RESPIRATORY ACTIVITIES OF *CHLORELLA ELLIPSOIDEA* IN VARIOUS NUTRIENT MEDIA

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Abstract

The respiratory activities of *Chlorella ellipsoidea* in the following nutrient media of algae: Bold's Basal Medium & modification (BBM), 5N-Bold's Basal Medium (5N-BBM), Kantz' modified BBM (KBBM) and Ott's were studied by Warburg technique. The O_2 uptake was found to be heighest in BBM with decreasing order in 5N-BBM, KBBM, and Ott's medium. The higher respiratory activity in BBM may be attributed to its nitrate components. The decreased respiration in KBBM is related to its lower potassium and phosphate contents. No growth took place in Ott's medium.

Introduction

Although most strains of *Chlorella* are able to grow in media whose compositions vary to a great extent (KUHL and LORENZEN 1964), we found that the population density of fresh-water algae in a batch system in small vessels depends on cultural conditions (TSCHEN and YIE 1971). A further study of respiratory activities of algae in different media may be helpful in selecting the optimal cultural media for the fresh-water algae.

In this experiment, *Chlorella elliposidea* was cultured in various nutrient media, and the respiratory activity of the algae was determined by using a Warburg respirometer.

Material and Methods

500 ml Erlenmeyer flasks were used as culture flasks. 300 ml of Chu's medium (**Table I**) were introduced into the flasks and each flask was fitted with a bubbler tube extending into the medium. The top ends of each bubbler tube were stoppered with cotton. The flasks were autoclaved and then cooled to room temperature. A pure culture of *Chlorella ellipsidea* was added to the

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medium and the culture flasks were placed in a greenhouse, 5% CO_2 (in air) which had been filtered and humidified was passed through each bubbler tube. The average temperature of greenhouse was 25°C, and the *Chlorella ellipsoidea* grew under nonsynchronous conditions.

Table I. *Compositions of Chu's medium and Ott's medium.*

Compounds	Chu's medium[2] (g/liter)	Ott's medium	
		Stock solution mg/100 ml	Amount of stock solution to be used[3]
$Ca(NO_3)_2$	0.04	—	—
K_2HPO_4	0.005	—	—
$MgSO_4\cdot 7H_2O$	0.025	10	60
Na_2SiO_3	0.025	1	1
$FeCl_3$	0.0008	—	—
Na_2CO_3	0.02	—	—
NaCl	—	25	85
$MgCl_2\cdot 6H_2O$	—	10	50
$CaCl_2\cdot 2H_2O$	—	10	10
KCl	—	10	8
NaBr	—	1	10
$NaHCO_3$	—	1	20
H_3BO_3	—	1	6
$Sr(NO_3)_2$	—	1	3
$NaNO_3$	—	1	20
Na_2HPO_4	—	1	2
Trace element[1]	+	+	+

(1) EDTA stock solution: 50 g EDTA+31 g KOH/1 deionized water. Iron stock solution: 49.8 g $FeSO_4\cdot H_2O$/1 deionized water. Boron stock solution: 11.24 g H_3BO_3/1 deionized water. Miscellaneous stock solution (g/1 acidified water): 8.82 g $ZnSO_4\cdot 7H_2O$, 1.44 g M_0O_3, 0.71 g $CuSO_4\cdot 5H_2O$, 1.57 g $CO(NO_3)_2\cdot 6H_2O$. add 4 ml of 4 kinds of stock solution (each one 1 ml) to the medium.
(2) pH=7.0.
(3) Add distilled water to 1000 ml, pH=7.6.

After two weeks, the cells were harvested by centrifugation. 100 ml solutions of the *Chlorella* culture were centrifuged at 3,000×g in two tubes for 15 minutes. After centrifugation, the nutrient solution was discarded. The precipitated cells in one tube was dried in an oven at 100°C for 5 hrs. to obtain dry weight and those in the other tube was suspend in 10 ml of dish H_2O to assay for respiration.

Gas exchange was measured by the manometric technique (UMBREIT *et al.* 1964). All experiments were carried out in a dark room at 30°C. In a Warburg Apparatus (MRK, Japan), each flask contained 2 ml of medium (The tested media were Bold's Basal Medium & modification (BBM), Kantz' modified BBM

(KBBM), 5N-Bold's Basal Medium (5N-BBM) (Bold 1967), Ott's; see Table I and II) 1 ml *Chlorella* suspension (in water) was placed in main compartment of Warburg flask and 0.2 ml of 20% KOH in center well. The average dry weight of *Chlorella* per flask was 5.5 mg and total volume is 3.2 ml. The test on each type of medium was repeated three times.

