# CHAPTER V

## CONCLUSION AND RECOMMENDATION

1. Dunaliella spp. are the dominant species in the high saline salt production ponds in Thailand. At salinity about 300 ppt (30% NaCl), D. salina was found in maximum number then water colour turned to orange. Clones isolation was accomplished by the single cell isolation technique.

2. The study on the nutrients concentration of the salt ponds found that nitrate and phosphate concentration increased with increasing water salinity while the pH decreased.

3. In this experiment, six isolated D. salina clones were studied. The result indicated that there were differences in growth and carotenoid accumulation capability among these clones. The clone number DS91008 was selected as it produced the highest carotenoid contents in three levels of salinity (10, 20 and 30% NaCl).

4. The intensity of light affected carotenoid content but showed no significant impact on the growth rate. Cell colour changed to orange if light intensity exceeded 10,000 lux (136 $\mu E/m^2/s$). At 15,000 lux (203 $\mu E/m^2/s$) 98% of carotenoid was $\beta$-carotene. The HPLC isomer separation showed that $\beta$-carotene

consisted of about equal amount of 9-cis and all-trans β-carotene.

5. D. salina which grew in low light intensity (low carotenoid content) had significant smaller size than growing in a bright light (high carotenoid).

6. Nitrate deficiency affected both growth and carotenoid content. Growth rate decreased with decreasing nitrate concentration while carotenoid content increased. The maximum carotenoid content was 137.2 pg/cell at 10% nitrate concentration in J/1 medium (0.1 g/l). The β-carotene content was 12% AFDW.

7. Phosphate concentration and pH affected the growth rate but did not significantly affected the carotenoid content.

8. The optimal conditions for D. salina to produce high β-carotene were prepared by reducing nitrate concentration to 10% of J/1 medium and increased light intensity and salinity.

9. D. salina clone number DS91008 could be cultivated in the outdoor raceway pond at a specific growth rate of 0.15 or doubling time 4.62 days. The maximum biomass was 12.04 g-AFDW/$m^2$ at day 22.

10. Freeze dried algal powder has higher β-carotene content (5.9% AFDW) than the 70°C oven dried algae (1.9% AFDW).

11. During the outdoor culture, transparent plastic sheet could be used for protecting the pond from rain. Photoinhibition was not detected in the outdoor culture with maximum light intensity of 135,000 lux (1,800 $\mu E/m^2/s$). the media temperature fluctuated form 29.2 to 40.7°C.

12. The protozoa Heteramoeba sp. and D. viridis was found to contaminate the outdoor pond especially when salinity dropped below 190 ppt. High salinity, i.e. exceed 250 ppt was recommended for D. salina outdoor production.

Recommendations

1. The field survey should be expanded to cover remote areas such as the salt fields in Pattani Province (southern part) or high saline lake in north-eastern part of Thailand in order to isolate the new strains.

2. To improve carotenoid accumulation capacity of the strain, the genetic engineering procedure such as genetic transformation, genetic recombination, and mutant induction should be tried in the future.

3. During the single cell isolation, medium which contain lower salinity than the initial salinity was recommended for a better survival rate.

4. The culture medium for the outdoor cultivation should be improved. The semi-continuous culture experiment should be done throughout the year to obtain annual productivity before

scaling up to a pilot plant. To control the contamination, high salinity and low nitrate concentration is recommended. However, other methods should also be explored.

5. As regard the outdoor cultivation, the economic study is necessary for selecting a proper culture system. Moreover, the simple and low operation cost harvesting method should be developed.

6. The photobioreactor system should be developed for the production of D. salina in a very high output rate and high β-carotene content in conjunction with the high β-carotene strain.