CHAPTER II

BACKGROUNDS AND LITERATURE REVIEW

2.1 Astaxanthin

Astaxanthin is widely used as a color additive in aquaculture and has recently attracted attention as nutraceutical food due to its high antioxidant activity. It is a red pigment commonly found in many marine animals which contributed to the pinkish color of their flesh. It also influences survival and growth of marine animals (Lorenz and Cysewski, 2000), and therefore it is used as a feed supplement in the production of salmon, trout, and shrimp (Boussiba et al., 1991 and Kobayashi et al., 1992b) and other applications such as cosmeceutical industries.

2.1.1 Chemical properties of astaxanthin

Astaxanthin is a keto-carotenoid with two asymmetric carbon atoms at the 3 and 3' positions, and therefore it can exist in four configurations, including the identical enantiomer (3S, 3S'; 3R, 3'R) and meso forms (3R, 3'S; 3'R, 3S) (Renstrom et, al, 1981). The configurations of astaxanthin are shown in Figure 2.1 and the properties of astaxanthin are shown in Table 2.1.

Table 2.1 Properties of astaxanthin

Name	: astaxanthin or 3,3'-dihydroxy-β-β-carotene-4-4' dione
Molecular formula	: $C_{40}H_{52}O_4$
Molecular weight	: 596.86
Melting point	: 224°C
Solubility	: dissolved at room temperature in non polar solvent (acetone, dimethyl sulfoxide)

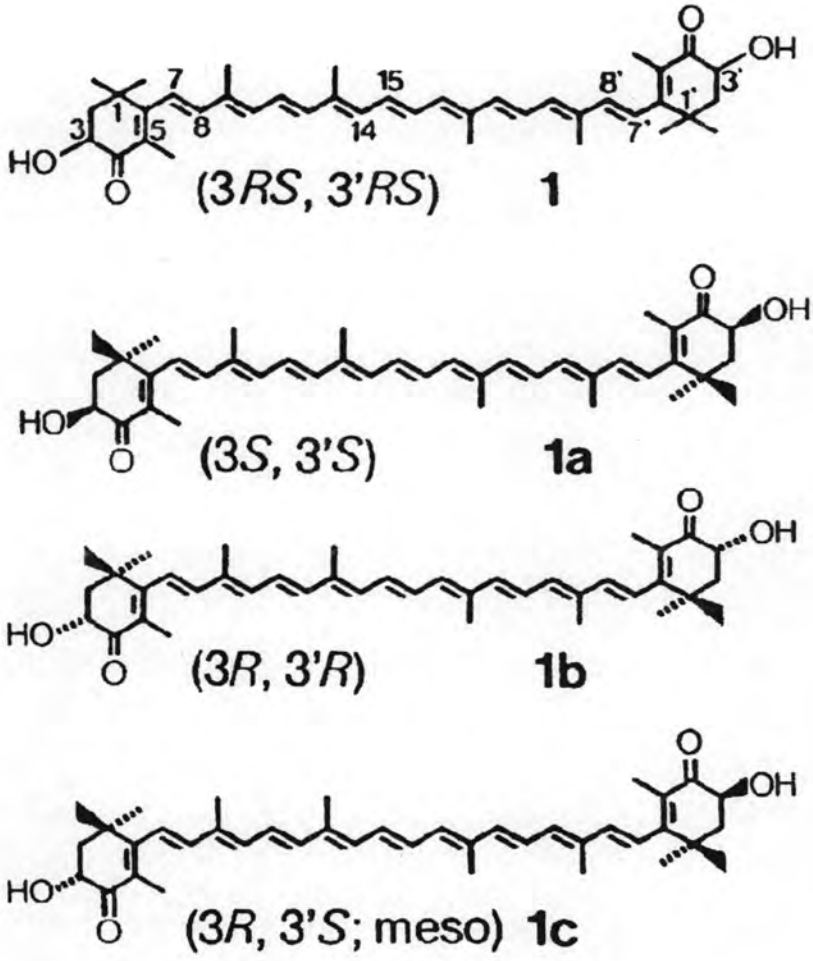

Figure 2.1 Four configulations of astaxanthin (Johnson and An, 1991)

Astaxanthin has an especially high propensity to absorb excess energy from singlet oxygen, releasing it as heat, and returning oxygen and itself back to the ground state. It is significantly more effective in neutralizing free radicals than beta-carotene and protects against peroxidation of unsaturated fatty acid methyl esters better than canthaxanthin, beta-carotene or zeaxanthin (about 10 times) (Terao, 1989; Miki, 1991; Jorgensen and Bille, 1993) and about 100-fold greater activity than vitamin E in inhibiting liquid peroxidation (Kurashige, 1990; Miki, 1991). It also has protection against UV light effect; immune response, pigmentation and improved reproduction (Lorenz and Cysewski, 2000).

