CHEMICAL ENGINEERING TRANSACTIONS

VOL. 78, 2020

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Jeng Shiun Lim, Nor Alafiza Yunus, Jiří Jaromír Klemeš

ISBN 978-88-95608-76-1; **ISSN** 2283-9216

DOI: 10.3303/CET2078004

Minimising the Cost of *Spirulina platensis* Culture Medium using Vinh Hao Natural Mineral Water

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This paper describes the use of a linear programming method to construct a formula for preparing culture medium for *Spirulina platensis* (*S. platensis*) algae using Vinh Hao natural mineral water (Binh Thuan Province, Vietnam) with a low cost. The results show that the cost of preparing the culture medium using Vinh Hao mineral water is less than 252.49 VND/L, which is approximately 13.05 % less than the cost of Zarrouk medium. To verify the optimised medium composition, *S. platensis* algae was cultured in the two media under the same conditions (25 to 40 °C; pH 8.5 to 10.2). The algae biomass concentration reached 1.0 g/L after 7 d of culturing. The experimental growth rates of *S. platensis* algae in Vinh Hao culture medium and Zarrouk medium had an average difference of 1.49 %. This study provides a basis for implementing *S. platensis* algae production on an industrial scale using media formed from Vinh Hao natural mineral water from Binh Thuan Province, Vietnam.

1. Introduction

The biomass of the Cyanophyta spirulina (*S. platensis*) has high nutritional value and is widely used for food and medicine (Le, 1999). In the presence of sufficient nutrients, including macro-minerals and micro-minerals, *S. platensis* algae synthesise biomass from carbon nutrient sources in the form of bicarbonate ionised HCO_3^- using sunlight energy (Gorbunova, 2013). Previous research has shown that *S. platensis* algae grows well in Zarrouk medium (Kedik et al., 2006). However, *S. platensis* is costly to culture on an industrial scale when using Zarrouk medium due to the cost of the necessary components used to prepare this nutrient medium (Henrikson R., 2010). Seawater enriched with a commercial compound fertilizer (NPK), $NaHCO_3$ and $FeSO_4$ was used as less expensive culture medium for *S. platensis* (Wu et at., 1993). Costa et at. (2004) used water from Mangueira Lagoon (Rio Grande do Sul State, Brazil) as a culture medium for *S. platensis* was low cost. Additionally, the use of wastewater materials (Kamyab et al., 2019) or the effluent from anaerobic digestion (Chaiklahan et al., 2010) for manufacturing algae products. However, these products have implications for food and pharmaceutical safety (Amha, 2008).
To overcome these disadvantages, it is necessary to find a cheaper and cleaner algae culture medium by taking advantage of clean and cheap natural mineral water sources in Vietnam (Trinh et al., 2017). By applying a linear programming method, a formula for preparing an *S. platensis* culture medium with the lowest cost was developed using Vinh Hao natural mineral water. The obtained medium was also assessed by comparing the growth rate of *S. platensis* algae cultured in Vinh Hao medium with that in Zarrouk medium under the same conditions.

2. Experimental methods

2.1 Minimising the cost of preparing the culture medium by using Vinh Hao natural mineral water

Vinh Hao natural mineral water was acquired from the natural water source at Vinh Hao commune, Tuy Phong District, Binh Thuan Province, Vietnam. The water mine code was 711 (depth of 71 m). The water composition is described in Table 1 (Vo, 1998). Zarrouk medium (Zarrouk, 1996) with the component substrates, macronutrients and micronutrients for *S. platensis* is shown in Table 2 and Table 3.

Paper Received: 21/03/2019; Revised: 26/07/2019; Accepted: 02/11/2019
Please cite this article as: Trinh D.V., Nguyen P.T.H., 2020, Minimising the Cost of Spirulina platensis Culture Medium using Vinh Hao Natural Mineral Water, Chemical Engineering Transactions, 78, 19-24 DOI:10.3303/CET2078004

The data in Table 1 and Table 2 indicate that many minerals are needed to compose *S. platensis* algae media using Vinh Hao natural mineral water so that it will have the same mineral content as Zarrouk medium, such as: HCO_3^-, NO_3^-, SO_4^{2-}, Cl^-, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+} and Fe^{2+}. The content of these ions in the mineral water is less than in Zarrouk medium; therefore, supplementation is required.

