Optimizing of Culture Medium for photosynthetic bacteria (PSB)

Author: Mohammad Arif Ahsas,

lecturer at the department of Biology, faculty of education, Ghor Institute of Higher Education, Ghor province, Afghanistan.

Abstract

Photosynthetic bacteria (PSB) have various applications but the culture cost is very expensive. To find an economic and efficient culture medium the effects of yeast extract, C/N ratio, and trace elements on the growth of PSB were highly studied. The results showed which the optimal condition for PSB growth was:

- *yeast extract of 100mg/l*
- *C/N of 12:1*
- *trace elements (in mol/L)*
  - *Mn2+ (0.009)*
  - *Fe3+ (0.0025)*
  - *Co2+ (0.0024)*
  - *Cu2 + (0.0024)*
  - *Zn2 + (0.0033).*

The trace element shortage could affect the growth of PSB. The order was Mn2 + > Fe3 + > Co2+ > Cu2+ > Zn2+.

The improved medium was named HCH, and the optimum medium components were (in g/l):

- *DL-malic acid: (4)*
- *MgSO4: (0.12)*
- *(NH4)2 SO4: (1)*
- *CaCl2: (0.075)*
- *KH2PO4: (0.5)*
- *K2HPO4: (0.3)*
- *Na2EDTA: (0.02)*
- *yeast extract: (0.1)*

Trace elements 1ml (in mol/l): Fe3+: (0.0025), Mn2+: (0.009), Zn2+: (0.0033), Co2+: (0.0024). pH: 6.8. Comparing with the traditional RCVBN medium, in HCH medium yield of PSB increased 1.2 times and the cost decreased 19.8 times.

Key Words:- *PSB ; Culture medium ; Optimization*

Introduction

Photosynthetic bacteria (PSB) contain abundant of nutrients [1, 2] and can be used as the supplement in medicine, cultivation, food industry and cosmetic [3, 4]. PSB have been widely used to treat food, manure, leather, starch, and soybean wastewater since 1960s [5]. The main PSB culture medium is RCVBN medium [6, 7], which is mainly used for rich, separation and preservation of bacteria. The disadvantage of use RCVBN medium culture PSB is that the PSB growth is slow and the cost is very high [8, 9]. In RCVBN medium one composition is biotin. The cost of biotin is very high and achieved 70% of RCVBN medium. Therefore, it is essential to find a new culture medium. In this paper we studied the effect of trace element, yeast extract, carbon-nitrogen ratio and the improved medium on the growth of PSB. The purpose is to culture PSB efficiently and economically.

Photosynthetic bacteria (PSB)

The most striking and common property of all purple and green phototrophic bacteria is their ability to carry out anoxygenic photosynthesis on the basis of bacteriochlorophyll mediated processes. The various anoxygenic phototrophic bacteria contain several types of bacteriochlorophylls and a variety of carotenoids as pigments, which function in the transformation of light into chemical energy and give cultures a distinct coloration varying with the pigment content from various shades of green, yellowish-green, brownish-green, brown, brownish-red, red, pink, purple, and purple-violet to even blue (carotenoidless mutants of some species containing maxima of bacteriochlorophylls and major carotenoid groups of anoxygenic phototrophic bacteria.

Photosynthesis in anoxygenic phototrophic bacteria depends on oxygen-deficient conditions, because synthesis of the photosynthetic pigments is repressed by oxygen (bacteria like *Erythrobacter longus* are exceptions to this rule). Unlike cyanobacteria (including *Prochloron* and related forms) and eukaryotic algae, anoxygenic phototrophic bacteria are unable to use water as an electron donor and oxygen is not produced. They use only one photosystem and require electron donors of lower redox potential than water. Most characteristically, sulfide and other reduced sulfur small organic molecules, are used as photosynthetic electron donors. Quite recently even growth with reduced iron as electron donor has been demonstrated with some phototrophic purple bacteria.

Besides this common theme of photosynthesis, anoxygenic phototrophic bacteria are an extremely heterogeneous eubacterial group, on the basis of morphological, physiological and molecular data. According to their phenotypic properties we distinguish between the green sulfur bacteria, the green nonsulfur bacteria, the purple sulfur bacteria, the purplenonsulfur bacteria and the heliobacteria. On the basis of 16S rRNA analyses major eubacterialgroups have been defined (Woese *et al*., 1985a). Among these, one is represented by the cyanobacteria, one by the green sulfur bacteria, one by *Chloroflexus* and 'relatives', and one by the phototrophic purple bacteria and their 'relatives'. On the basis of their 16S rRNA, the recently discovered *Heliobacteriumchlorum* and *Heliobacillusmobilis* do not fit into the aforementioned groups, but are related to certain Gram-positive bacteria.