2.1.2 Sources of astaxanthin

There have been two major industrial sources of astaxanthin; chemical (synthetic) and biological (natural) sources:

2.1.2.1 Synthetic source

F. Hoffman-La Roche, Basal Switzerland accomplished the synthesis of trans-astaxanthin which has been marketed as "carophyll pink". This contains 8% astaxanthin (Sommer et al., 1992). Synthetic astaxanthin is presently the principle source in aquaculture with more than 95% of this market being consumed in feed to produce coloration (Lorenz and Cysewski, 2000). However, this synthetic astaxanthin is expensive, unnatural configurational and involves potentially harmful process (Parker, 1992).

2.1.2.2 Natural sources

A. Crustacean meals

Astaxanthin composition in Tiger shrimp (*Panaeus monodon*) was studied for potential applications in natural colorants (Wu and Hwang, 1993). However, crustacean meals have relatively low contents of astaxanthin and high level of moisture, ash, chitin (Johnson and An, 1991) and minerals, causing several practical problems in feed formation that limits their usefulness in animal feed (Bubrick,1991).

B. Yeast

Phaffia rhodozyma contains astaxanthin in wild strain, but this only presents in small amount, i.e. 200 to 300 mg/kg yeast (0.02-0.03%). The content of astaxanthin depends on strain and method of culture (Johnson and An, 1991). However, the use of yeast as a source of astaxanthin for aquaculture is limited since the cell wall is difficult to be digested by some microorganisms (Andersson et al., 2003). Only free astaxanthin is found in the yeast which has lower antioxidant activity than esterified forms (Choi et al., 2003).

C. Algae

Astaxanthin can be produced from algae such as *Ankistrodesmus branuii*, *Chlorella zofinglensis* and *Dunaliella salina* (Borowitzka, 1989), *Euglena rubida* (Czeczuga, 1974). However, the quantities of astaxanthin generated from these microorganisms are relatively low and not suitable for mass production. The green

alga, *Haematococcus pluvialis*, provides the most concentrated natural source of astaxanthin known, from 10,000-40,000 ppm (mg/kg). As a comparison, the fleshes of wild Atlantic salmon on average contain 5 ppm (mg/kg) of astaxanthin, Coho salmon about 14 ppm (mg/kg) astaxanthin and sockeye salmon average 40 ppm (mg/kg) (Turujman, 1997).

D. Other microorganisms

Some bacteria such as *Mycobacterium lacticola* and *Brevibacterium sp.* and fungi in genus Penicphora and Copepod were also reported to be able to accumulate astaxanthin (Borowitzka et al., 1989). However, carotenoid level of this organism was low and growth was also slow (Droop, 1995 and Anderssen et.al., 2003). Some examples of sources of astaxanthin producing microorganisms are shown in Table 2.2.

2.2 *Haematococcus pluvialis*

Haematococcus pluvialis, also referred to as *Haematococcus lacustris* or *Sphaerella lacustris*, is a ubiquitous green alga of the order *Volvocales*, family *Haematococcus*. The taxonomy of *Haematococcus pluvialis* is as follows (Smith, 1950):

Taxonomy of *Haematococcus pluvialis*

Phylum : Chlorophyta
Class : Chlorophyeeae
Order : Volvocales
Family : Haematococcaceae
Genus : Haematococcus
Species : *Haematococcus pluvialis*

Table 2.2 Biological sources of astaxanthin (Simpson et al., 1981; Borowitzka, 1989; Harker et al., 1996)

Source	Astaxanthin (μg/g cell)	Astaxanthin (%dry cell)	Referance
Bacteria			
Brevibacterium sp.	30		Simson et al., 1981
Mycobacterium lacticola	30		Simson et al., 1981
Holobacterium salinarium	265		Simson et al., 1981
Yeast			
Phaffia rhodozyma	200-300	0.02-0.03	Borowizka et al., 1989
Mold			
Peniophora (Hymenomycetes)	<50		Borowizka et al., 1989
Algae			
Chlamydomonas nivalis	<50		Harker et al., 1996
Euglena rubida	<50		Harker et al., 1996
Haematococcus pluvialis	7,000-55,000	1.5-3	Lorenz and Cysewski ., 2000

It is now known that the alga occurs in nature worldwide, where environmental conditions for its growth are favorable. No toxicity associated with *Haematococcus* has ever been reported in the literature. The physical characteristics of *Haematococcus pluvialis* is as follows:

Physical characteristics of *Haematococcus pluvialis*

Color	:	red to dark red	
Particle size	:	5-25 microns	
Moisture	:	4-9%	
Bulk density			
Loose value		0.303-0.345	g/ml
Tapped value		0.370-0.435	g/ml
Astaxanthin	1%		

2.2.1 Characteristics and reproduction of *Haematcoccus pluvialis*

Motile cells of *Haematcoccus pluvialis* are solitary, biflagellate and enclosed by a wall that is broadly ellipsoid to ovoid. The protoplasm lies some distance inward from the wall and this is connected with it by numerous delicate strands of cytoplasm. The intervening space between the wall and the cytoplasm is filled with a watery gelatinous substance. There are flagella at the interior end of a cell and portion of each flagellum between the wall and proportion and protoplasm lies within a gelatinous canal. There is an eyespot in the equatorial region, contractrile vacuole here and there beneath the plasma membrane and several pyrenoid within chloroplast (Smith, 1950).