Table 1: Composition of Vinh Hao natural mineral water

Anion	mg/L	mge/L	Cation	mg/L	mge/L
HCO_3^-	2445.64	40.080	Li^+	0.74	0.107
Cl^-	31.45	0.890	Na^+	905.0	393.65
F^-	8.00	0.421	K^+	32.1	0.821
SO_4^{2-}	1.44	0.030	Ca^{2+}	14.83	0.740
NO_3^-	0.08	0.001	Mg^{2+}	6.08	0.500
PO_4^{3-}	0.21	0.04	Fe^{2+}	0.20	0.007
SiO_3^{2-}	-	-	NH_4^+	0.01	0.001

mge/L = milligrams of ion per litre

Table 2: Composition of Zarrouk medium

Chemical formula	Zarrouk, g/L
$NaHCO_3$	16.8
$NaNO_3$	2.5
NaCl	1.0
K_2SO_4	1.0
K_2HPO_4	0.5
$MgSO_4.7H_2O$	0.2
$CaCl_2.2H_2O$	0.04
$FeSO_4.7H_2O$	0.01
EDTA	0.08
Micronutrient solution 1	1.0 mg/L
Micronutrient solution 2	1.0 mg/L

Table 3: Composition of micronutrient solution

Chemical formula	Micronutrient solution 1, mg/L	Chemical formula	Micronutrient solution 2, mg/L
H_3BO_3	2.86	NH_4VO_3	0.023
$MnCl_2.4H_2O$	1.81	$NiSO_4.7H_2O$	0.048
$ZnSO_4.7H_2O$	0.22	Na_2WO_4	0.018
$CuSO_4.5H_2O$	0.08	$Ti_2(SO_4)_3$	0.040
MoO_3	0.01	$Co(NO_3)_2.6H_2O$	0.044

The problem of minimising the cost of the culture medium using Vinh Hao mineral water is set out as follows: the mineral content of the culture medium prepared with the natural mineral water must be equivalent to Zarrouk medium but at a lower cost.
The mathematical model of the problem is described as Eq(1):

$$Minf(X) = min(c_1x_1 + c_2x_2 + \cdots c_nx_n) = min\sum_{i=1}^{8} c_ix_i \tag{1}$$

where x_i corresponds to the weight of 8 substances to be added (g/L): $NaHCO_3$, $NaNO_3$, NaCl, K_2SO_4, K_2HPO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$ and $CaCl_2.6H_2O$, and c_i are the prices of these substances (VND/g).
The amount of each mineral in the medium prepared from Vinh Hao natural mineral water cannot be smaller than the amount available in Zarrouk medium; this condition is shown as Eq(2):

$$\begin{cases} \sum_{i=1}^{8} a_{ji}x_i \geq b_j \\ x_i \geq 0 \quad i = 1 - 8 \end{cases} \tag{2}$$

where a_{ji} is the ratio between the molecular weight of ion j (M_j) and the molecular weight of compound i (M_i), and the amount is added x_i (shown in Table 4 and Table 5); b_j is the amount to be added to Vinh Hao mineral water and is described as Eq(3). Therefore, the culture medium has an amount of mineral j that not less than that in Zarrouk medium.

$$b_j = m_{j(Zar.)} - m_{j(V.H)} \quad (3)$$

The ratio of a_{ji} mineral composition of 10 minerals (HCO_3^-, NO_3^-, Na^+, K^+, HPO_4^{2-}, SO_4^{2-}, Cl^-, Ca^{2+}, Mg^{2+}, Fe^{2+}) from 8 basic compounds, which are shown in Table 4, is determined as Eq(4):

$$a_{ji} = \frac{M_j}{M_i} \quad (4)$$

As shown in Table 6: $M_{HCO_3^-} = 61$; $M_{NaHCO_3} = 84$; $a_{11} = \frac{M_{HCO_3^-}}{M_{NaHCO_3}} = \frac{61}{84} = 0.7262$

