การกระตุ้นแอสตาแซนตินของสาหร่าย *Haematococcus pluvialis* ในถังปฏิกรณ์ชีวภาพแบบอากาศยกชนิดแนวระนาบ

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ASTAXANTHIN INDUCTION OF *Haematococcus pluvialis* IN FLAT PANEL AIRLIFT PHOTOBIOREACTORS

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วรเดช พูนคำ : การกระตุ้นแอสตาแซนตินของสาหร่าย *Haematococcus pluvialis* ในถังปฏิกรณ์ชีวภาพแบบอากาศยกชนิดแนวระนาบ. (ASTAXANTHIN INDUCTION OF *Haematococcus pluvialis* IN FLAT PANEL AIRLIFT PHOTOBIOREACTORS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.ประเสริฐ ภวสันต์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ดร.สรวิศ เผ่าทองศุข, 100 หน้า.

งานวิจัยนี้ได้ศึกษาการกระตุ้นแอสตาแซนทินในจุลสาหร่าย *H. pluvialis* (NIES-144) ในเครื่องปฏิกรณ์ชีวภาพแบบอากาศยกชนิดแบนภายใต้สภาวะกลางแจ้งและในร่ม โดยขั้นแรกได้ทำการศึกษาหาสภาวะที่เหมาะสมสำหรับการกระตุ้นแอสตาแซนทินในเครื่องปฏิกรณ์ชีวภาพแบบหออากาศขนาด 1.5 ลิตรในร่มก่อน โดยสภาวะที่เหมาะสมที่ได้รับคือ การใช้น้ำเปล่าที่ไม่ใส่สารอาหาร การใช้สัดส่วนของคาร์บอนไดออกไซด์เป็น 3 เปอร์เซ็นต์โดยปริมาตร การใช้ความเข้มแสง 35,000 ลักซ์ และการใช้อุณหภูมิกระตุ้นที่ 33 องศาเซลเซียส โดยสภาวะดังกล่าวสามารถกระตุ้นแอสตาแซนทินได้เท่ากับ 18.21 มิลลิกรัมต่อลิตร (3.63 เปอร์เซ็นต์โดยน้ำหนักแห้ง) และที่สภาวะเดียวกันนี้เมื่อทำการทดลองในเครื่องปฏิกรณ์ชีวภาพแบบอากาศยกชนิดแบนขนาด 17 ลิตร โดยใช้อัตราส่วนของพื้นที่ให้อากาศต่อพื้นที่ที่ไม่ให้อากาศเป็น 0.4 และความเร็วของอากาศเป็น 0.4 เซนติเมตรต่อวินาที พบว่าสามารถกระตุ้นแอสตาแซนทินได้เท่ากับ 26.63 มิลลิกรัมต่อลิตร (5.32 เปอร์เซ็นต์โดยน้ำหนักแห้ง) และสำหรับการทดลองการกระตุ้นในระบบสองขั้นตอนในเครื่องปฏิกรณ์อันเดียวพบว่าสภาวะที่เหมาะสมในการกระตุ้นแอสตาแซนทินคือ การลดไนเตรดและลดฟอสเฟตเป็น 4 และ 6 เท่า ตามลำดับ โดยที่สภาวะนี้สามารถกระตุ้นแอสตาแซนทินได้เท่ากับ 28 กรัมต่อลูกบาศก์เมตร (5.24 เปอร์เซ็นต์โดยน้ำหนักแห้ง) สำหรับกรณีของการกระตุ้นแอสตาแซนทินในระบบกลางแจ้งพบว่าสามารถกระตุ้นแอสตาแซนทินได้น้อยกว่าระบบในร่ม เนื่องจากผลกระทบของความเข้มแสง โดยที่สภาวะการคลุมตาข่ายกรองแสง 1 ชั้น ใน 3 วันแรกของการกระตุ้นเป็นสภาวะที่สามารถกระตุ้นแอสตาแซนทินได้มากที่สุดโดยมีค่าเท่ากับ 20.9 มิลลิกรัมต่อลิตร (4.45 เปอร์เซ็นต์โดยน้ำหนักแห้ง) นอกจากนี้เมื่อพิจารณาการขยายขนาดเครื่องปฏิกรณ์พบว่ามีผลต่อการกระตุ้นแอสตาแซนทินค่อนข้างน้อยมาก สุดท้ายนี้เมื่อคำนวณราคาต้นทุนการผลิตรายปีพบว่าที่สภาวะการทดลองกลางแจ้งมีราคาถูกที่สุดเนื่องจากที่สภาวะนี้ไม่จำเป็นต้องใช้แสงจากหลอดฟลูออเรสเซนต์เพื่อกระตุ้นแอสตาแซนทิน

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This work studied astaxanthin induction from *H. pluvialis* (NIES-144) using flat panel airlift photobioreactors (FP-APBRs) under indoor and outdoor conditions. Preliminary experiments in 1.5 L bubble column photobioreactors (BC-PBR) revealed that sterilized clean water with 3% CO_2 aeration (1.47 cm^3 s^{-1} CO_2 loading), 35,000 LUX and 33^{0}C could best encourage astaxanthin accumulation at 18.21 mg L^{-1} (3.63% by weight). Operating 17 L FP-APBR with these bubble column parameters under the downcomer to riser cross sectional area ratio (A_d/A_r) of 0.4 and superficial gas velocity of 0.4 cm s^{-1} could further enhance astaxanthin to 26.63 mg L^{-1} (5.34% by weight). For the operation of the two-step single reactor system, reducing nitrate and phosphate at 4 and 6-fold of that in the original F1 medium could best encourage astaxanthin accumulation at 28 mg L^{-1} (5.24% by weight). However, similar operation under outdoor condition exhibited slightly poorer performance due to the light inhibition effect. The best outdoor performance was obtained with the FP-APBR covered with 1-layer shading net on the first few days (1-3 days) where 20.9 mg L^{-1} (4.45% by weight) of astaxanthin was resulted. In addition, the various sizes of FP-APBRs exhibited similar performance implying a potential scale-up opportunity. In case of annual cost estimations of astaxanthin induction, results indicated that outdoor system charges were cheaper because this system did not require the use of fluorescent lamps as a light source for astaxanthin induction.