Asexual reproduction of *Haematococcus pluvialis* may take place by division of free swimming cells into two or four daughter cells (macrospores). Sexual reproduction, gamatic union isogamous, and the colonies are homotallic, but apparently with a fusion of gametes from different cells. Four or eight gametes are formed within a cell. A pair of fusion gametes becomes apposed at the anterior ends, and the flagella persist after fusion is completed (Donkin, 1976; Gudin and Chaumont, 1991; Zlonik et al., 1993).

2.2.2 Life cycle of *Haematococcus pluvialis*

Life cycle of *Haematococcus pluvialis* is divided into four stages as described below (Figure 2.2):

1. Vegetative motile cell

Under optimal environment conditions, *Haematococcus pluvialis* produces chlorophyll *a* and *b* and primary carotenoid, especially β-carotene and lutein (Rockette, 1970). The cells are green and ellipsoid, two flagella provide motility and growth rate is high (Figure 2.2 (a)).

2. Encystment

Under condition of growth limitation such as nitrogen limitation, phosphorous limitation, light induction etc., the alga produces secondary carotenoids such as echinenone canthaxanthin and primary carotenoids (Droop, 1954; Goodwin and Jamikorn, 1954; Lee et al., 1991 and Zlotnik et al., 1993). In this stage, the cell decreases its growth rate significantly, acquires spherical shape, loses flagella and motility and builds a new thick cell wall, transforming into aplanospore (Santos and Mesquita, 1984) (Figure 2.2 (b)).

3. Maturation

At this stage the cells begin the massive accumulation of astaxanthin. Astaxanthin deposition is first noted around the nucleus immature cyst and proceeds radically until the entire protoplast is red (Mature cyst). The two processes, encystment and maturation, while usually coupled, are in fact distinct processes which can be experimentally separated in time. Fully mature cysts contain up to 5% by weight astaxanthin, predominantly in the form of monoesters of fatty acids (Renstrom et al., 1981; Bubrick, 1991; Bidigare et al., 1993; Lee and Ding, 1992) (see Figure 2.2 (c)).

4. Germination

At this stage, the cells begin the synthesis of chlorophyll and protein, and the degradation of carotenoid is occurred as shown in Figure 2.2 (a).

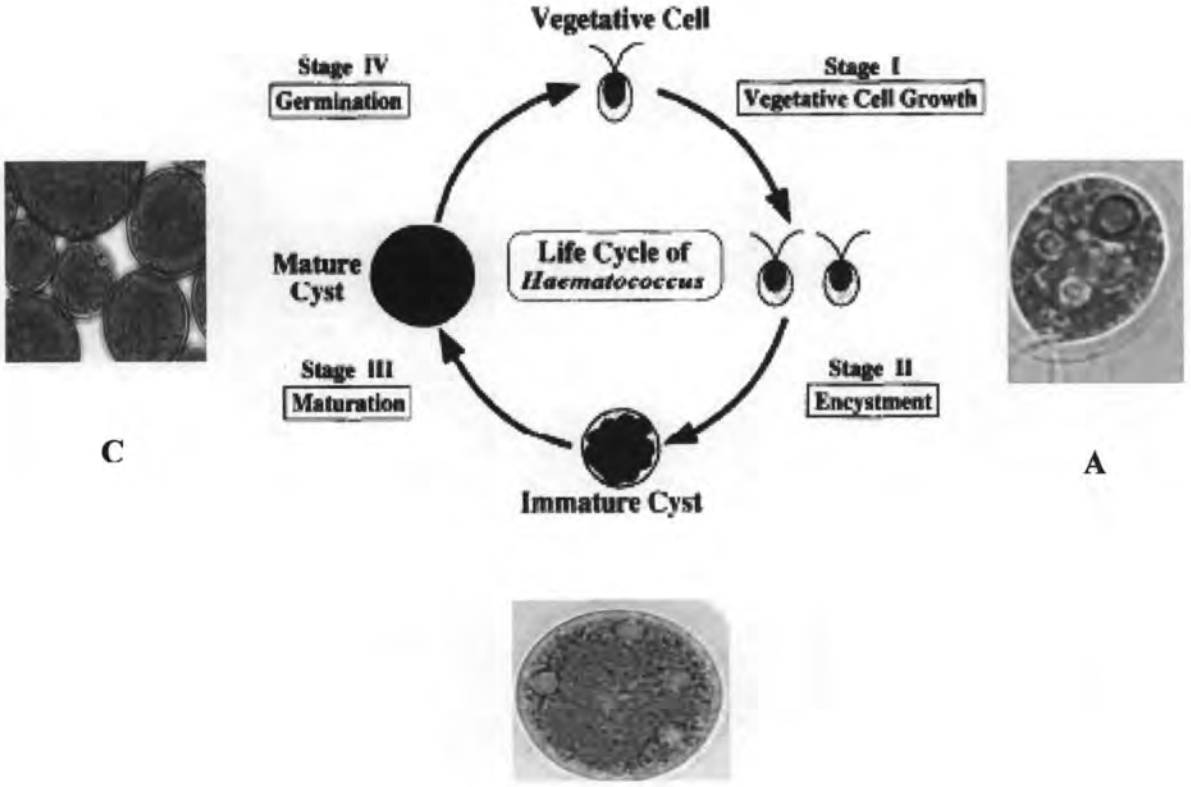

Figure 2.2 Life cycle of *Haematococcus pluvialis*

(a) vegetative motile cells (The morphology has flagellate)

(b) immature cyst cell (The morphology has not flagellate and the astaxanthin accumulation is initiated.)