Table II. *Compositions of medium of BBM, KBBM, and 5N-BBM.*

	BBM(1) g/400 ml (stock soluton)	KBBM(3) (stock solution) g/400 ml	5N-BBM(1) (stock solution) g/400 ml
$NaNO_3$	10	10	—
$CaCl_2 \cdot 2H_2O$	1	1	1
K_2HPO_4	3	3	3
KH_2PO_4	7	—	7
$MgSO_4 \cdot 7H_2O$	3	3	3
NaCl	1	1	1
Tris	—	12.5	—
ES(2)	—	+	—
$(NH_2)_2CO$	—	—	50
Trace element	+	+	+

(1) each trace element 1 ml+10 ml of each different stock solution +940 ml H_2O pH= 6.3.
(2) Erdschreiber solution (ES): 0.1 mg vitamin mixture, 0.1 mg biotin, 20 mg thiamine /HCl, 100 ml distilled water, two drops of vitamin B_{12} (conc. mg/ml).
(3) 10 ml ES+5 ml of each stock solution+900 ml distilled water, make the volume to 996 ml, pH=7.5, and add the trace element.

Results and Discussion

When algae are cultured in a good medium, their metabolism is expected to be active and their respiration rate high. In **Table III** and **Table IV**, their respiration rates in various media were compared with those in H_2O. When the relative efficiency in H_2O is 100%, that in BBM is 404%, 5N-BBM 389%, KBBM 275%, but only 91% in Ott's medium. The time courses of O_2 uptake were shown in Fig. 1 and 2. Thus it seems that BBM and 5N-BBM are

Table III. *The respiration rate of* ***Chlorella ellipsoidea*** *in H_2O, BBM and KBBM.* (μl $O_2/100$ *mg dry wt/hr*)

Exp. No.	H_2O	BBM	KBBM
1	15.35	50.83	40.80
2	12.60	48.90	34.99
3	15.43	67.20	44.73
Total efficiency	100%	404%	275%

Table IV. *The respiration rate of* ***Chlorella ellipsoidda*** *in H_2O, 5N-BBM and Ott's medium.* (μl O_2/100 *mg dry wt/hr*)

Exp. No.	H_2O	5N-BBM	Ott's
4	28.45	110.2	24.43
5	2.00	88.1	23.20
6	23.30	98.5	22.10
Total efficiency	100%	389%	91%

excellent media for *C. ellipsoides.* No growth took place in Ott's medium. The results are in good agreement with those of population studies (Tschen and Yie 1971).

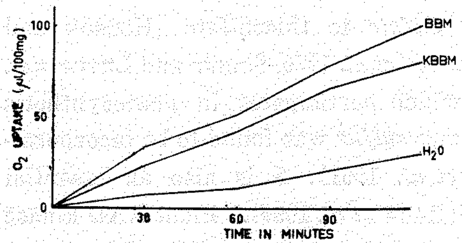

Fig. 1: Respiration curve of *Chlorella ellipsoidea* in H_2O and in media (BBM and KBBM).

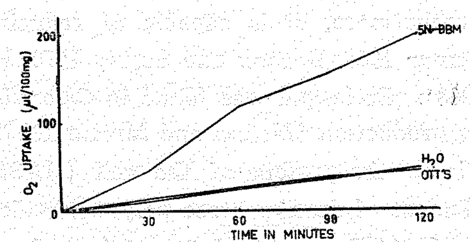

Fig. 2: Respiration curve of *Chlorella ellipsoidea* in H_2O and in other media (5N-BBM and Ott's).

Nitrogen is the most important element for *Chlorella ellipsoidea* growth. It is found in molecules such as porphyrins, some coenzymes, purines, pyrimidines and amino acids (Chorin-Kirsh and Mayer 1964, Hattori 1957, Yamamoto 1965). The phorphyrin structure is found in chlorophyll and cytochrome enzymes (Bogorad 1966, Iveish 1936). In other words, nitrogen is essential in respiration and photosynthesis and in the synthesis of purines and pyrimidines of RNA and DNA which are essential for protein synthesis. In BBM nitrogen source in $NaNO_3$ while in 5N-BBM urea is used in place of $NaNO_3$. Many studies have shown that urease was not detected in *Chlorella ellipsoidea* (Hattori 1957). So urea can not be hydrolyzed to CO_2 and NH_3 which is the essential compound to be utilized by plant. This should be the reason why BBM is superior to 5N-BBM for *Chlorellaella ellipsoidea* culture.

