MASS CULTIVATION TECHNIQUES AND COMMERCIAL APPLICATION OF MICROALGAE DUNALIELLA

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Abstract: Dunaliella is a halotolerant unicellular microalga, with high photosynthetic efficiency and faster growth, thus easy to be cultivated in the pilot scale. This paper describes the mass cultivation techniques and commercial application of Dunaliella in aspect of function test of soft capsule, diversity of products and their nutritional assessment. The prospect of Dunaliella and natural carotene industry is also discussed.

Keywords: Dunaliella salina, Mass cultivation technique, Commercial application

INTRODUCTION

Dunaliella is a halotolerant unicellular microalga, with high photosynthetic efficiency and faster growth, thus easy to be cultivated in the pilot scale. The abundance of the bioactive components existed in this microalgae, with various structures and differential functions, leads to its therapeutic applications, as preventive agents in coronary heart disease, anti-cancer and anti-irradiance. Therefore Dunaliella is of increasing interest for scientific research and industry, which mainly focus on the improvement of cultivation efficiency and development of healthy protection product.

MASS CULTIVATION TECHNIQUES OF DUNALIELLA

Selection of production site

Dunaliella is an extremophile, which survives in the medium ranging from seawater and high salinity brine. Maximizing the algal biomass and cellular carotene content in the unit volume are needed for commercial purpose. It is well recognized that optimal culture temperature of 25-32℃ and high light intensity and long irradiance leads to higher carotene accumulation. On the other hand, mass culture of Dunaliella also requires large volume of brine with high salinity. Therefore the saltworks with high solar irradiance, year-round windy climate and less rainfall (daily precipitation is < 30mm) is preferred for Dunaliella cultivation.

Algae species

The selection of Dunaliella species should depend on the production purpose. The main Dunaliella species that are used for carotene production are D. salina and D. bardawill. Both can be obtained through purchasing the commercial colonial strain or selecting from the local brine water or production ponds.

Culture medium

The composition of the culture medium is of importance in large-scale algal cultures. To produce Dunaliella in purpose of β-Carotene production, large volume of high salinity brine is needed. This can be achieved by adding salt in fresh water or seawater, which undoubtedly adds considerably to the cost of operation. Alternative

method is to add salt in the brine from salt lake, which has been proved to be more cost-effective by Australian. In SRI, the bittern brine is used as the basic medium. The ion composition of the brine used in our cultivation ponds in different years is given in Table 1. We found that various ions are present in sufficient quantities, which satisfy the growth of Dunaliella. However, it is necessary to supplement certain amount of nitrogen, phosphorous and carbon source in order to promote the cell growth and carotene accumulation in Dunaliella.

Table 1 Salinity and ion composition of Brine

Date for sampling	Salinity (°Be')	Ion (mg/l)					Ion (g/l)				
		Cu^{2+}	Zn^{2+}	Fe^{3+}	Mn^{2+}	Co^{2+}	Ca^{2+}	Mg^{2+}	K^{+}	Cl^{-}	SO_4^{2-}
1988.4.14	18.5	0.004	0.259	0.244	0.112	0.004	1.00	9.42	2.62	122.63	10.81
1988.5.19	15	0.006	0.132	0.003	0.299	0.004	1.11	7.66	2.82	104.49	11.62
1988.6.24	9	0.0046	0.080	5.050	0.850	0.002	1.01	4.30	5.33	240.49	6.32
1989.3.24	20.4	0.0032	0.072	0.146	0.394	0.004	0.76	12.61	3.60	132.32	20.66
1989.9.24	18	0.0028	0.038	0.254	0.128	0.002	1.52	9.07	3.49	114.20	15.19
1990.4.12	20.6	0.006	0.042	0.38	0.31	0.006	1.10	10.22	3.01	136.90	16.99
1990.5.20	21.1	0.011	0.090	0.019	0.306	0.002	0.98	10.78	3.14	148.58	20.51

Facilities for outdoor cultivation

The facilities for outdoor cultivation include algae stock ponds, intermediate culture ponds (Fig.1)and production ponds(Fig. 2). The algae stock ponds are used for stocking algae inoculum on site, while the intermediate culture pond is used to provide large volume of inoculum at the beginning of the growth season and is kept stocked throughout the growing season. Production pond is equipped with mechanical stir, and is connected to a common over-flow ditch. The bottom of the pond is usually lined to prevent from leakage. Fig.3 shows the view of the Dunaliella cultivation basis in SRI.