(c) mature cyst cell (astaxanthin accumulation is completed)

2.2.3 Astaxanthin in *Haematococcus pluvialis*

The green algae *Haematococcus pluvialis* provides the most concentrated natural source of astaxanthin, from 7,000-55,000 mg/kg cell astaxanthin in addition to other important carotenoids such as beta-carotene, lutein and canthaxanthin (70% monoesters of astaxanthin, 10% diesters of astaxanthin, 5% free astaxanthin, and the remaining 15% consists of a mixture of β-carotene, canthaxanthin, lutein and other carotene). Carotenoid content in *Haematococcus pluvialis* is shown in Figure 2.3 (www.cyanotech.com/pdfs/bioastin/axbul/62.pdf).

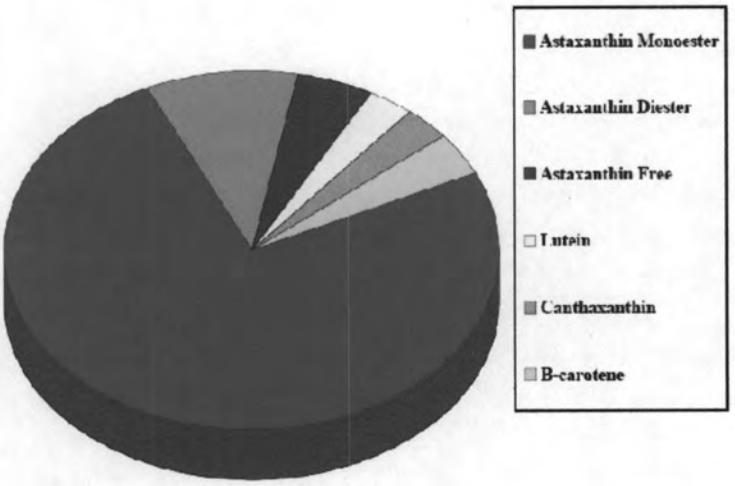

Figure 2.3 Carotenoid content in *Haematococcus pluvialis*

Astaxanthin is biosynthesized though the isoprenoid pathway which is also responsible for the vast array of liquid soluble molecules such as sterols, steroid, prostaglandins, hormones, vitamins D, K and E. The pathway initiates at acetyl-co-A and precedes though phytoene, lycopene, β–carotene, and canthaxanthin. The astaxanthin biosynthetic pathway of *Haematococcus pluvialis* is described in Figure 2.4. Fatty acids are esterified onto the 3' hydroxyl group(s) of astaxanthin after biosynthesis of the carotenoid, and allow it to have more solubility in the cellular environment.