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Field of Study:	Chemical Engineering	Advisor's Signature .....
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CONTENTS

Page

ABSTRACT IN THAI iv
ABSTRACT IN ENGLISH v
ACKNOWLEDGEMENTS vi
CONTENTS vii
LIST OF TABLES xi
LIST OF FIGURES xiii

CHAPTER I INTRODUCTION 1
1.1 Motivations 1
1.2 Objectives 2
1.3 Scopes of this work 3

CHAPTER II BACKGROUNDS AND LITERATURE REVIEW 4
2.1 Astaxanthin 4
2.1.1 Chemical properties of astaxanthin 4
2.1.2 Sources of astaxanthin 5
2.1.2.1 Chemical sources 5
2.1.2.2 Natural sources 6
2.2 *Haematococcus pluvialis* 7
2.2.1 Life cycle of *Haematococcus pluvialis* 7
2.2.2 Astaxanthin in *Haematococcus pluvialis* 11
2.3 Astaxanthin induction step in *Haematococcus pluvialis* 13
2.4 Photobioreactors for the induction of astaxanthin from *H. pluvialis* 17

CHAPTER III EXPERIMENTAL 28
3.1 Algal strain and inoculums culture preparation 28
3.2 Set up of 1.5 L bubble column for studying astaxanthin induction in both indoor and outdoor conditions 30
3.3 Experimental procedure for indoor 1.5 L bubble column 32
3.3.1 Effect of diluted nutrient to astaxanthin induction in 1.5 L bubble column 32
3.3.2 Effect of light intensity to astaxanthin induction in 1.5 L bubble column 32

Page

3.3.3 Effect of carbon dioxide to astaxanthin induction in 1.5 L bubble column 32

3.3.4 Effect of temperature to astaxanthin induction in 1.5 L bubble columns 33

3.4 Set up of large scale bioreactors for indoor and outdoor experiments 34

3.5 Experimental procedure in indoor flat panel airlift photobioreactor 37

3.5.1 Effect of ratio between downcomer per riser cross sectional area (A_d/A_r) of flat panel airlift photobioreactor in indoor system to induction of astaxanthin 37

3.5.2 Effect of Superficial gas velocity (u_{sg}) of flat panel airlift photobioreactor in indoor system to induction of astaxanthin 37

3.5.3 Effect of up-scale of flat panel airlift photobioreactor in indoor system to induction of astaxanthin 38

3.6 Experimental procedure of outdoor flat panel airlift photobioreactor 38

3.6.1 Effect of reducing light intensity to astaxanthin induction in outdoor flat panel airlift photobioreactor 38

3.6.2 Effect of up-scale of flat panel airlift photobioreactor in outdoor system 39

3.7 Experimental procedure by operating of two-step single reactor process 41

3.7.1 Effect of reducing nitrate of sterilized F1 medium to astaxanthin induction by operating of two-step single reactor process 41

3.7.2 Effect of reducing phosphate of sterilized F1 medium to astaxanthin induction by operating of two-step single reactor process 41

Page

3.7.3 Effect of reducing both nitrate and phosphate of sterilized F1 medium to astaxanthin induction by operating of two-step single reactor process 42
3.7.4 Effect of up-scale of flat panel airlift photobioreactor to astaxanthin induction in *H. pluvialis* by operating of two-step single reactor process 43
3.8 Determination of growth 44
3.8.1 Determination of cell density 44
3.8.2 Determination of cell dry weight 46
3.9 Determination of astaxanthin 47
3.10 Determination of astaxanthin productivity 48
CHAPTER IV RESULTS AND DISCUSSION 49
4.1 Determination of suitable indoor conditions for astaxanthin induction from *H. pluvialis* (NIES-144) 49
4.1.1 Effect of diluted nutrient on astaxanthin induction in 1.5 L bubble columns 50
4.1.2 Effect of light intensity on astaxanthin induction in 1.5 L bubble columns 53
4.1.3 Effect of carbon dioxide on astaxanthin induction in 1.5 L bubble columns 55
4.1.4 Effect of temperature on astaxanthin induction in 1.5 L bubble columns 57
4.2 Determination of suitable conditions for astaxanthin induction from *H. pluvialis* (NIES-144) in flat panel airlift photobioreactor systems 59
4.2.1 Effect of ratio between downcomer and riser cross sectional area (A_d/A_r) of flat panel airlift photobioreactor on induction of astaxanthin 60
4.2.2 Effect of superficial gas velocity (u_{sg}) of flat panel airlift photobioreactor on induction of astaxanthin 61
4.2.3 Effect of up-scale of indoor flat panel airlift photobioreactor on induction of astaxanthin 63

Page

4.2.4 Effect of up-scale of flat panel airlift photobioreactor in outdoor system 65
4.3 Determination of suitable conditions for astaxanthin induction from *H. pluvialis* (NIES-144) with a two-step single reactor process 74
4.3.1 Effect of reducing nitrate in sterilized F1 medium on astaxanthin induction with two-step single reactor process 74
4.3.2 Effect of reducing phosphate in F1 medium on astaxanthin induction with two-step single reactor process 77
4.3.3 Effect of reducing nitrate and phosphate in sterilized F1 medium on astaxanthin induction with two-step single reactor process 80
4.3.4 Effect of up-scale in flat panel airlift photobioreactor on astaxanthin induction in *H. pluvialis* with two-step single reactor process 82
4.4 Annual costs estimation for astaxanthin induction from *H. pluvialis* 84
CHAPTER V CONCLUSIONS AND RECOMMENDATIONS 89
5.1 Conclusions 89
5.2 Contributions 90
5.3 Recommendations 91
REFERENCES 92
APPENDIX 98
BIOGRAPHY 100