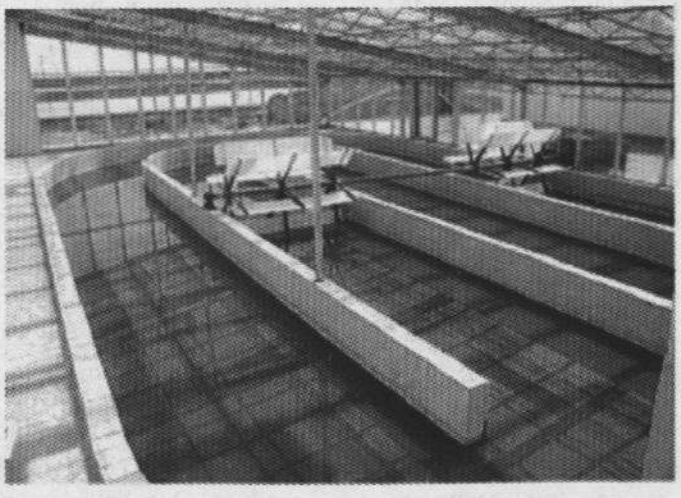

Fig. 1 Outdoor intermediate cultivation pond of Dunaliella

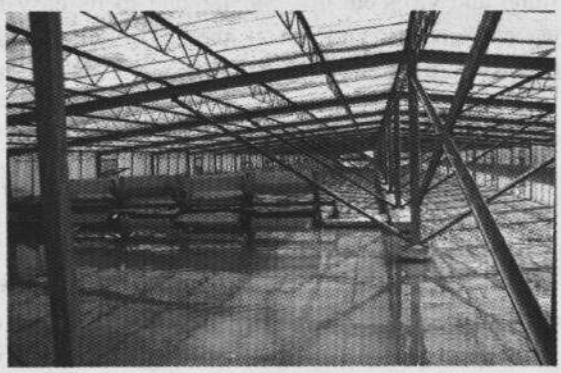

Fig. 2 Outdoor production pond of Dunaliella

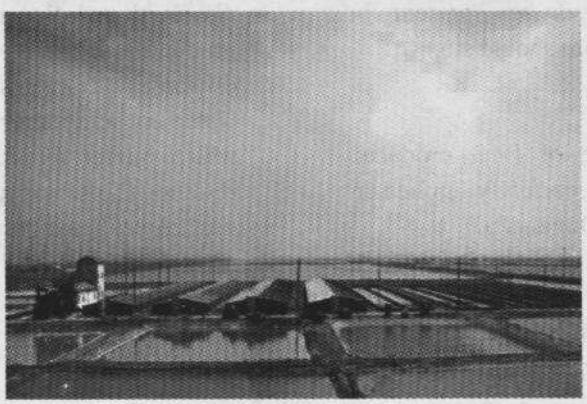

Fig. 3 View of the Dunaliella cultivation basis in SRI

Outdoor cultivation

Cultivation methods

Two separate processes must be considered in the culturing of Dunaliella for carotene production: the growth of cells and the accumulation of carotene. Dunaliella can be cultured in batches, or maintained in the semi-continuous and continuous culture system. The last two methods are usually applied in large-scale Dunaliella production.

Control of culture conditions

Since the effects of various environmental factors on the cell growth and accumulation of carotene are different, thus under the open air conditions, the following main factors, such as salinity, nutrients and pH should be controlled.

------ Salinity: Dunaliella is able to grow in salinities ranging from 0.3M to saturated NaCl solution, but the best growth is obtained at 1-2M.

------ Nitrogen and phosphorous: Dunaliella can utilize sodium nitrate, ammonia nitrate or urea as nitrogen sources. The nitrogen concentration of 0.5-5mM promotes the cell growth, while 0.5M facilitates the accumulation of carotene. The phosphorous concentration of 0.1-0.25M promotes the cell growth, protein synthesis and carotene accumulation of Dunaliella.

------ Carbon: Dunaliella can utilize carbon either from CO_2 and HCO_3^-. The optimum concentration of $NaHCO_3$ for cell growth is 10mM; whereas at higher concentration, carotene accumulation is promoted.

------ pH: The optimum pH of Dunaliella culture medium ranges from 7 to 9.