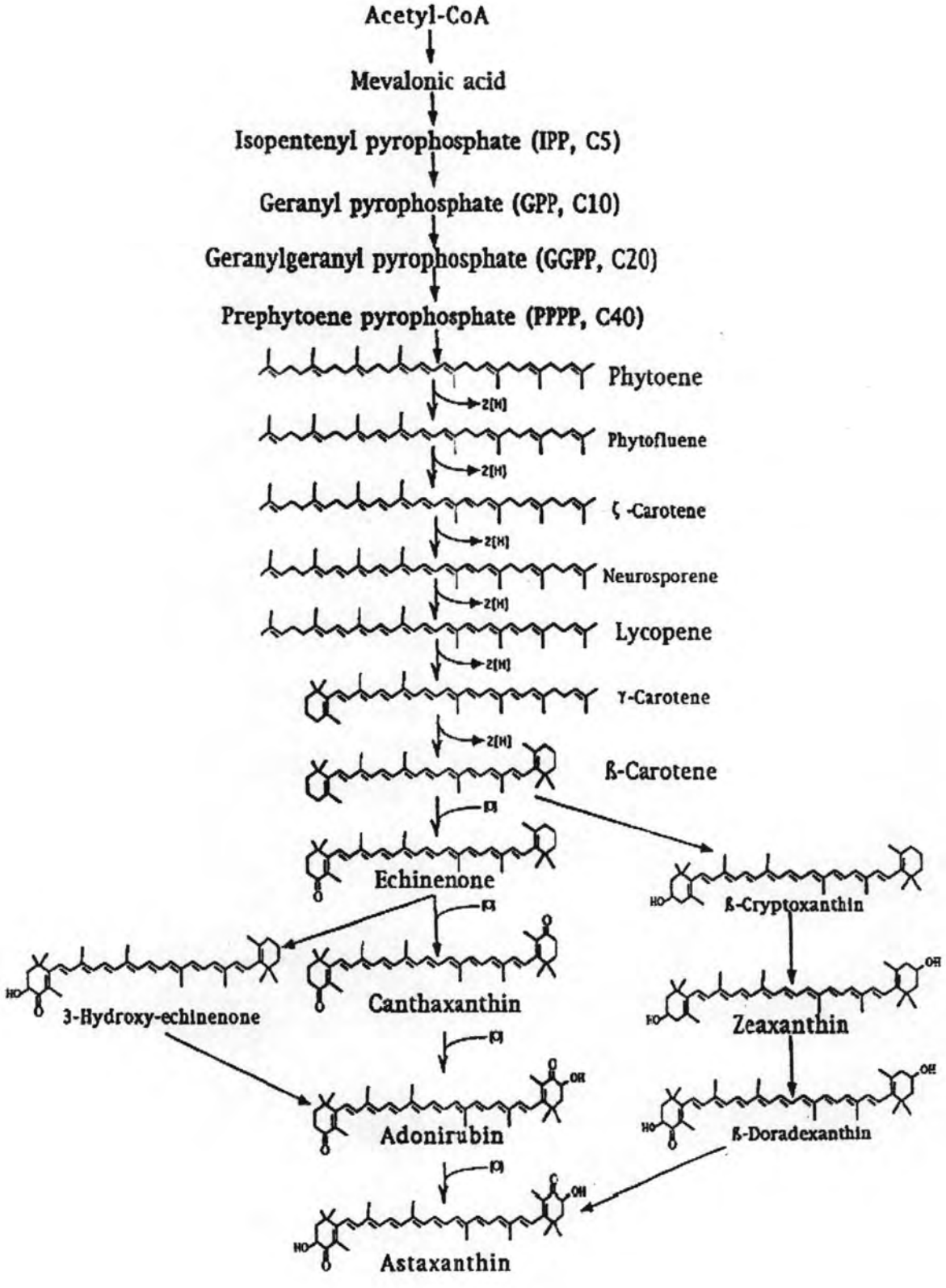

Figure 2.4 Astaxanthin pathways of *Haematococcus pluvialis*

2.2.4 Physical changes during astaxanthin accumulation

Physical changes occurring during astaxanthin accumulation could be divided into 2 major catagories: i) changes in cell constituents and ii) changes in cellular activities.

i) Changes in cell constituents

The conversion of green algae to red aplanospores is accompanied by a massive increase in carbohydrate content which may reach up to 63% of the cell dry weight (Boussiba and Vonshak 1991). This reflects entrance to a resting stage and the synthesis of compounds requiring less energy than proteins whose synthesis requires higher energy input and whose content decrease during this process. An increase in lipid content with correlates with the pigment accommodation in the lipid globules, suggesting that the accumulation of astaxanthin is closely related to the synthesis of fatty acids. Carotenoids are hydrophobic compounds dissolved in oil. This astaxanthin esters to float and accumulate throughout the cytoplasm (Lang 1968, Santos and Mesquita, 1984).

ii) Changes in cellular activities

The amount of cellular astaxanthin is inversely related to cellular photosynthetic activities, although the amount of chlorophyll and PSII reaction center remain stable during astaxanthin accumulation (Tan et al., 1995; Boussiba et al., 1999). Tan et al. (1995) attributed the decline in photosynthetic activity mainly to the lack of cytochrome f and expected absence of linear electron flow from PSI to PSII and, to a lesser extent, to decrease of some of the PSI and PSII components.

2.3 Processes for astaxanthin production

The accumulation process for astaxanthin is generally different from the growth process. Thus, a two stage batch cultivation is commonly applied for the production of astaxanthin from *Haematococcus pluvialis*. The first-stage is conducted with the main purpose of attaining a high cell concentration, whereas the second stage is designed to enhance the carotenoid formation. The second-stage could be achieved by several means, e.g. the injection of ferrous ion with acetate and sodium chloride into the middle of the first-stage culture (Schoefs et al., 2001), by limiting nutrients

such as nitrate starvation, phosphate starvation, iron starvation, or by controlling other growth environments, such as temperature, light, pH (Harker et al., 1996) or oxidative stress (Kobayashi et al., 2001).

2.3.1 Cultivation of *Haematococcus pluvialis*

The cultivation of *Haematococcus pluvialis* aims to achieve high *Haematococcus pluvialis* growth rates in vegetative form. This can be done through a well control of several physiological parameters such as light and temperature, as well as nutritional and other environmental factors that could potentially affect the growth of the cell. The optimal conditions for the growth of *Haematococcus pluvialis* is summarized in Table 2.3 whereas the complete review of the reactors for the cultivation of *Haematococcus pluvialis* could be found in Kaewpintong (2006).