$m_{j(Zar.)}$ is the content of mineral *j* in Zarrouk medium and is calculated as Eq(5):

$$m_{j(Zar.)} = \sum_{i=1}^{8} a_{ji}.x_j \quad (5)$$

$m_{j(V.H)}$ is the content of mineral *j* in Vinh Hao natural mineral water and is calculated as Eq(6):

$$m_{j(V.H)} = \sum_{i=1}^{8} a_{ji}.x_j \quad (6)$$

Table 4: Molecular weights of substances used in modelling and experimentation

Chemical formula	$NaHCO_3$	$NaNO_3$	NaCl	$K_2HPO_4.3H_2O$	K_2SO_4	$CaCl_2.6H_2O$	$MgSO_4.7H_2O$	$FeSO_4.7H_2O$
M_i. g/mol	84	85	58.5	228	174	219	246	278

Table 5: Molecular weight of minerals used in modelling and experimentation

Chemical formula	HCO_3^-	NO_3^-	Na^+	K^+	HPO_4^{2-}	SO_4^{2-}	Cl^-	Ca^{2+}	Mg^{2+}	Fe^{2+}
M_j. g/mol	61	62	23	39	96	96	35.5	40	24	56

2.2 Experimental verification with optimised medium

To evaluate the results of the mathematical model, two similar samples of *S. platensis* algae were experimentally cultivated in the optimised medium and in Zarrouk medium. The experiment was conducted with the following tools: precision balance; Erlenmeyer flasks (500 mL and 250 mL); volumetric flasks (500 mL and 100 mL); measuring pipettes (10 mL and 2 mL); measuring cylinders (250 mL and 10 mL).
Other tools: alcohol thermometer, measure scale 0 - 100 °C (France); pH meter (Hanna, Model HI98172); Beckman Coulter DU 750 spectrophotometer (USA), measuring at 750 nm.
The concentration of *S. platensis* algae is calculated with Eq(7):

$$C = k.OD_{750} \quad (7)$$

where k is the conversion factor for *S. platensis*; k = 0.73.
The *S. platensis* algae used in the experiment was purchased from the experimental biology room, Research Institute for Aquaculture No. 2, No. 116 Nguyen Dinh Chieu St., Da Kao Ward, District 1, Ho Chi Minh City.
The experimental verification for the optimal Vinh Hao natural mineral water media was carried out as follows. Two parallel experimental samples of nutrient medium were prepared according to the optimised results using Vinh Hao natural mineral water (S_1 and S_2) and a sample using Zarrouk medium (Table 6). Every hour throughout the day, the following parameters were measured: temperature of the air and of the algae culture medium, pH of the medium, and the optical density using a spectrophotometer at a wavelength of 750 nm (OD_{750}). The results were analysed by analysis of variance (ANOVA) with a confidence probability greater than 95% ($p < 0.05$).

Table 6: S. platensis *algae cultures for comparison of the optimised medium and Zarrouk medium*

Medium	S_1	S_2	Zarrouk
Vinh Hao medium, mL	150	150	0
Zarrouk medium, mL	0	0	150
S. platensis (1 g/L), mL	15	15	15

3. Results and discussion

3.1 Minimising the cost of preparing the algae culture medium

To minimise the cost of the algae medium, (Eq(1) was applied, and the coefficients a_{ji} *and* b_j were calculated according to Eq(3) to Eq(6) using the data in Table 4 andTable 5. The results are presented in Table 7.