LIST OF TABLES

Page

Table 2.1 Properties of astaxanthin 5
Table 2.2 Scientific classification of *Haematococcus pluvialis* 7
Table 2.3 General properties of *H. pluvialis* microalgae 11
Table 2.4 Carotenoid content in *H. pluvialis* 11
Table 2.5 Suitable conditions for astaxanthin induction in *H. pluvialis* 14
Table 2.6 Summarizing light intensity/supply conditions for high astaxanthin yield 15
Table 2.7 Effect of nutritional factors for high astaxanthin yield 16
Table 2.8 Advantages and disadvantages of reactor each type 19
Table 2.9 Literature review for astaxanthin induction in *H. pluvialis* 20-27
Table 3.1 Compositions of F1 medium 29
Table 3.2 Geometric details of flat panel airlift photobioreactors 35
Table 3.3 Analyzing conditions of high performance liquid chromatography (HPLC) for astaxanthin content 48
Table 4.1 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction of the culture with sterilized RO water, 0.5x, 0.25x, and 0.1x F1-medium dilution conditions 53
Table 4.2 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration under various light intensity conditions 55
Table 4.3 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under carbon dioxide supplemental conditions 57
Table 4.4 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under various temperature conditions 59
Table 4.5 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration various ratios between downcomer per riser cross sectional area 61
Table 4.6 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration of superficial gas velocities (u_{sg}) 63

Page

Table 4.7 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration in 17, 50 and 90 L FP-APBRs under indoor conditions 65
Table 4.8 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration in 17 L FP-APBR under outdoor conditions 70
Table 4.9 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction in 17, 50 and 90 L FP-APBRs under outdoor conditions with 1-layer shading net on 1-3 days 73
Table 4.10 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under reducing nitrate in TSSRP in 17 L FP-APBR 77
Table 4.11 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under reducing phosphate in TSSRP in 17 L FP-APBR 79
Table 4.12 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under reducing nitrate and phosphate in TSSRP in 17 L FP-APBR 82
Table 4.13 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under reducing nitrate and phosphate in TSSRP with 17, 50 and 90 L FP-APBRs 84
Table 4.14 Annual costs estimation for astaxanthin production from *H. pluvialis* using 17, 50 and 90 L FP-APBRs with indoor system 86
Table 4.15 Annual costs estimation for astaxanthin production from *H. pluvialis* using 17, 50 and 90 L FP-APBRs with outdoor system 87
Table 4.16 Annual costs estimation for astaxanthin production from *H. pluvialis* using 17, 50 and 90 L FP-APBRs with TSSRP 88

LIST OF FIGURES

Page

Figure 2.1 Structure of astaxanthin and numbering scheme 4
Figure 2.2 Life cycle of *H. pluvialis* 8
Figure 2.3 Vegetative motile cell 8
Figure 2.4 *H. pluvialis* in encystment stage 9
Figure 2.5 Immature cyst 10
Figure 2.6 Mature cyst 10
Figure 2.7 Astaxanthin pathways of *H. pluvialis* 12
Figure 3.1 Flow chart of works involved in Part I 30
Figure 3.2 Bottle of 1.5 L for astaxanthin induction 31
Figure 3.3 Schematic diagram of 1.5 L bubble column 31
Figure 3.4 Flat panel airlift photobioreactors 33
Figure 3.5 Work Flow Diagram of Part II 34
Figure 3.6 Schematic diagram of flat panel airlift photobioreactor 36
Figure 3.7 Flow Diagram of all the works in Part III 40
Figure 3.8 (a) Side view of the cell counting chamber showing the cover glass and the space beneath it that holds a microalgae suspension. (b) Top view of the chamber.The chamber had two grids located in the center of the side. (c) An enlarged view of the grid. The microalgae in the squares 1, 2, 3 and 4 were used for cell count. 45
Figure 3.9 Counting cell densities 46
Figure 4.1 Growth behavior of *H. pluvialis* (NIES-144) in 5 and 10 L BC-PBRs and 17 L FP-ALPBR 49
Figure 4.2 Growth behavior of *H. pluvialis* (NIES-144) induced with clean sterilized RO water, 0.5x, 0.25x, and 0.1x F1-medium dilution conditions 51
Figure 4.3 Astaxanthin concentrations from culture induced with sterilized RO water, 0.5x, 0.25x, and 0.1x F1-medium dilution conditions 52
Figure 4.4 Contaminations happened under F1-medium dilution conditions 52
Figure 4.5 Growth behavior of *H. pluvialis* (NIES-144) under various light intensity conditions 54
Figure 4.6 Astaxanthin concentrations under various light intensity conditions 55
Figure 4.7 Growth behavior of *H. pluvialis* (NIES-144) under carbon dioxide induction conditions 56

Page

Figure 4.8 Astaxanthin concentrations under carbon dioxide induction conditions 57
Figure 4.9 Growth behavior of *H. pluvialis* (NIES-144) under temperature induction conditions 58
Figure 4.10 Astaxanthin concentrations under various temperature induction conditions 58
Figure 4.11 Growth behavior of *H. pluvialis* (NIES-144) under various ratios between downcomer per riser cross sectional area 60
Figure 4.12 Astaxanthin concentrations under various ratios between downcomer per riser cross sectional area 61
Figure 4.13 Growth behavior of *H. pluvialis* (NIES-144) at various superficial gas velocities (u_{sg}) 62
Figure 4.14 Astaxanthin concentrations at various superficial gas velocities (u_{sg}) 63
Figure 4.15 Astaxanthin concentrations in 17, 50 and 90 L FP-APBRs under indoor conditions 64
Figure 4.16 Productivity of astaxanthinin 17, 50 and 90 L FP-APBRs under indoor conditions 65
Figure 4.17 Time courses of temperatures and light intensities during the induction under outdoor condition with no shading net (sunlight) in 17 L FP-APBR 66
Figure 4.18 Time courses of temperatures and light intensities during the induction under outdoor condition with 1-layer shading net in 17 L FP-APBR 67
Figure 4.19 Time courses of temperatures and light intensities during the induction under outdoor condition with 2-layer shading net in 17 L FP-APBR 67
Figure 4.20 Time courses of temperatures and light intensities during the inductionunder outdoor condition with 1-layer shading net on 1-3 day in 17 L FP-APBR 68
Figure 4.21 Astaxanthin induction in 17 L FP-APBR under outdoor conditions without shadings 69
Figure 4.22 Productivity of astaxanthin in 17 L FP-APBR under outdoor conditions without shadings 69