2.3.2 Induction of astaxanthin

This stage is generally adjusted for the astaxanthin accumulation in *Haematococcus pluvialis*. The accumulation of astaxanthin could be induced by controlling several environmental parameters, e.g. light, aeration, nutrient and other factors. These induction conditions are summarized in Table 2.4 where each of the important factors is being described below:

1. Light

Light is an important factor for growth and astaxanthin accumulation in a wide variety of organisms. According to Kobayashi et al. (1997), optimal light intensity for the most of specific growth rate of *H. pluvialis* was 1.5 klux and the optimal light intensity for astaxanthin accumulation of *Haematococcus pluvialis* was ranged within 3-18 klux (Qinglin et al., 2007). Higher light intensity, the morphology of *Haematococcus pluvialis* changed from vegetative cell to cyst and tended to take place together with carotenoids formation. It was reported that the carotenoids formation was more efficiently enhanced under blue light than under red light (Kobayashi et al., 1992a). According to Lababpour et al. (2005), the highest astaxanthin concentration of 60 μg/ml was obtained when the light intensity was 0.19 klux. Table 2.5 provides detail summary of literature regarding the effect of light on astaxanthin induction.

2. Temperature

Temperature is a factor that affects growths and the accumulation of astaxanthin in a wide strain of algae. According to Borowitzka et al. (1989), Harker et al. (1996) and Kobayashi et al. (1997), the optimal temperature for growth in *Haematococcus pluvialis* lied in the range of 15 and 27°C and the optimal temperature for astaxanthin accumulation was reported to be higher 30°C (0.5μg/ml at 33°) by Fan et al. (1994) and Tjahjono et al. (1994a).

3. pH

pH is important for growths and accumulation of astaxanthin. The optimal pH for growth in *H. pluvialis* was approximately 7.0 (Hata et al., 2001), and when the pH was lower than 5 or higher than 9, there would be no growth (Sarada et al., 2001). The optimal pH for astaxanthin accumulation was 7 (the astaxanthin concentration was 5.4 μg/ml) and significantly lowers at pH 6 (Sarada et al., 2001). Table 2.6 is the summary of literature on the effect of pH.

4. Nutritional factors

Essential elements are usually divided into macronutrients and micronutrients. Macronutrients are required in relatively high quantity for the growth, i.e. nitrogen phosphorous, calcium, etc. whereas micronutrients, although essential, are only required in relatively low quantities, Examples of micronutrients included iron, boron, manganese, copper and vitamins.

4.1 Macronutrients

A. Nitrogen

Haematococcus pluvialis grows rapidly at high nitrogen concentration, but the accumulation of astaxanthin is high when nitrogen is limited. In *Haematococcus pluvialis* cultures, nitrogen limitation is a key factor for the accumulation of astaxanthin (Kobayashi et al., 1991). For instance, Fabregas et al. (1998) reported that the astaxanthin accumulation was high in culture grown without potassium nitrate medium, where the astaxanthin concentration was 49.5 μg/ml. For more information of the effect of nitrogen starvation, please consult Table 2.7.

B. Phosphorous

Phosphate starvation is a trigger for the accumulation of astaxanthin (Boussiba and Vonshak, 1991) but the reduction in phosphate levels did not inhibit

growth as severely as nitrogen starvation in this alga (Harker et al., 1995). According to Boussiba et al. (1999), the induction of astaxanthin accumulation by starvation of phosphorous was 13.75 μg/ml. Table 2.8 gives complete summary on the literature of phosphorous limitation.

4.2 Micronutrients

A. Iron

The astaxanthin formation of *Haematococcus pluvialis* was enhanced in Fe^{2+} rich medium (Kobayashi et al., 1992b). It was shown that Fe^{2+} would possibly function as an OH^- generator via an iron-catalyzed Fenton reaction that plays an essential role in the enhanced carotenoids function in the algal cyst cells. The astaxanthin in the medium with Fe^{2+} and acetate medium was 0.09 μg/ml (Kobayashi et al., 1992b).

B. Vitamin

In addition to inorganic nutrient, some *Haematococcus pluvialis* strains require exogenous supplies of vitamins such as cyanobalamin (B12), thyamin (B1) (Pringsheim, 1966). Furthermore, cell division inhibitor such as vinblastine or dichlorophenyl dimethyl urea can cause cessation of growth followed by an increase in the accumulation of astaxanthin in the resting cells (Boussiba et al., 1992). The astaxanthin in the medium with dichlorophenyl dimethyl urea was 0.09 μg/ml (Brinda et al., 2004).

5. Salt Stress

The carotenoids formation increases in the salinity of culture medium but this is accompanied by high rates of cell mortality. The carotenoids formation became more efficiently enhanced in the culture with sodium chloride (25 μg/ml) than with potassium chloride (17.5 μg/ml) (Harker et al., 1995) μg/ml. The summary of the effect of salt stress on the induction of astaxanthin in *Haematococcus pluvialis* is given in Table 2.9.

2.4 Photobioreactors for the induction of accumulation of astaxanthin

During the astaxanthin production stage, green cells are generally exposed to stress conditions such as high irradiance, nitrate and/or phosphate deprivation, high temperature, etc., in order to induce the accumulation of astaxanthin. This step is often conducted in close systems to prevent the contamination by bacteria, fungi and other faster growing algae, as well as protozoan predators which have been reported to eliminate 90% of the algal biomass within 72 h (Spencer, US patent no. 4871551, 1989). Advantages and disadvantages of open and closed systems are shown in Table 2.10.

