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AN INEXPENSIVE INORGANIC MEDIUM FOR THE MASS CULTIVATION OF FRESHWATER MICROALGAE*

PETER POHL, MANFRED KOHLHASE, SABINE KRAUTWURST and KLAUS-HINNERK BAASCH

Institut für Pharmazeutische Biologie, Universität Kiel, Grasweg 9, D-2300 Kiel, F.R.G.

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Abstract—A new and inexpensive inorganic medium ('DS medium') for the mass cultivation of freshwater blue-green algae (Cyanobacteria) and green algae has been developed. It consists basically of distilled or demineralized water (90%) and seawater (10%) and requires only little addition of pure purchasable chemicals (phosphate, trace elements, if necessary nitrate). No addition of macronutrients (NaCl, $MgCl_2$ or $MgSO_4$, KCl or K_2SO_4, $CaCl_2$) and of boron is required because they are sufficiently provided by the seawater. Decalcified water may also be suitable instead of demineralized water. For the cultivation of green algae, a higher trace element concentration is recommended than for blue-green algae. Because of its low total salt concentration the DS medium is freshwater-like. It is easy to prepare and effects rapid algal growth. It may be of special value for algal mass culture in regions which are close to the sea.

INTRODUCTION

During the last 6 years our laboratory has been carrying out experiments on the axenic mass culture of blue-green algae (Cyanobacteria) and other microalgae [1–4]. Since these studies are now being extended from laboratory scale (20–300 l.) to pilot scale (3000 l.) the costs for the medium will become a major factor. We have therefore initiated investigations for cheap inorganic media for algal mass cultivation. Our previous studies with several microalgae had shown that they are able to grow in mixtures of freshwater and seawater [5]. However, further experiments with several sources of tap water indicated that in such mixtures only freshwater with a relatively low calcium level (less than *ca* 40–80 mg CaO/l.) was useful for algal mass culture (unpublished results). Consequently, we also included water without any calcium, i.e. distilled water. The results appeared so promising that we decided to systematically develop a culture medium on the basis of a distilled water/seawater mixture. This medium is henceforth referred to as 'DS medium' (*d*istilled water plus *s*eawater). It has now been employed successfully in our laboratory for the mass cultivation (250–3000 l.) of microalgae during the past 3 years. We report here on its composition and preparation.

RESULTS

The DS medium is composed of 90% distilled or demineralized water, 10% seawater, $K_2HPO_4 \cdot 3H_2O$ (25 mg/l.), and trace elements. The latter are added as two separate trace element solutions A and B (see Table 1). Furthermore, non-N_2-fixing blue-green and green algae require addition of a nitrogen source. We recommend 300 mg KNO_3 per l. (= 0.03%) [1].

Preparation of trace element solutions A and B

$MnCl_2$, $ZnSO_4$, $CoSO_4$, Na_2MoO_4 and $CuSO_4$ were dissolved in DS trace element solution A as indicated in Table 1A. In order to avoid precipitation, $FeCl_3$ had to be dissolved together with Na_2EDTA as a chelating agent in a separate trace element solution B as indicated in Table 1B. No boron addition appeared to be necessary since seawater contains about 4600 μg boron/l. [6]. A 10% addition of seawater to the DS medium corresponds to a boron concentration of about 460 μg/l. Usually a boron concentration of about 200–500 μg/l. is used in artifical media [7, 8].

The preparation of the DS medium is described in Table 2.

According to analyses carried out in our laboratory, green algae appear to have higher concentrations of trace elements in their biomass than blue-green algae (unpublished). From these data and from cultivation

Table 1. Compositions of the trace element solutions A and B

A: Trace element solution A	Final concentration in 1 l. of DS medium
$MnCl_2 \cdot 4H_2O$: 20 mg/l.	100 μg
$ZnSO_4 \cdot 7H_2O$: 5 mg/l.	25 μg
$CoSO_4 \cdot 7H_2O$: 5 mg/l.	25 μg
$Na_2MoO_4 \cdot 2H_2O$: 5 mg/l.	25 μg
$CuSO_4 \cdot 5H_2O$: 0.5 mg/l.	2.5 μg
H_2O: 1000 ml	
B: Trace element solution B	
$FeCl_3 \cdot 6H_2O$: 200 mg/l.	1 mg
$Na_2EDTA \cdot 2H_2O$: 275 mg/l.	1.375 mg
H_2O: 1000 ml	

* Part 3 in the series "Preparatory Experiments for the Axenic Mass Culture of Microalgae".

experiments with various green algae we recommend to add 20 ml each of the trace element solutions A and B (instead of 5 ml as indicated in Table 2) to 1 l. of DS medium when green algae are to be cultivated.

Approximate ion composition of the DS medium

The ion composition of the DS medium is shown in Table 3. It is calculated from the ion composition of seawater with a salinity of 35–38°/oo [6] and from the compositions of the trace element solutions.

DISCUSSION

The DS medium presented here has been designed specifically for the mass cultivation of microalgae in order to lower the total costs. When these organisms are grown under controlled conditions in larger volumes of artifical medium (for example more than 1000 l.) the costs for the chemicals required for the medium become a major factor. Among these, the macronutrients (NaCl, $MgCl_2$ or $MgSO_4$, KCl or K_2SO_4, $CaCl_2$) can account for more than 50% of the total costs for all chemicals. One main advantage of the DS medium lies in the fact that due to its seawater component (10%) no addition of the above macronutrients is required.