Figure 4.23 Time courses of temperatures and light intensities during the induction under outdoor condition with 1-layer shading net on 1-3 days in 50 L FP-APBR 71
Figure 4.24 Time courses of temperatures and light intensities during the induction under outdoor condition with 1-layer shading net on 1-3 days in 90 L FP-APBR 72
Figure 4.25 Astaxanthin inductions in 17, 50 and 90 L FP-APBRs under outdoor conditions with 1-layer shading net on 1-3 days 73
Figure 4.26 Astaxanthin inductions in 17, 50 and 90 L FP-APBRs under outdoor conditions with 1-layer shading net on 1-3 days 73
Figure 4.27 Growth behavior of *H. pluvialis* (NIES-144) under reducing nitrate for TSSRP in 17 L FP-APBR 76
Figure 4.28 Astaxanthin induction under reducing nitrate for TSSRP in 17 L FP-APBR 76
Figure 4.29 Growth behavior of *H. pluvialis* (NIES-144) under reducing phosphate for one-step process in 17 L FP-APBR 78
Figure 4.30 Astaxanthin inductions under reducing phosphate for TSSRP in 17 L FP-APBR 79
Figure 4.31 Growth behavior of *H. pluvialis* (NIES-144) under reducing nitrate and phosphate for TSSRP in 17 L FP-APBR 81
Figure 4.32 Astaxanthin induction under reducing nitrate and phosphate for TSSRP in 17 L FP-APBR 81
Figure 4.33 Growth behavior of *H. pluvialis* (NIES-144) under reducing nitrate and phosphate for TSSRP with 17, 50 and 90 L FP-APBRs 83
Figure 4.34 Astaxanthin induction under reducing nitrate and phosphate for TSSRP with17, 50 and 90 L FP-APBRs 83
Figure 5.1 Possible reactor configuration for a more effective use of light 91

CHAPTER I

INTRODUCTION

1.1 Motivations

Carotenoids are natural pigments found in animals and microorganism such as fungi, bacteria, algae and fruits with red, orange, green and yellow color such as carrots, cantaloupe, spinach and pumpkin etc. Carotenoid are lipid compounds divided into two groups constituting carotenes having red or orange pigment and xanthophylls having have yellow or brown pigment. In plants and algae, carotenoids are vital components in the photosynthetic process along with chlorophyll and other light-harvesting pigments, with antioxidants properties which are important factors of cells and organs degeneration.

Astaxanthin (3, 3′-dihydroxy-crotene-4, 4′-dione) is a natural red pigment in the xanthophyll group/carotenoid family. It is commonly found in some natural aquatic lives such as salmon, trout, caviar, crab shell, shrimp shell and microalga *Haematococcus pluvialis* (Turujman, 1997). In aquaculture, astaxanthin is one of the major carotenoids contributed to the enhancement of colors in aquatic animals. In addition, astaxanthin has ability to protect cell membranes from damage by free radicals which cause cancer. Other benefits of astaxanthin include the prevention of deterioration of kidneys and blood vessels to diabetic patients, restoring of brain cells and blood vessels to brain attack patients, maintaining eyes, adding moisture to dry skin, and increasing muscle strength. At present, astaxanthin is red ketocarotenoid which has the ability of very highly efficient anti-oxidants. It has been reported that astaxanthin has the anti-oxidant ability of more than 6000 times vitamin C, 800 times co-enzyme Q ten, 550 times vitamin E alpha tocopherol, 550 times green tea, 75 times alpha lipoic acid, 40 times beta carotene, and 17 times grape seed extract (Lorenz and Cysewski, 2008).

Currently, astaxanthin can be produced in two ways. The first is the chemical synthesis. This chemical astaxanthin product is called carophyll pink which contains 8% of astaxanthin (Sommer et al., 1992). However, this method is relatively complex and unnatural which poses serious questions regarding the health safety issue, not to mention its relatively high price. In addition, it is believed that astaxanthin from chemical synthesis is less efficient than natural astaxanthin which is produced via bio-synthesis. The second route for producing astaxanthin is generally through the

bio-synthesis in certain microorganisms such as some yeasts and microalgae. The latter is a more preferable technique because this type of astaxanthin is a natural product which is considered safe and believed to be more effective than the synthetic one (Choir et al.,2003; Droop, 1995 and Andersson et al. 2003). Among all of the astaxanthin accumulated microorganisms, *H. pluvialis* has been reported to accumulate the richest amount of astaxanthin with an accumulation of astaxanthin up to 10000-40000 ppm (equivalent to 2-5 % of dry weight) (Lorenz and Cysewski, 2008).

Astaxanthin-rich production in a large system from *H. pluvialis* microalgae has been achieved by the two-step process (Qinglin et al. 2007; Lorenz and Cysewski, 2008). In the first step, *H. pluvialis* vegetative cells are cultured in closed photobioreactor with a well control environment (for pH, temperature, light and nutrient levels). The second step is the induction of astaxanthin of the active vegetative cells by stress conditions such as increasing salinity in culture nutrients, high temperatures, high light intensity, deprivation of nitrate and phosphate and types of the reactor which is important hydrodynamics aspects (Limpanyalert 2008; Panitchakarn 2006; Issarapayup 2009, Lorenz and Cysewski, 2008)

Unfortunately, there have not been reports on a successful large scale cultivation of *H. pluvialis* and nor has the large scale induction system, despite an existence of large scale production plants in a few places in the Israel and Hawaii. In particular, there have not a single report of success in the cultivation of such alga in Thailand and all astaxanthin must be imported (estimated cost of astaxanthin is 2,500-3,000 $/kg) (Lorenz and Cysewski, 2008). Hence, this work aims to study optimal conditions for astaxanthin induction in *H. pluvialis* NIES-144 in a large system by controlling a number of various parameters such as light intensity, nutrient concentration, air flow rate and types of the reactor etc.