Pneumatic reactors such as bubble column bioreactors and tubular bioreactors have been used in the astaxanthin production stage because the shear stress level is lower than that in stirrer tank reactors, and because it is generally cheaper than other designs. This type of bioreactor has been extensively proposed as outdoor closed photobioreactors in the industrial production of microalgae (Tredici and Materassi., 1992; Richmond et al., 1993; Molina et al., 1994; Acien et al., 1998 and Garcia et al., 1999).

Kim et al. (2005) enhanced production of astaxanthin by flashing light in bubble column 2 L. The condition for astaxanthin production is continuous fluorescent light and flashing light at an intensity of 220.6 $\mu Em^{-2}s^{-1}$ or 11.03 klux in modified bold basal medium. The astaxanthin production is 0.466 mg/l in 30 days.

Lopez et al. (2005) compared the induction of astaxanthin using different types of bioreactors. The tubular reactor (9.23 μg astaxanthin/ml) was more efficient than the bubble column photobioreactor (1.54 μg astaxanthin/ml). Ranjbar et al. (2007) reported that bubble column photobioreactors could reach an extraordinary high astaxanthin content at 390 μg/ml.

A summary of the astaxanthin production in photobioreactors is given in Table 2.11.

Table2.3 Optimum conditions for the cultivation of green vegetative cells of *Haematococcus pluvialis*

Factors	Range
Light	2-24 klux
Temperature	15-28°C
pH	6-8
Nutrients	
1) Macronutrients	
Carbon	1-4 g/l CH_3COONa
Nitrogen	0.25-1.5 g/l $NaNO_3$
Phosphorous	0.075-4 g/l K_2HPO_4
2) Micronutrients	
Iron	2.5-5mg/l $FeCl_3.6H_2O$
Boron	2.8-5.7mg/l H_3BO_4
Manganese	0.73-3.6mg/l $MnCl_2.4H_2O$
Copper	8-770μg/l$CoSO_4.5H_2O$
Vitamins	
B_1	0.336-26.7 mg/l
B_{12}	0.004-0.27 mg/l

Table 2.4 Optimum conditions for the induction of astaxanthin in *Haematococcus pluvialis*

Factor	Ranges	Refferance
Light	3-18 klux	Qinglin et al.,2007
	0.01-0.6 klux	Katsuda et al., 2006
	3.28-11.03 klux	Kim et al., 2005
	2-11.5 klux	Fabregas et al., 1998
	0.1-4.45 klux	Harker et al., 1995
	3.4-14.05 klux	Kobayashi et al., 1992a
N-deficient	0-300 mg/l $NaNO_3$	Qinglin et al.,2007
	0-1000 mg/l $NaNO_3$	Orasa et al., 2004
	4.97-12.4 mg/l $NaNO_3$	Sarada et al.,2001
	100-1,640 mg/l $C_2H_3O_2Na$	Gong and Chen, 1997
	0-510 mg/l $NaNO_3$	Harker et al., 1995
P-deficient	147.9-591.6 mg/l K_2HPO_4	Harker et al.,1995
Salinity	2.5-20 mg/l NaCl	Sarada et al., 2001
	0-5.85 g/l NaCl	Harker et al.,1995
	0-7.45 g/l KCl	Harker et al.,1995
pH	6.8-11	Lababpour et al., 2005
	5-9	Sarada et al.,2001
iron	5-20.02 mg/l $FeSO_4.7H_2O$	Harker et al.,1995

Table 2.5 Effect of light intensity on the production of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^4$ cells/ml)	Condition				Solvent	Final cells ($x10^4$ cells/ml)	Astaxanthin (μg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Qinglin et al., 2007	Flask 500 ml	BBM[a]	-	18	-	-	23.8	methanol+ 5% NaOH	-	56	13
Katsuda et al., 2006	Flask 200 ml	Kobayashi's basal medium	1.33	0.6	Continu-ous light	6.8	20	methanol	50	29	12.5
Lababpour et al., 2005	Flask 200 ml	Kobayashi'basal medium	0.2	0.19	Blue LEDs	6.8	20	methanol	-	70	16.67
Kim et al., 2005	Bubble column	MBBM[b]	5	11.03	Flashing light	6.5	23	acetone	4.5	0.466	30

Table 2.5 (continued) Effect of light intensity on the production of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^4$ cells/ml)	Condition				Solvent	Final cells ($x10^4$ cells/ml)	Astaxanthin (µg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Dominguez-Bocanegra et al., 2003	Flask 200 ml	BAR medium	-	17.25	Continu-ous light	-	28	hexane:acetone:alcohol (100: 70:70)	-	9.8	8
Choi et al., 2003	Bubble column 2 l	FBBM[c]	1	7	Continu-ous Light	-	25	-	15	40	17
Fabregas et al., 1998	Mini-reactor 70ml	Fabregas 's medium	10	11.5	Day light 12 hr.	7.2-7.5	25	acetone: methanol (1:2)	-	19.05	7
Olaizola et al., 2000	airlift reactor	MBBM[b]	1.87	-	Sun light	-	-	-	-	8.4	-

