Mg^{2+} results in a proportional decrease in the trace metal activities due to the release of EDTA. In such situations, the chemical speciation of the medium should be recalculated.

A freshwater recipe ("Fraquil") similar to Aquil but with a background salt composition following that of the WC medium (16) and of the Chu No. 10 recipe (8) has been used in our laboratories for the study of freshwater algae. However, as discussed by Provasoli and Pintner (28), due to the great variability in the composition of continental waters, one cannot expect to define a general freshwater culturing medium as one can a seawater one. For example, we have found it necessary to modify the ratios of the major salts to that found in the Allen medium (2) in order to grow some species of blue green algae.

Changes of trace metal concentrations. Probably the most common variations of the medium Aquil are in the total concentrations (or the activities) of metals for the purpose of trace metal studies. When the concentration of a given trace metal is modified by addition of the appropriate stock to the medium (in which the metal may have been omitted), the reequilibration of the medium chemistry is not always rapid. For example, the reaction of Cu^{2+} + Ca-EDTA→Cu-EDTA + Ca^{2+} takes a few hours to attain equilibrium (24). This creates no difficulty when the metal additions are made in flasks ca. 24 h before inoculation, but it can lead to large errors when the additions are made to the cultures themselves (3). If direct additions to the cultures are necessary, a solution of metal-EDTA has to be used and the resulting changes in the chelator concentrations of the final solution have to be considered.

When the concentration of one metal is changed, its free ion activity is changed proportionally and other metal activities are left unchanged as long as the chelator concentration remains in great excess of that of the metal. However, when a metal concentration becomes comparable to that of the chelating agent, a "titration" phenomenon takes place in which the metal ion activity rises sharply with small increments of that metal and other metal ion activities also increase due to competition for the complexing ligand. Such systems near the titration region of the chelating agent(s) are poorly buffered with respect to metal ion activities and require a complete equilibrium calculation for each modification of the medium. Use of weaker complexing agents than EDTA permits an increase in the range of metal concentrations in which the activities are buffered. Tris (tris (hydroxmethyl)aminomethane) has been used for this purpose in Cu toxicity experiments (3,32).

When the concentrations of a complexing agent is varied, the activities of all complexed metals vary concomitantly. To modify the activity of only one metal in this manner, it is necessary to alter the total concentrations of all other complexed metals to maintain their activities constant.

EPILOGUE

The medium Aquil described here is not the ultimate phytoplankton culturing medium, but it represents an attempt at quantifying completely the chemistry of the phytoplankton's external milieu for the purpose of physiological studies. In this respect it belongs to a long tradition, started by Miquel (22) who developed an enrichment for natural seawater in 1890, by E. J. Allen (1) who worked with a completely artificial recipe in 1914, and continued by the various investigators whose papers we have quoted. As defined, Aquil is convenient for study of the requirement for, or the toxicity of, metals such as Cu, Zn, Ni, Co, Hg, Pb and Cd, which remain primarily in cationic form in aquatic systems. However, particular organic complexes (e.g., vitamin B_{12} with Co) should be considered. Metals that form stable oxyanions (notably, V, Se, Sn, Cr) are are not particularly well controlled in Aquil (Chelex 100 removes only cations) and their study necessitates other procedures. Aquil may be useful for physiological studies other than those directly related to trace metals because many cellular processes (e.g., nutrient uptake or cell division) may be affected by the trace metal chemistry of the medium.

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