Culture medium optim ·ation for the halotolerant alga *Dunaliella salina*.

Ramses Riad ABDALLA

National Institute of Oceanography and Fisheries, ALEXANDRIA (EGYPT)

Introduction

The unique adaptability of the green alga *Dunaliella* to grow in a wide range of salt concentrations, has made it a favorite subject for detailed physiological and biochemical investigations, aimed at its utility as a source of some fine chemicals such as β-carotene and glycerol or a source of single cell protein for rearing rotifers and brine shrimp, *Artemia*. It is well known that the concentrations and the ratios of the essential nutritive elements, strongly affect algal growth and photosynthetic rates (FISHER *et al* ., 1981). The main target of this work is to maximize *Dunaliella salina* growth with the most economical culture component supply.

Material and methods

The methodology of experimental design, namely, the random balance, the fractional factorial and the steepest ascent designs (SATTERTHWAITE, 1959; COCHRAN and COX, 1957) were used to develop a new synthetic optimized medium for the growth of *Dunaliella salina*. All major cations (Na, K, Mg and Ca) were added as chloride /salts and anions (HCO_3, SO_4, NO_3 and PO_4) as sodium salts. The trace metals (Zn, Mn, Mo, Co, Cu and Fe) were added in chelated form with EDTA. Vitamin B_{12} was added to all media at a level of L µg/l. Culture media were inoculated under sterile conditions with actively growing *Dunaliella salina*, adjusting its initial concentration to 10^4 cell/l. Experiments were performed in triplicates. Cultures were grown in incubator at light intensity 4 k Lux and temperature of 25 ± 1°C. Experiment duration lasted for 9 days. Population density was estimated by cell count on a hemacytometer.

Results and discussion

The random balance design was used first to evaluate the main effect of 15 nutritive elements as mentioned previously plus the effect of H_3BO_3 at 2 levels of concentrations (+1) and (-1), which were chosen to express the highest and the lowest element concentrations used in the known artificial sea water media (ASP-2, ASP-6, ASP-12, ASM and Muller media). Data gained using this design showed that alga was tolerant to a wide range of macroelement concentration changes. Concerning micro- elements and H_3BO_3 the best algal yield was achieved at the levels given in table 2. For optimizing the major cations and anions concentrations in relation to algal yield the 2^{8-4} fractional factorial design was used, where the concentrations of the media on which the alga attained its maximum yield in the previous design was taken as a middle point in defining the (+1) and (-1) levels for this design. Results are given in table 1. After statistical treatment of the data we can conclude that algal yield was only significantly affected by the concentration changes of K, Mg, PO and CO_3. For optimizing their concentrations, a set of experiments was done using the steepest ascent method, where the composition of medium N° 15 on which the algal yield was maximum (table 1) was taken as original point. The highest mean algal yield (18 x10^6 cell/ml) was achieved on growth medium N°8. This is about 9-10 times greater than those recorded in the literature at about the same conditions of cultivation used in our experiments. In conclusion we are recommending a new medium for best *Dunaliella salina* growth as given in table 2.

Table I . 2^{8-4} fractional factorial design and steepest plan.

Factors	NaCl X_1	KCl X_2	$MgCl_2$ X_3	$CaCl_2$ X_4	Na_2SO_4 X_5	$NaHCO_3$ X_6	$NaNO_3$ X_7	NaH_2PO_4 X_8	culture density after 9 days 10^6cell/ml
Level mM/l									
-1 level	1000	8	25	8	15	1.5	2	0.05	
+1 level	2000	16	55	18	25	2.5	5	0.10	
0 level	1500	12	40	13	20	2.0	3.5	0.075	
Variation unit (λ)	500	4	15	5	5	0.5	1.5	0.025	
Experiment									
1	-1	-1	-1	-1	-1	-1	-1	-1	4.39
2	+1	-1	-1	-1	+1	+1	+1	-1	3.51
3	-1	+1	-1	-1	+1	+1	-1	+1	6.86
4	+1	+1	-1	-1	-1	-1	+1	+1	4.56
5	-1	-1	+1	-1	+1	-1	+1	+1	6.97
6	+1	-1	+1	-1	-1	+1	-1	+1	4.61
7	-1	+1	+1	-1	-1	+1	+1	-1	4.63
8	+1	+1	+1	-1	+1	-1	-1	-1	3.47
9	-1	-1	-1	+1	-1	+1	+1	+1	0.28
10	+1	-1	-1	+1	+1	-1	-1	+1	5.09
11	-1	+1	-1	+1	+1	-1	+1	-1	4.44
12	+1	+1	-1	+1	-1	+1	-1	-1	4.64
13	-1	-1	+1	+1	+1	+1	-1	-1	4.07
14	+1	-1	+1	+1	-1	-1	+1	-1	4.59
15	-1	+1	+1	+1	-1	-1	-1	+1	7.66
16	+1	+1	+1	+1	+1	+1	+1	+1	5.17
Regre. coef.(b_i)	-0.23	0.5	0.46	-0.19	0.26	-0.46	-0.41	0.47	
b_i λ		2.0	6.9			-0.23		0.012	
Initial level.	1000	16	55	18	15	1.5	2	0.100	
Level on path.									
1	1000	18	62	18	15	1.25	2	0.112	12.90
2	1000	20	69	18	15	1.00	2	0.124	14.93
3	1000	22	76	18	15	0.75	2	0.136	14.09
4	1000	24	83	18	15	0.50	2	0.148	13.76
5	1000	26	90	18	15	0.25	2	0.160	15.43
6	1000	28	97	18	15	0.00	2	0.172	13.55
7	1000	30	104	18	15	0.00	2	0.184	14.49
8	1000	32	111	18	15	0.00	2	0.169	18.10
9	1000	34	118	18	15	0.00	2	0.208	15.91
10	1000	36	125	18	15	0.00	2	0.220	16.01
11	1000	38	132	18	15	0.00	2	0.232	17.19
12	1000	40	139	18	15	0.00	2	0.244	16.24
13	1000	42	146	18	15	0.00	2	0.256	15.92
14	1000	44	153	18	15	0.00	2	0.268	16.17
15	1000	46	160	18	15	0.00	2	0.280	17.24

Table 2 . Mineral composition of medium No.8.

Salt	NaCl	KCl	$MgCl_2$	$CaCl_2$	Na_2SO_4	$NaNO_3$	NaH_2PO_4
Conc. mM/l	1000	32	111	18	15	2	0.196
Salt	Fe	Zn	Mn	Mo	Co	Cu	H_3BO_3
	2	1	1	6	0.1	0.02	400

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