Pest control and prevention

One of the chief problems in large-scale outdoor algae cultures is the presence of contaminators (e.g. bacteria, fungi and virus etc.) and predators (e.g. protozoa, crustaceans and rotifers, etc.). Though the Dunaliella cultivation is operated in high salinity brine, the main organisms that may compete with or predate Dunaliella are other undesired Dunaliella species, Artemia and protozoa, etc.

The protocols preventing Dunaliella culture from crashes includes:

------Maintaining clean and axenic stock culture

------Sterilization of culture medium prior to inoculation

------Increasing salinity of culture medium, to eliminate most contaminators and predators

------Frequent inspection during the culturing process, whenever the pests are present in unacceptable levels, the culture should stop.

Harvest

According to the type of final products, following two protocols are currently applied in the harvesting procedure of Dunaliella.

Centrifugation

The harvest of Dunaliella is done continuously using high-speed disk centrifuge. The Dunaliella paste is then processed into powder through desalting and drying. Centrifugation is considered as a better method so far due to its easy manipulation, no pollution to the environment and less injury to the algae cell. However, the costs of the equipments and electricity are higher in this kind of operation.

Dissolved gas flocculation

Dunaliella paste can also be obtained via dissolved gas flocculation, which is then used for carotene extraction after desalting, dewatering and drying. The vegetable oil extraction is used to produce oil suspension of carotene. And the supercritical CO_2 extraction and solvent extraction are used to produce carotene extract, from which carotene crystal can be further produced. The techniques of dissolved gas flocculation are stable and the products are applied in wide scope. The disadvantages of this technique are high amount water consumption and the flocculants residue which hinder the further application of algae residue. The schematic diagram of the dissolved gas flocculation and carotene extraction is shown in Figure 4.

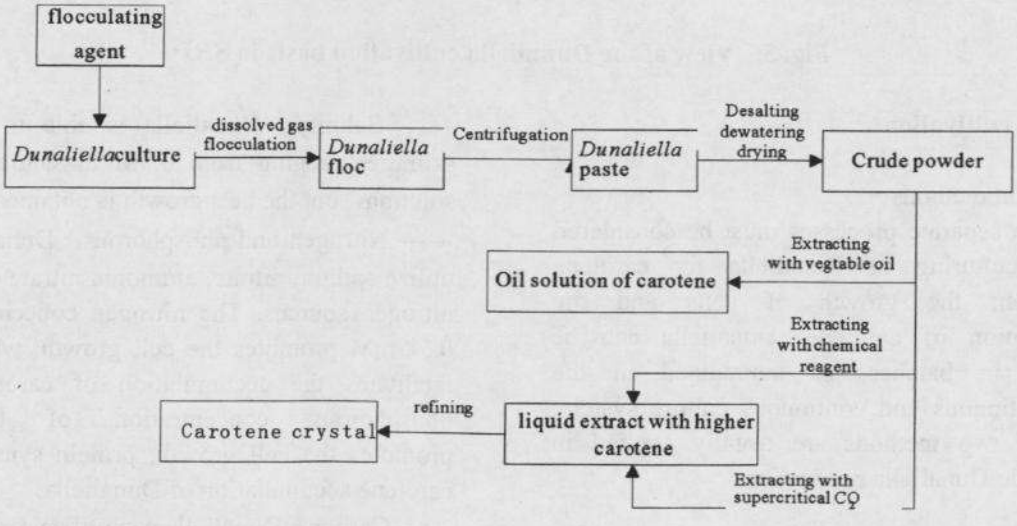

Fig. 4 Schematic diagram of the dissolved gas flocculation and carotene extraction

COMMERCIAL APPLICATION OF DUNALIELLA

Composition of Dunaliella dry powder

Dunaliella dry powder contains abundant amount of natural carotene, protein, polysaccharides, lipid, chlorophyll, as well as a variety of vitamins and minerals, which make it more valuable. Furthermore, Dunaliella dry powder contains 15-30% protein and many kinds of essential amino acids. Table 2 shows the proximate composition of Dunaliella dry powder produced by SRI. Table 3 shows the amino acid composition of Dunaliella dry powder produced by SRI.