Table 2.5 (continued) Effect of light intensity on the production of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^4$ cells/ml)	Condition				Solvent	Final cells ($x10^4$ cells/ml)	Astaxanthin (µg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Kobayashi et al., 1997	Flask 250 ml	Fe^{2+} Rich medium	5	14.05	Day light 24 hr.	-	-	acetone	-	2.5	8
Harker et al, 1995	Flask 250 ml	BBM	8	4.45	Cool white fluores-cent	-	22	Acetone 100%	6.5	25	20

[a] Bold basal medium

[b] Modifiled bold basal medium

[c] Fortified bold basal medium

Table 2.6 Effect of pH on the production of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^4$ cells/ml)	Condition				Solvent	Final cells ($x10^4$ cells/ml)	Astaxanthin (µg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Lababpour et al., 2005	Flask 200 ml	Kobayashi' basal medium	2	0.4	Floures-cent lamp	6.8	20	0.1 ml methanol in NaOH 5mM	-	0.08	-
Sarada et al., 2001	Flask	BM[d] (0.25%NaCl in 4.4mM Sodium acetate)	-	-	-	7	25	acetone	-	5.4	10

[d] Basal medium

Table 2.7 Effect of nitrogen deficiency on the production of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^4$ cells/ml)	Condition				Solvent	Final cells ($x10^4$ cells/ml)	Astaxanthin (µg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Qinglin et al., 2007	Flask 500 ml	BBM	-	18	-	-	23.8	methanol+ 5% NaOH	-	33	13
Orasa et al., 2004	Mini-reactor 400 ml	BBM	-	3.5	Daylight 12 hr.	7	18	acetone	-	12.05	9-11
Sarada et al., 2001	Flask	BM	-	1.5	-	-	25	acetone	-	8	10
Boussiba et al., 1999	-	BG-11	35	5	-	-	-	DMSO	30	7.5	12
Fabregas et al., 1998	Mini-reactor 70ml	Fabregas 's medium	10	11.5	Day light 12 hr.	7.2-7.5	25	acetone: methanol (1:2)	-	49.52	14

Table 2.7 (continued) Effect of nitrogen deficiency on the production of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^4$ cells/ml)	Condition				Solvent	Final cells ($x10^4$ cells/ml)	Astaxanthin (μg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Harker et al, 1995	Flask 250 ml	BBM (nitrate =0)	1	1.75	Cool white fluores-cent	-	22	Acetone 100%	5	17.5	30

Table 2.8 Effect of phosphate deficiency on the production of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^4$ cells/ml)	Condition				Solvent	Final cells ($x10^4$ cells/ml)	Astaxanthin (μg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Boussiba et al., 1999	-	BG-11	35	5	-	-	-	DMSO	30	13.75	12
Harker et al, 1995	Flask 250 ml	BBM (nitrate =0)	1	1.75	Cool white fluores-cent	-	22	Acetone 100%	5	8.75	30

Table 2.9 Effect of salinity on the production of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^4$ cells/ml)	Condition				Solvent	Final cells ($x10^4$ cells/ml)	Astaxanthin (µg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Sarada et al., 2001	Flask	BM	-	1.5	-	-	25	Acetone	-	1.25	12
Harker et al., 1995	Flask 250 ml	BBM (nitrate =0)	1.25	1.75	Cool white fluores-cent		22	Acetone 100%	5	25	30

Table 2.10 Advantages and disadvantages of open and closed systems

Parameter	Open pond	Closed system
Contamination	Extremely high	Low
Space required	high	Low
Water losses	Extremely high	Almost none
CO_2 losses	high	Almost none
Weather dependence	absolute	Insignificant, because closed configuratuions allow production also during bad weather
Species	Are restricted to few algal varieties	May be cultivated
Biomass concentration	low	high
Efficiency of treatment process	Low	high

Table 2.11 Photobioreactor for the induction of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^5$ cells/ml)	Condition				Solvent	Final cells ($x10^5$ cells/ml)	Astaxanthin (μg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Ranjbar et al, 2007	Bubble column 1L	Standard Inorganic medium	-	2.43	Fluorescent lamps	7.5	20	-	$6x10^6$	390	35
Garcia-Malea et al., 2006	Bubble column 55L	Inorganic medium free of acetate	10	2-100	Daylight	8	20	acetone	25	1.54	16
Garcia-Malea et al., 2006	Tubular reactor 55 L	Inorganic medium free of acetate	10	2-100	Daylight	8	20	acetone	45	9.23	16

Table 2.11 (continued) Photobioreactors for the induction of astaxanthin

Ref.	Reactor	Medium	Initial cell ($x10^4$ cells/ml)	Condition				Solvent	Final cells ($x10^4$ cells/ml)	Astaxanthin (μg/ml)	Time (days)
				Light Intensity (klux)	Light source	pH	Temp (°C)				
Kim et al., 2005	Bubble column 2L	Modifiled bold basal medium	50	11.03	Continuous and flashing light	6.5	23	acetone	45	0.466	30
Zhang et al., 1999	Stirrer tank 3.7 L 350 rpm	Hong Kong medium	0.22	-	-	7	30	-	15	64.4	20
Harker et al., 1996	Bubble column 30L	Bold basal medium + NaCl	1.25	2.5	Fluorescent light	7	-	acetone	1.5	18	55