Furthermore, the DS medium has low phosphate and trace element concentrations and does not require the addition of boron because this element is provided in sufficient quantities by the seawater added to the medium (see Table 3). The amount of seawater added to the medium may be varied because all algae so far tested in our laboratory tolerate a rather wide range of seawater addition (5–20%). So it is of minor importance whether seawater with a lower salinity (*ca* 35°/oo) or higher salinity (38–40°/oo) is used. Despite this seawater addition, the DS medium is freshwater-like due to its low total salt concentration and is suitable for the cultivation of freshwater algae. Although the seawater used for the preparation of the DS medium is diluted by a factor of 10, one should still take into consideration whether it is likely to be contaminated with inorganic and/or organic materials such as heavy metals, pesticides etc.

The DS medium leads to rapid algal growth. This has been tested in our laboratory with numerous N_2-fixing and non-N_2-fixing blue-green algae and green algae

Table 2. Preparation of the DS medium*

H_2O (distilled or demineralized)†: 790 ml Seawater (*ca* 35–38°/oo salinity): 100 ml Trace element solution A: 5 ml	Preparation: Mix and autoclave; cool to room temperature
Trace element solution B: 5 ml $K_2HPO_4 \cdot 3H_2O$: 25 mg H_2O (dist. or demin.)†: 100 ml KNO_3 (for non-N_2-fixing microalgae): 300 mg pH: *ca* 7.5	Mix and autoclave; cool to room temperature. Add to the upper solution.

*This medium is recommended for the cultivation of N_2-fixing blue-green algae (Cyanobacteria). For the cultivation of green algae, addition of 20 ml each of the trace element solutions A and B may be more suitable (see remarks in the text).

†According to our experience decalcified water may also be used instead of distilled or demineralized water. This, however, should be tested in each laboratory because of the varying contents of calcium and other ions in the local tap water.

Table 3. Approximate ion composition of the DS medium (1 l.) containing 10% seawater (100 ml). No KNO_3 added

	Seawater* (35–38% salinity) (100 ml)	Trace element solutions A and B (5 ml each) + $K_2HPO_4 \cdot 3H_2O$ (25 mg)
Cl	1900.0–2063 mg	0.43 mg
Na	1072.1–1164 mg	0.005 mg
Mg	135.0–146.6 mg	—
S	90.1–97.8 mg	0.006 mg
Ca	41.0–44.5 mg	—
K	39.8–43.2 mg	8.57 mg
Br	6.7–7.3 mg	—
C	2.8–3.04 mg	—
Sr	0.77–0.84 mg	—
B	0.46–0.5 mg	—
Si	0.3–0.33 mg	—
F	0.13–0.14 mg	—
Ar	0.06–0.065 mg	—
N	0.05–0.054 mg	—
Li	0.017–0.018 mg	—
Rb	0.012–0.013 mg	—
P	0.007–0.0076 mg	3.39 mg
J	0.006–0.0065 mg	—
Ba *ca*	0.003 mg	—
In *ca*	0.002 mg	—
Al *ca*	0.001 mg	—
Fe *ca*	0.001 mg	0.2066 mg
Mo *ca*	0.001 mg	0.0099 mg
Zn *ca*	0.001 mg	0.0057 mg
Cu *ca*	0.0003 mg	0.00064 mg
As *ca*	0.003 mg	—
U *ca*	0.0003 mg	—
Mn *ca*	0.0002 mg	0.0278 mg
Ni *ca*	0.0002 mg	—
Vd *ca*	0.0002 mg	—
Ti *ca*	0.0001 mg	—
Sn *ca*	0.00008 mg	—
Sb *ca*	0.00005 mg	—
Cs *ca*	0.00005 mg	—
Se *ca*	0.00004 mg	—
Y *ca*	0.00003 mg	—
Kr *ca*	0.00003 mg	—
Cd *ca*	0.00001 mg	—
Co *ca*	0.00001 mg	0.00524 mg
Ne *ca*	0.00001 mg	—
W *ca*	0.00001 mg	—
Xe *ca*	0.00001 mg	—

*The data are based on the composition of seawater containing 19 000 mg Cl/l. which corresponds to a salinity of 35°/oo [6]. All ions or elements present in concentrations lower than 0.00001 mg per 100 ml seawater have been omitted. The elements, C (*ca* 0.44 mg), Na (*ca* 0, 17 mg) and N (*ca* 0.1 mg) of Na_2 EDTA $\cdot 2H_2O$ (chelating agent for Fe^{3+}; 1.375 mg/l.) have not been considered in this table.

grown in 250 and 3000 l. bioreactors under axenic conditions [4, 9]. The medium may be of special value for algal mass cultivation in those regions of the world which are close to the sea and where demineralized water (from desalination plants) or decalcified water is available.

Although it is speculative there may be a reason for the good growth of the algae in the DS medium: it contains practically all existing inorganic ions or elements because they are all present in seawater although mostly in very low concentrations.

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