Table 2 Proximate composition of Dunaliella dry powder

Composition	Content	Composition	Content
Carbohydrate	43.3%	Minerals	
Sugar	0.8%	Calcium	4850
Starch	41.8%	Phosphorous	15200
		Magnesium	18200
Protein	16.3%	Zinc	370
Total lipid	13.4%	Potassium	10500
Linolic acid	25.0%	Ferric	1940
Oleic acid	13.0%	Copper	20.8
Linoleic acid	9.0%	Selenium	0.23
Natural carotene	5.18%	Manganese	402
Cellulose	0.05%	Strontium	161
Vitamins (mg/100g)			
Vitamin C	186		
Vitamin E(α)	68.6 m		
Vitamin E(γ)	16.7		
Vitamin B_{12}	0.5		
Folic acid	19.1		

Table 3 Amino acid composition of Dunaliella dry powder (mg/100g sample)

Amino acids	Content	Amino acids	Content
ASP	1359.8	ILE	873.7
THR	800.1	LEU	1662.1
SER	702.4	TYR	421.9
GLU	1068.5	PHE	609.7
GLY	842.5	LYS	791.8
ALA	1030.9	HIS	271.3
CYS	91.8	TRP	85.8
VAL	903.6	ARG	1265.7
MET	363.7	PRO	1022.8
Taurine	354		
		Total	16803.5

The content of natural carotene in Dunaliella dry powder can be archived at level of >12%. Among which, cis-β-carotene accounts for 50% of total carotene, thus the biological function of carotene in Dunaliella dry powder is preferable than that of the chemically synthetic product. Dunaliella dry powder contains 10% polysaccharides. Among which, sulfuric polysaccharide (mainly consisted of xylose, rhamnose and hexulosonic acid) has therapeutic functions of anti-virus, anti-inflammation, anti-cancer and immuno-system improvement.

Health-protection function of soft capsule of Dunaliella dry powder

Due to the fact that Dunaliella contains several kinds of bio-active compounds, SRI has developed Soft Capsule of Dunaliella Dry Powder. The function test on the product proved that it has therapeutic functions of anti-irradiance, immuno-modulation and anti-cancer. Therefore it is a preferable health-protection product.

Products containing carotene from Dunaliella

A series of products containing Dunaliella carotene have been developed and produced by SRI, such as:

Oil solution of carotene

Oil solution or suspension are obtained through vegetable oil extraction, which contains 0.5 ~ 20% carotene. This product can be supplemented into healthy protection products (e.g. oral solution) or oil-based food, and can also be used as raw material for carotene crystal production.

Carotene crystal

Carotene crystal is used to produce micro-encapsulated products. However, because of its high price and inconvenience in use, less application is performed in the food and medicine industry so far.

Micro-encapsulation of carotene

Carotene crystal is covered by β-cyclodextrin by means of micro-encapsulation. Trough encapsulation, the stability, solubility and bioavailability of carotene can be enhanced. This product can be applied in the beverage, cake and medicine products.

Carotene extract using supercritical CO_2 extraction techniques

Carotene can be extracted from Dunaliella by supercritical CO_2 extraction technique. The advantages of this technique are high extraction rate and fast process, and no solvent residue or sting odor remained in the product. The extraction rate can reach a level of above 95%, among which cis-β-carotene accounts for about 80%. The quality of down stream products based on this product can be improved remarkably.

Carotene extracted from Dunaliella has a high color index and better therapeutic functions. Several international organizations, such as FAO, WHO and Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommend β-carotene as a non-poisonous and nutritious food additive. Food and Drug Administration of the US (FDA) certificates it as a healthy protection product. Natural carotene has been recognized as a food additive in China, and has been applied in a variety of food stuffs, including the oral solution, artificial butter, cheese, fruit and vegetable juice, beverage and salt.

DISCUSSION

The limitation of outdoor Dunaliella cultivation is the algae density, which is hardly over 8×10^5cells/ml in practice. Therefore the quality of the algae strain plays a key role in Dunaliella cultivation. By means of biotechnique and genetic engineering, the quality of algae strain can be improved; hence the high biomass production and high carotene content can be archived. On the other hand, the application of bioreactor also improves the algae biomass production.

The location of cultivation area should be selected according to meteorological condition. The inferior meteorological conditions result in low production and benefit.

Harvest is a limit factor that influences the overall production efficiency. Research on the harvest technique has critical influence on the development of mass culture of Dunaliella.

PROSPECTS

In Summary, microalgae Dunaliella has been widely applied in the field of healthy protection, medicine and aquaculture, due to its multi-function of anti-oxidation, cancer prevention and immuno-system enhancement. Yet there are still many researches need to be done in aspect of product development and mass culture techniques. In present, the most critical issue is to further improve the culture techniques and production efficiency. The techniques of mass Dunaliella cultivation have great application potential from economic point of view.