A quite remarkable group of bacteria, containing bacteriochlorophyll, but unable to grow phototrophically under anaerobic conditions, is represented by a number of Gram-negative aerobic marine bacteria. The best studied of these bacteria is *Erythrobacter longus* which can synthesize bacteriochlorophyll *a*, form intracytoplasmic membranes, and has reaction center complexes similar to those of other purple bacteri. In contrast to all previously known phototrophic purple bacteria, synthesis of bacteriochlorophyll ***a*** and carotenoids in aerobic phototrophs is stimulated by oxygen. It was demonstrated that *Roseobacterdenitrificans* (formerly designated as *Erythrobacter* strain OCH 114) effectively uses light to increase the cellular ATP level and the rate of incorporation of CO_2.

According to 16S rRNA analyses, *Erythrobacterlongus* belongs to the alpha subgroup of the Proteobacteria, and appears distantly related to other bacteria of this group, such as *Rhodobacter* and *Rhodopseudomonas* species. These bacteria and also a recently discovered bacteriochlorophyll containing *Rhizobium* sp. (Evans *et al*., 1990).

Materials and Methods

The bacteria which were obtained, the characteristic absorption of PSB was 660nm.The relationship between PSB dry weight and OD660 was: biomass= 850.11* OD660. Artificial soybean wastewater was stimulated with soybean milk and the COD (chemical oxygen demand) was around 8300 mg/L. The RCVBN medium formula was shown in Table 1. Modified RCVBN medium without biotin was used unless stated otherwise. The carbon (C) source was DL-malic acid and the nitrogen source was ammonium sulfate. Five trace elements [10] Fe3+ (0.0025 mol/L), Mn2+ (0.009 mol/L), Zn2+ (0.0033 mol/L), Co2+ (0.0024 mol/L), Cu2+ (0.0024 mol/L) were added into the modified RCVBN medium as control. For trace element lack test, each time one element was missing from the medium. The medium was added into a 50ml flask, which was bandaged with 8 layers of gauze and sterilized in 121°C for 20 min. 80mg/l PSB were inoculated into medium and cultured under 30°C, 120 r/min, and natural light.

COD was measured by a COD detector; trace elements were measured by an Inductive Coupled Plasma Emission Spectrometer (ICP).

Results and Discussion

Effect of C/N on PSB growth Fig. 1 showed the effect of C/N on the growth of PSB. Clearly, the growth of PSB under different C/N ratio had a similar trend and reached the peak after 1 d. The best C/N was12.0.

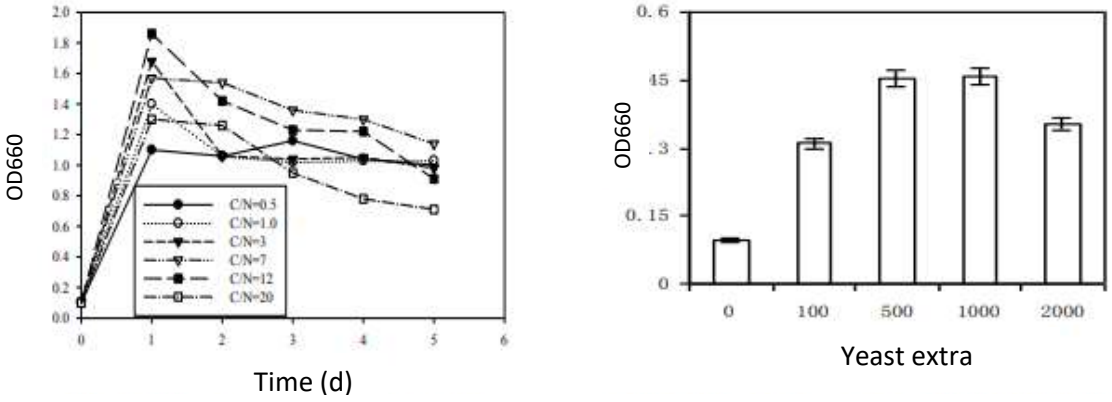

Fig. 1 Effect of C/N on PSB growth Fig. 2 Effect of yeast extract on PSB growth, 2 d Effect of yeast extract on PSB growth Fig. 2 showed clearly that the growth of PSB was enhanced significantly by adding yeast extract. But the enhancement diminished when yeast extract was over 500 mg/l. Considering both cost and PSB growth, the optimum concentration of yeast extract was 100mg/l.

HCH medium compared with RCVBN medium

On the basis of above findings, a new culturing medium named HCH medium was proposed and the compositions were shown in Table 2. PSB growth in HCH medium was compared with RCVBN medium. In these two mediums PSB growth had a similar trend: rapid growth for 2 days and then recession. The maximal OD660 in HCH medium was 1.8, which was 1.2 times of that RCVBN medium (1.5).