1.2 Objectives

The objectives of this work are to:

1. Study the most suitable conditions for the induction of astaxanthin from *H. pluvialis* (NIES-144) in 1.5 L bubble column
2. Study scale-up for the induction of astaxanthin from *H. pluvialis* (NIES-144) in indoor and outdoor systems with flat panel airlift photobioreactor 17, 50 and 90 L, respectively

3. Study astaxanthin induction from *H. pluvialis* (NIES-144) by two-step single reactor process

1.3 Scopes of this work

- Experimental parameters used to determine the suitable conditions for the induction of astaxanthin from *H. pluvialis* (NIES-144) in 1.5 L bubble column are indicated below:

  1.3.1 Study the suitable conditions for induction of astaxanthin from *H. pluvialis* (NIES-144) such as F1 medium nutrients concentration, light intensity, carbon dioxide addition and temperature in indoor systems

- Experimental parameters used to determine the induction conditions of astaxanthin from *H. pluvialis* (NIES-144) in large scale of flat panel airlift bioreactor as indicated below.

  1.3.2 Compare the induction and accumulation of astaxanthin from *H. pluvialis* (NIES-144) in flat panel airlift photobioreactor 17, 50 and 90 L in indoor systems

  1.3.3 Compare the induction and accumulate of astaxanthin from *H. pluvialis* (NIES-144) in flat panel airlift photobioreactor 17, 50 and 90 L in outdoor systems

- Experimental parameters used to determine the induction conditions of astaxanthin from *H. pluvialis* (NIES-144) by two-step single reactor process (TSSRP)

  1.3.4 Study suitable conditions for astaxanthin induction from *H. pluvialis* (NIES-144) by operating of TSSRP in 17 L flat panel airlift photobioreactor by reducing nitrate and phosphate

  1.3.5 Compare astaxanthin induction from *H. pluvialis* (NIES-144) by operating of TSSRP in 50 and 90 L flat panel airlift photobioreactor

CHAPTER II

BACKGROUNDS AND LITERATURE REVIEW

2.1 Astaxanthin

Astaxanthin is one of the major carotenoids found in microalgae, yeast, salmon, trout, krill, shrimp and crayfish (Lorenz and Cysewski, 2008). It is a red pigment which contributes to the color enhancement in aquatic animals and it has been used as a supplement for maintaining complexion and eyesight for people. In addition, it has high antioxidant ability which protects cell membranes from damage by free radicals which cause cancer (Palozza et al., 2009).

2.1.1 Chemical properties of astaxanthin

Astaxanthin or 3, 3'-dihydroxy-ß-carotene-4, 4'-dione is organic chemical which has molecule formula: $C_{40}H_{52}O_4$ (596.86 g/mol molar mass). In standard state (at 25 °C, 100 kPa), its melting and moiling points are 216^{0}C and 774^{0}C, respectively. Therefore, it is solid with a density of 1.071g/mL of density at room temperature and has appearance as red solid powder. Astaxanthin can not be dissolved in aqueous and most organic solvents but can be dissolved in some non-polar solvents such as acetone, dimethyl-sulfoxide and methanol etc. Structure of astaxanthin molecule has two asymmetric carbons located at the 3 and 3′ positions of the benzenoid rings on either of the molecule (Johnson and An, 1991). Properties and structure of astaxanthin are shown in Table 2.1 and Figure 2.1, respectively.

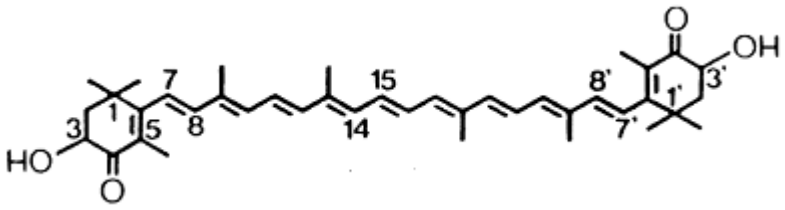


Figure 2.1 Structure of astaxanthin and numbering scheme

Table 2.1 Properties of astaxanthin

Properties	Details
Molecular formula	$C_{40}H_{52}O_4$
Molar mass	596.84 g/mol
Appearance	Red solid powder
Density	1.071 g/mL
Melting point	216^0C
Boiling point	774^0C

2.1.2 Sources of astaxanthin

Chemical and biological syntheses are two sources of astaxanthin production at present. Production with chemical route is rather expensive because it involves potentially harmful process, and the product has unnatural configuration (Parker, 1992). Therefore this chemical synthesis is not popular. The biological synthesis is a lower-priced process, and the product form is of natural configuration. The biological synthesis can be conducted with several microorganisms such as algae, yeast, crustacean meals and other microorganisms. Sources of astaxanthin are shown as follows:

2.1.2.1 Chemical sources

Astaxanthin compounds which is synthesized via chemical methods has been called in market as “carophyll pink”. Its prominent characteristic is flesh concentration which contains minimum 8% astaxanthin by weight. It is different from natural sources synthesis which has less flesh concentration. However, chemical synthesis involves potentially harmful process and the safety procedure requirement raises the price of the product (Sommer et al., 1992, Parker, 1992). Consequently, carophyll pink is more expensive astaxanthin received natural source.

2.1.2.2 Natural sources

A. *Crustaceans products*

Astaxanthin compounds are found in the shell part of crustaceans such as shrimp, crab, krill and crayfish etc. At present, it is not popular to use crustaceans for astaxanthin production because they have relatively low astaxanthin contents and high in moisture, ash, chitin and minerals which cause several practical problems in feed formation that limits their usefulness in animal feed (Bubrick, 1991, Johnson and An, 1991).