Table 7: Calculation results of a_{ji} matrix coefficient components, vector b_j

Substance	$NaHCO_3$ a_{j1}	$NaNO_3$ a_{j2}	$K_2HPO_4.3H_2O$ a_{j3}	K_2SO_4 a_{j4}	NaCl a_{j5}	$MgSO_4.7H_2O$ a_{j6}	$FeSO_4.7H_2O$ a_{j7}	$CaCl_2.6H_2O$ a_{j8}	b_j
HCO_3^-	0.7262	0	0	0	0	0	0	0	9.7544
NO_3^-	0	0.7294	0	0	0	0	0	0	1.8234
Na^+	0.2738	0.2706	0	0	0.3932	0	0	0	4.7646
K^+	0	0	0.3421	0.4483	0	0	0	0	0.5872
HPO_4^{2-}	0	0	0.4211	0	0	0	0	0	0.2103
SO_4^{2-}	0	0	0	0.5517	0	0.3902	0.0007	0	0.6283
Cl^-	0	0	0	0	0.6068	0	0	0.3242	0.5882
Ca^{2+}	0	0	0	0	0	0	0	0.1826	0.0148
Mg^{2+}	0	0	0	0	0	0.0976	0	0	0.0134
Fe^{2+}	0	0	0	0	0	0	0.0397	0	0.0002

Cost minimisation was achieved through a simplex algorithm, calculated in Microsoft Excel (Trinh, 2016), shown in Table 8. The prices of the components were provided by South Chemical Import Export Joint Stock Company (SOUTHCHIMEX JSC; Vietnam). The results in Table 8 show that preparing the medium with Vinh Hao natural mineral water will achieve a cost reduction of 252.59 VND (13.05 %) compared with Zarrouk medium.

Table 8: Minimising the cost of preparing S. platensis *culture medium using Vinh Hao natural mineral water*

Chemical formula	$NaHCO_3$	$NaNO_3$	NaCl	$K_2HPO_4.3H_2O$	K_2SO_4	$MgSO_4.7H_2O$	$FeSO_4.7H_2O$	$CaCl_2.6H_2O$	$\sum c_i x_i \rightarrow Min$
x_i, g/L	13.43	2.50	1.04	0.50	0.93	0.30	0.00	0.08	1,682.71
Zarrouk, g/L	16.80	2.50	1.00	0.50	1.00	0.20	0.01	0.04	1,935.3
Price c_i, VND/g	76	150	62	104	148	90	70	70	Δ=252.59

3.2 Changes in temperature and pH of the culture medium

The temperature of the *S. platensis* culture medium was taken hourly, from 5:00 am (at sunrise) to 18:00 (at sunset). The amount of sun received was highly dependent on the weather, which affected the temperature. The average temperatures of the culture medium are shown in Table 9 and included 1 rainy day, 5 sunny days and 2 shady days.

Table 9: Change in the average temperature of the culture medium (Binh Thanh District, HCM City)

Day	τ (h)	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Rain		25.0	27.0	33.0	34.0	34.0	35.0	33.0	34.5	35.5	38.0	39.0	40.0	37.5	28.0
Shade	T (°C)	24.0	26.0	28.0	32.0	36.0	37.0	37.5	37.0	35.0	36.0	34.5	31.0	31.5	29.0
Sun		25.0	30.0	35.5	37.0	36.5	40.0	39.0	38.5	40.0	42.0	37.5	38.0	36.0	30.0

The temperature increased rapidly from 6:00 am to 8:00 am, while from 8:00 am to 16:00 the temperature changed more slowly. On sunny days (and even rainy days), the temperature of the medium exceeded the optimal temperature range for *S. platensis* algae (30 to 38 °C) and reached dangerous temperatures (above 40 °C), but only for a short time.

Changes in the pH of two parallel experimental samples (S1 and S2) and a sample with Zarrouk medium are described in Table 10.These results show that the pH of all three samples varied similarly. They gradually increased from 8.5 to 10.2, which is within the suitable pH range for *S. platensis*; the photosynthesis of *S. platensis* produces OH^- ions that raise the pH, described as Eq(8):

$$n.HCO_3^- + n.H_2O + h.\nu \rightarrow (CH_2O)_n + n.OH^- + n.O_2 \quad (8)$$

where n is the number of reacted ions, h is the Planck constant and ν is the light frequency.

Table 10: pH changes in the culture medium

Day	S_1	S_2	Zarrouk
1	8.50	8.55	8.50
2	8.70	8.80	8.70
3	9.20	9.30	9.30
4	9.45	9.50	9.60
5	9.60	9.65	9.70
6	9.70	9.80	9.80
7	9.90	9.95	9.90
8	10.15	10.25	10.25

3.3 Biomass growth rate

Figure 1 shows that the biomass growth rate of *S. platensis* algae in Vinh Hao medium was similar to that in Zarrouk medium. The biomass increased throughout the first 7 d, but on the 8th day it decreased slightly.