Potassium is accumulated in the cytoplasm of *Chlorella* mainly by diffusion (Schaedle and Jacobson 1964). It is essential an element as an activator for enzymes in certain peptide bond synthesis, and thus potassium may affect the respiration and photosynthesis (Nason and McElroy 1963). *Phosphorus* is found in many important coenzymes such as ATP, ADP, and NADP. These coenzymes are very important in photosynthesis, glycolysis, TCA cycle and fatty acid synthesis. Furthermore, ATP is very important to chloroplasts (BALDRY *et al.* 1966, BUCKE *et al.* 1966, HATTORI 1957). Therefore, Phosphorus is a very important element to *Chlorella ellipsoidea.* Phosphorus and potassium content in KBBM are less than those in BBM and 5N-BBM. This may be the reason why the respiration rate in KBBM is less than that in BBM and 5N-BBM.

There is a high *vitamin* content in *Chlorella* sp. (MORIMURA 1957). When *Chlorella* sp. grow in a greenhouse, cells carry out photosynthesis with enough trace elements, vitamins should be produced. In KBBM there are enough trace elements for *Chlorella ellipsoidea* to induce vitamins synthesis. Therefore, adding vitamins to the cultured algae is not necessary.

Chlorella is known to utilize only thiosulfate (HODSON and SCHIFF 1968). Furthermore, it is capable of reducing *sulfate* to thiosulfate (HODSON and SCHIFF 1968, HODSON and SCHIFF 1970, HODSON *et al.* 1970, SCHIFF and LEVINTHAL 1968). Sulfolipid was found in *Chlorella* which participates in photosynthetic O_2 production (MIYCHI and MIYCHI 1965), and *sulfur* was found to be incorporated into the protein of the cell (HASE *et al.* 1961); S is also an essential element of deoxypentose polynucleotides (HASE *et al.* 1959). Plant NAD kinase needs ATP and Mg^{++}, to catalyze formation of NADP from NAD and the concentration of Mg^{++} required to produce maximum activity is 10^{-2}M; Co^{++} or Mn^{++} could replace Mg^{++} in this system (YAMAMOTO 1965). Study of *Chlamydomonas snowiae* revealed that the ATPase is affected by both Mg^{++} and Ca^{++} (CHORINKIRSM and MAYER 1964); Mg^{++} is very important element in chlorophyll synthesis (BOGORAD 1966) and its defficiency leads to chlorosis in *Chlorella* (EQSTER *et al.* 1958). Cu^{++} plays a role as function metal in the NADPH-plastocyanin reductase (KATOH 1961) and serves an important function in photosynthesis (NEISH 1939). Thus all of these ions play important roles of *Chlorella ellipsoidea* growth.

Ott's medium contains so many ions, yet the fresh-water algae do not grow well in it, only an effieiency of 91% can be measured.

Acknowledgments

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綠藻營養生理之研究

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在臺中附近，採集之薄膜細肋球藻（*Chlorella ellipsoidea*）經人工繁殖後使用各種藻類培養液：Bold's Basal Medium & Modification (BBM) 5N-Bold's Basal's Medium (5N-BBM), Kantz' modified BBM (KBBM) 和 Ott's Medium 等，以 Warburg 氏呼吸器測定該綠藻在短期間培養之呼吸量。

試驗結果顯示：*C. ellipsoidea* 在 BBM 培養期間呼吸量爲最高，其次爲 5N-BBM 和 KBBM，而以 Ott's 爲最低。該綠藻在短期間培養之下，利用 5N-BBM 培養液中所含尿素爲氮源，不如 BBM 中所含之硝酸鈉有效。由於 KBBM 培養液中，所含鉀和磷成份不高，而影響該綠藻之呼吸作用。Ott's 培養液中含鹽份很高，故不適合一般淡水藻類之生長。

C. ellipsoidea 在不同培養液中新陳代謝強度差異之反應，依試驗結果與藻類營養生理觀點討論之。

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