B. *Yeast*

Yeast, *Phaffia rhodozyma*, contains astaxanthin in wild strain, but this only presents in small amount, i.e. 200 to 300 mg/kg dry weight (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 (Choir et al., 2003).

C. *Algae*

Astaxanthin can be produced from algae such as *Ankistrodesmus branuii*, *Chlorella zofinglensis*, *Dunaliella salina* (Borowitzka, 1989) and *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).

2.2 *Haematococcus pluvialis*

Haematococcus pluvialis is a freshwater species of Chlorophyta division, Haematococcaceae family. It is referred to as *Haematococcus lacustris* or *Sphaerella lacustris* which is well known for its high content of the strong antioxidant astaxanthin. The scientific classification of *Haematococcus pluvialis* is shown in Table 2.2.

Table 2.2 Scientific classification of *Haematococcus pluvialis* (Smith, 1950)

Scientific	Classification
Domain	Eukaryota
Kingdom	Viridiplantae
Division	*Chlorophyta*
Class	*Chlorophyeeae*
Order	*Volvocales*
Family	Haematococcaceae
Genus	Haematococcus
Species	*Haematococcus pluvialis*

2.2.1 Life cycle of *Haematococcus pluvialis*

H. pluvialis has four stages in its life cycle which are composed of (i) vegetative motile growth stage, (ii) encystment stage, (iii) maturation stage, and (iv) germination stage. Various conditions such as light intensity, temperature, nutrient etc. are induction conditions which affects the transformation stages of *H. pluvialis*. This life cycle of *H. pluvialis* is illustrated in Figure 2.2 and a brief detail of each stage is given as follows:

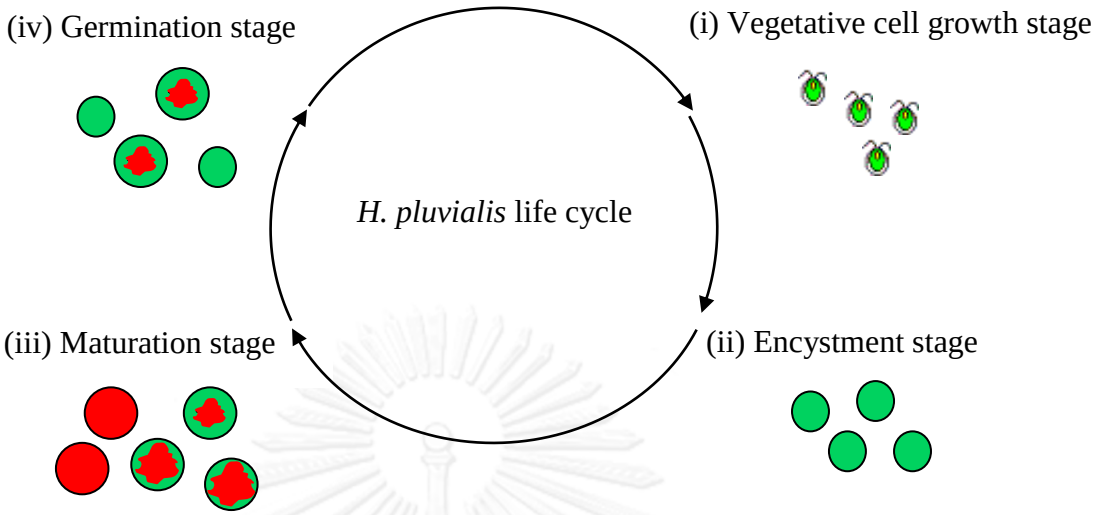


Figure 2.2 Life cycle of *H. pluvialis*

A .Vegetative motile growth stage

The cells enter their vegetative motile growth stage when the cultivation condition is suitable for the growth of *H. pluvialis*. In this stage, cells create chlorophyll A, B and carotenoids which are composed of β-carotene and lutein. Vegetative cells are oval in shape and have two flagella used for movement. In addition, vegetative cells also contain higher protein content than the other stage (Lorenz and Cysewski, 2008). Figure 2.3 is the picture of vegetative motile cell of *H. pluvialis*.

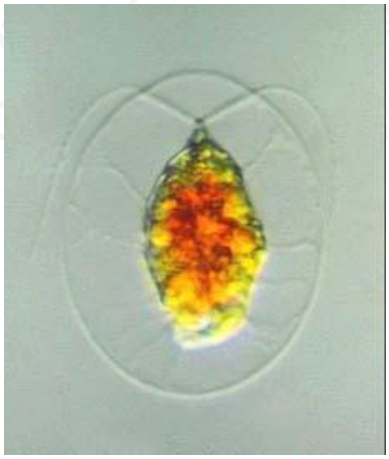


Figure 2.3 Vegetative motile cell
(http://www.biol.tsukuba.ac.jp/~inouye/ino/g/chl/haemato1.jpg)

B .Encystment stage

When vegetative cells are stimulated with stress cultivation conditions such as high light intensity, high saltiness and high temperature etc, it forms a thick skin and disposes of its two flagella, ready to enter its protective stage. In this stage, canthaxanthin carotenoids is increasingly accumulated and appearance of cell is changed from oval to round type. In addition, the cell size becomes larger and no longer moves. *H. pluvialis* in the encystment stage is displayed in Figure 2.4.