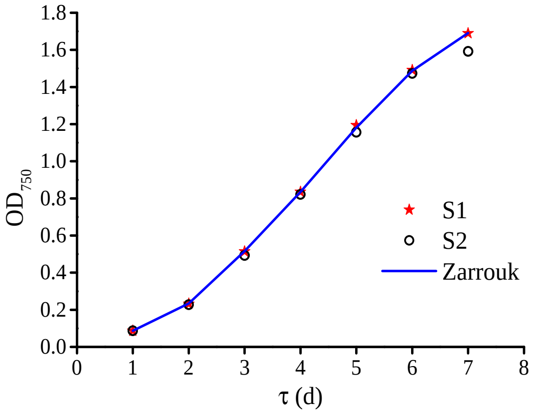

Figure 1: Biomass growth rate

The growth time of *S. platensis* algae is 7 d, including the lag, log, and stationary phases. On the 8th day, the equilibrium phase is reached. At this point, the algae begin to decline (OD_{750} is reduced) because the nutrients in the medium are gone and the pH has increased to greater than 10.5. The algae become inhibited, and they grow slowly and begin die. *S. platensis* algae can be harvested on the 7th day, when their concentration (calculated by Eq(7)) is 1.161 g/L, which is greater than 1 g/L.

3.4 Comparison of the optimised and Zarrouk media

The optical densities at OD_{750} of Vinh Hao and Zarrouk medium are shown in Figure 2.

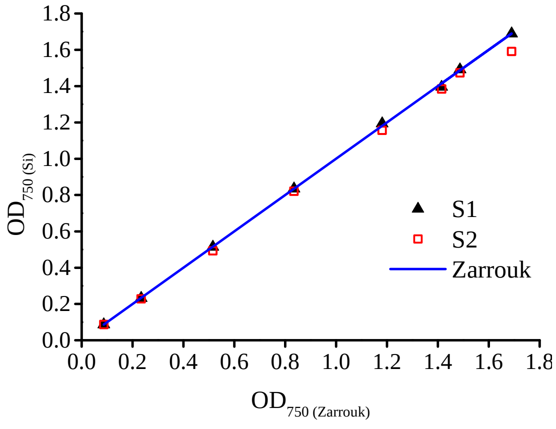

Figure 2: Optical density (OD_{750}) of Vinh Hao and Zarrouk media

The average relative deviation between $OD_{750\,(Si)}$ and $OD_{750\,(Zar.)}$ ($\varepsilon_{average}$) is calculated as Eq(9):

$$\varepsilon_{average} = \frac{1}{16}\left(\sum_{l=1}^{8}\frac{|S_{l1}-Z_l|}{Z_l} + \sum_{l=1}^{8}\frac{|S_{l2}-Z_l|}{Z_l}\right).100\% = 1.49\% \quad (9)$$

4. Conclusions

By a linear programming method, the formula for preparing an *S. platensis* culture medium using Vinh Hao natural mineral water was determined. The cost of preparation according to this formula was less than 252.49 VND/L, approximately 13.05 % less than the cost of Zarrouk medium. The culture growth results are shown in Figure 2. The obtained formula was evaluated by experimentally cultivating *S. platensis* in the Vinh Hao and Zarrouk media under the same conditions (25–40 °C; pH 8.5–10.2). The biomass growth rate of *S. platensis* algae in two media were equivalent to each other, reaching 1.0 g/L after 7 d. The average relative deviation of the biomass growth rate (OD_{750}) in these media was 1.49 %, indicating the reliability of the results. This study provides a basis for furthering algae culturing technology using Vinh Hao natural mineral water, which has a low cost in Vietnam.

Acknowledgments

This research was funded by the Vietnam National University, Ho Chi Minh City (VNU-HCMC) under grant number B2019-20-03.

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