Furthermore, costs of these two mediums were compared (Tables 1 and 2). The cost for RCVBN medium was 1370 Yuan/t while the cost for HCH medium was only 65.8 Yuan/ t, showing a decrease of 19.8 times.

Item	Weight (g)	Unit price (Yuan/t)	Total price (Yuan/t)
DL-malic acid	4	6000	24
MgSO4	0.12	350	3.25
(NH4)2 SO4	1	900	9
CaCl2	0.075	1200	0.09
Niacin	0.001	24/25g	0.96
T.M reserve liquid	1 ml		7.397
KH2PO4	0.5	6500	3.25
K2HPO4	0.3	4000	1.2
Na2EDTA	0.02	22500	0.05
VB1	0.001	15/5g	3
Biotin	0.015	87.85/g	1318
Total			1370

Table 1 Composition and cost of RCVBN medium

Table 2 Composition and cost of HCH medium

Item	Weight(g)	Unit price (Yuan/t)	Total price (Yuan/t)
DL-malic acid	4	6000	24
MgSO4	0.12	350	3.25
(NH4)2 SO4	1	900	9
CaCl2	0.075	1200	0.09
Trace Elements	1ml		23
KH2PO4	0.5	6500	3.25

K2HPO4	0.3	4000	1.2
Na2EDTA	0.02	22500	0.05
yeast extract	0.1	20000	2
Total			65.8

Conclusion

The advantage of PSB culture medium was10mg/l yeast extract, C/N of 12, trace elements: Mn2 +: (0.009 mol/L), Fe3+: (0.0025 mol/L), Co2+: (0.0024 mol/L), Cu2 +: (0.0024 mol/L), Zn2 +: (0.0033 mol/L). HCH medium comparing with RCVBN yield of PSB increased 1.2 times and the cost decreased 19.8 times. All these indicated that the optimized culture medium can culture PSB efficiently and economically.

References

[1] A Cordova, FJ Navas. Effect of training on zinc metabolism: changes in serum and sweat zinc concentrations in sportsmen. Ann Nutr Metab. Vol. 42 (1998), p. 274-282

[2] Daniel, Graham; Rui, Pereira; Dominic, Barfield; Don, Cowan. Nitrile biotransformations using free and immobilized cells of a thermophilic Bacillus spp. Enzyme and Microbial Technology. Vol. 5-6 (2000), p. 368-373

[3] Evans WR, Fleischmann DE, Calvert HE, Pyati PV, Alter GM and Rao NSS (1990) Bacteriochlorophyll and photosynthetic reaction centers in Rhizobium strain BTAi 1. Appl Environ Microbiol 56: 3445–3449

[4] J Mariet, W Vander, Z J Gregory. 5-aminolevulinate production by E.coli containing the Phodobact erspaeroides hem A Gene. Applied and Environmental Microbiology. Vol. 10 (1990), p. 3560-3566

[5] H. Michel, K. A. Weyer, H. Gruenberg, I. Dunger, D. Oesterhelt, F. Lottspeich. The 'light' and 'medium' subunits of the photosynthetic reaction centre from Rhodopseudomonas viridis: isolation of the genes, nucleotide and amino acid sequence. The EMBO Journal. Vol. 5-6 (1986), p. 1149-1158

[6] Lambelet Pierre, Loliger Jurg, Saucy Fragcoise, Bracco Umberto. Antioxidant Propertites of Coenzyme Q10 in Food Systems. Agricultural Food Chemical. Vol. 40 (1992), p. 581-584

[7] M. Nishioka, H. Nishiuma, M. Miyake. Metabolic flux analysis of a poly-β-hydroxybutyrate producing cyanobacterium, Synechococcus sp. MA19, grown under photoautotrophic conditions. Biotechnology and Bioprocess Engineering. Vol. 5 (2002), p.295-302

[8] R. J. Cogdell, H. A Frank. How carotenoids function in photosynthetic bacteria. Biochim et Biophysica Acta. Vol. 2 (1987), p. 63-79

[9] R. H. Shipman, I. C. Kao, L. T. Fan. Single-cell protein production by photosynthetic bacteria cultivation in agricultural by-products. Biotechnology and Bioengineering. Vol. 17 (1975), p. 1561-1570

[10] S. Sirianuntapiboon, M. Srikul. Reducing Red Color Intensity of Seafood Wastewater in Facultative Pond. Bioresource Technology. Vol. 97 (2006), p. 1612-1617

[11] Woese CR, Stackebrandt E, Macke TJ and Fox GE (1985a). The phylogenetic definition of the major eubacterial taxa. Syst Appl Microbiol 6: 143–151

[12] Yu JA, Ye Y J, Lin Z X. Effect of carotenoids in photosynthetic bacteria on inhibiting lipid per-oxidation. Shanghai Jiaotong University. Vol. 3 (1998), p. 107-109