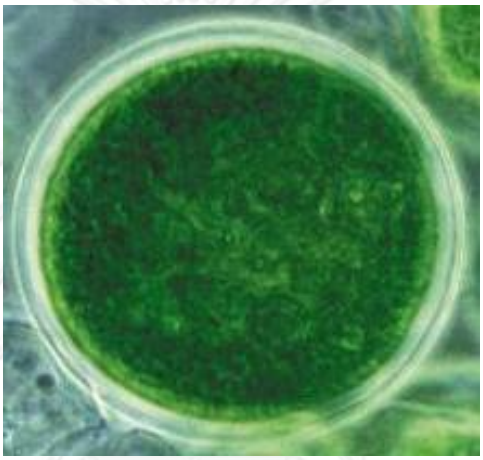


Figure 2.4 *H. pluvialis* in encystment stage
(http://www.biol.tsukuba.ac.jp/~inouye/ino/g/chl/haemato2.jpg)

C. Maturation stage

A long exposure to stress cultivation conditions results in the formation of thick cell skin and astaxanthin carotenoids are increasingly accumulated at nucleus boundary leading to an appearance of red color from the cell center. This character of *H. pluvialis* is called immature cyst (Figure 2.5). After that, astaxanthin is intensively accumulated starting from nucleus boundary to entire body. This consequently leads to a larger and heavier cell. In addition, there have been reports that astaxanthin accumulation in this mature cyst may be able to stimulate astaxanthin more than 3% by weight (Lorenz and Cysewski, 2008). This character of *H. pluvialis* is called mature cyst as shown in Figure 2.6.

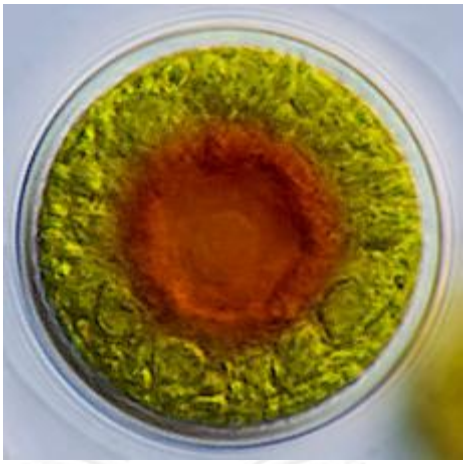


Figure 2.5 Immature cyst

(http://www.biol.tsukuba.ac.jp/~inouye/ino/g/chl/haemato2.GIF)

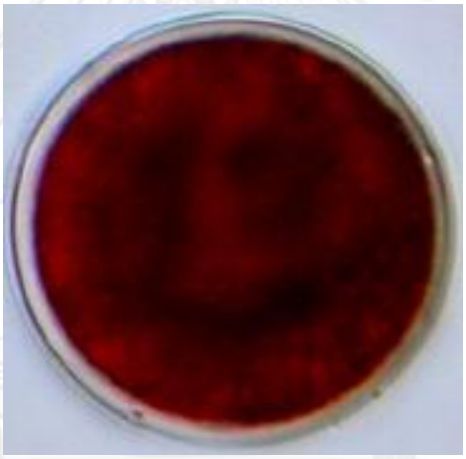


Figure 2.6 Mature cyst

D. Germination stage

Mature cyst cells can be returned to vegetative motile cell again when the environmental condition becomes suitable for growth. Cells start to disintegrate carotenoids and increasingly create protein and chlorophylls A and B. In addition, two flagella which are once fallen off, are again created for movement. After that, cell completely reaches vegetative motile cell.

2.2.2 Astaxanthin in *Haematococcus pluvialis*

The general composition of *H. pluvialis* algae consists of total carotenoids, proteins, carbohydrates, fats, ash, and minerals as shown in Table 2.3. Their main compositions consist of carbohydrates, proteins and fats which assemble more than 70% of the cell weight. For total carotenoids, it consists of 4% lutein, 5% canthaxanthin, 6% β-carotene and 85% astaxanthin (70% monoester, 10% diester, 5% free). These esterified compositions are similar to crustaceans and the natural dietary source of salmon. Total carotenoid content in *H. pluvialis* is shown in Table 2.4 (www.cyanotech.com/pdfs/bioastin/axbul/62.pdf).

Table 2.3 General properties of *H. pluvialis* microalgae

Compositions	Percents
Astaxanthin	>1.5%
Total catrotenoids	>1.75%
Proteins	20-30%
Carbohydrates	30-40%
Fats	7-25%
Ash	5-15%
Moisture	4-9%

Table 2.4 Carotenoid content in *H. pluvialis*

Carotenoids	Percents
Lutein	4%
Canthaxanthin	5%
β-carotene	6%
Astaxanthin monoester	70%
Astaxanthin diester	10%
Astaxanthin free	5%

Astaxanthin is biosynthesized through 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 phytoene and proceeds through lycopene, β–carotene, and canthaxanthin. The astaxanthin biosynthetic pathway of *H. pluvialis* is described in Figure 2.7. 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 (Han et al, 2013).

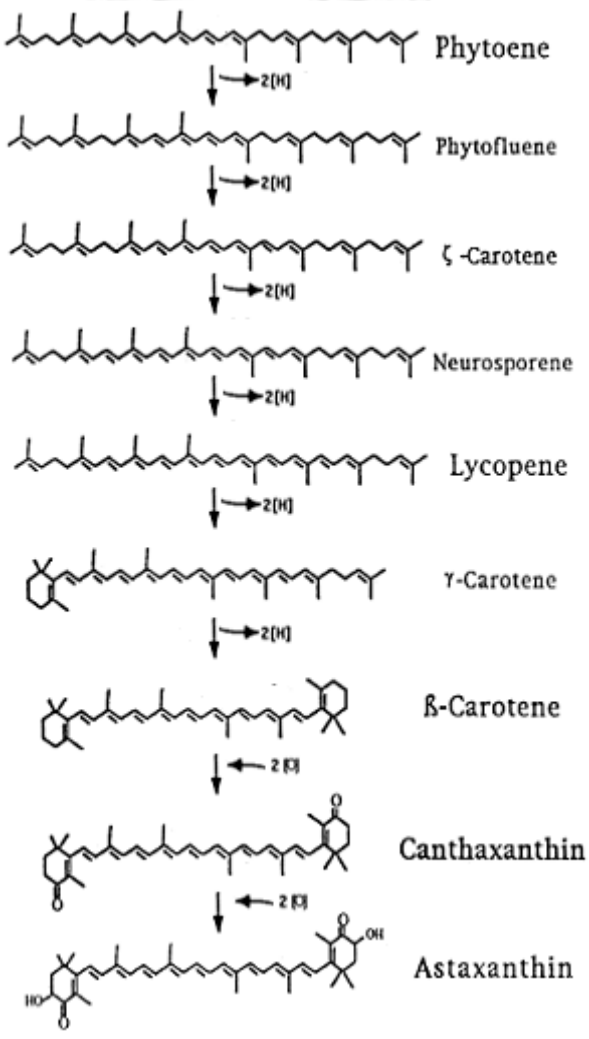


Figure 2.7 Astaxanthin pathways of *H. pluvialis*

2.3 Astaxanthin induction step in *Haematococcus pluvialis*

Stress cultivation conditions can be achieved in several ways such as increasing saltiness, high temperature (Heepchantree, 1997), high light intensity (Qinglin et al., 2007; Zhang et al.; 2009 and Imamoglu et al., 2009), carbon dioxide addition (Kang et. al., 2006) and reduction of major and minor nutrients (He et al., 2007, Imamoglu et al., 2009). In addition, reactor types may involve astaxanthin accumulation due to the differences in hydrodynamic properties (López et al. 2006; Lorenz and Cysewski, 2008; Ranjbar et al., 2008). A summary of suitable conditions for astaxanthin induction in *H. pluvialis* is given in Table 2.5 where details of each parameter are given below.

A. Light intensity

Light is important for algal growth, however, excess light intensity can also be harmful to cells. There are always optimal light intensity and light frequency which could maximize the cell growth. Most research findings reveal that high light intensity affected astaxanthin induction more significantly than low light intensity which was more suitable for vegetative cell growth. However, excess light intensity (> 40,000 LUX) was often found to harm cells (Imamoglu et al., 2009). When analyzed the wave length of lights to astaxanthin induction, blue light (390-550 nm wave length) was the best frequency range because cell could well absorb light in this wave length period for most carotenoids accumulation (Katsuda et al., 2004). However, under some nutrient limiting condition, astaxanthin induction occurred more at low light intensity (7,400 LUX) than at high light intensity (12,580LUX) (He et al., 2007). The summarizing conditions with high astaxanthin yield for effect of light intensity is shown in Table 2.6.

Table 2.5 Suitable conditions for astaxanthin induction in *H. pluvialis*

Factor	Ranges	References
Light	3-18 klux	Qinglin et al.,2007
	0.01-0.6 klux	Katsuda et al., 2006
	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., 1992
N-deficient	0-300 mg/l $NaNO_3$	Qinglin et al.,2007
	0-1000 mg/l $NaNO_3$	Orosa 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
Temperature	28-30 °C	Heepchantree,1997
	29-33 °C	Tjahjono et al., 1994
	29-34°C	Fan et al., 1994
Carbon dioxide	0-5% CO_2	Kang et al., 2006
	0-7% CO_2	Sarada et al., 2001
	0-8% CO_2	Hata et al. , 2001
	0-4 %CO_2	Kaplan and Reinhold, 1999

Table 2.6 Summarizing light intensity/supply conditions for high astaxanthin yield

Reference	Conditions with high astaxanthin yield
Qinglin et al. (2007)	- High light intensity (26,640 LUX) was better than low light intensity (2,520 LUX)
Imamoglu et al. (2009)	- High light intensity (40,404 LUX) was better than low light intensity (32,930 LUX) - Excess light intensity (40,404 LUX) harms cells
Katsuda et al. (2006)	- Vegetative motile cell grows better with red light (567-700 nm wave length) - Astaxanthin induction in cyst was better with blue light (390-550 nm wave length)
He et al. (2007)	- Low light intensity (7,400 LUX) with no phosphorus in the medium was better than high light intensity (12,580LUX) with full medium

B. Nutritional factors

Nutrient manipulation is a popular technique for inducing astaxanthin from *H. pluvialis*. Several nutrients such as N, P, S, and Fe are reported to affect the induction of astaxanthin. For instance, nitrogen depletion could encourage astaxanthin more than phosphorus depletion (Imamoglu et al. 2009 and Brinda et al., 2004) but the growth of *H. pluvialis* decreased more with nitrogen starvation. He et al. (2007) reported that effect of nutrient starving could encourage more astaxanthin than the effect of light intensity. Literature suggests that major nutrient (N and P) reduction could encourage astaxanthin better than minor nutrient (S, Fe) reduction (as 25 pg/cell of phosphorus deprivations). When consider reduction between major nutrients (N and P), N reduction could encourage astaxanthin better than P reduction (Brinda et al., 2004). However, excess N reduction also led to a lower growth. Orosa et al. (2005) indicated that cell stopped growth when medium was without N. Meanwhile, cells could still grow but at a slow rate when medium was without P. In addition, there has been report that distilled water without nutrients reduction could encourage astaxanthin in the similar level with the medium without N and P. The

summarizing conditions with high astaxanthin yield for effect of nutritional factors is shown in Table 2.7.

Table 2.7 Effect of nutritional factors for high astaxanthin yield

Reference	Conditions with high astaxanthin yield
Imamoglu et al. (2009)	- Distilled water without nutrients and medium without N and P condition could similarly encourage astaxanthin.
He et al (2009)	- Major nutrient reduction (N and P) could encourage astaxanthin better than minor nutrient reduction (S, Fe)
Orosa et al. (2005)	- When medium is without N, cell growth stopped and astaxanthin was accumulated. - When medium is without P, cell grows slightly and astaxanthin was accumulated but not as high as that with N starvation.
Brinda et al. (2004)	- N reduction could encourage astaxanthin better than P reduction

C. Temperature

Temperature is one of the major growth parameters for algae. For the growth of vegetative motile cell of *H. pluvialis*, suitable temperatures were in the range of 15-28°C (Kaewpintong, 2006). For mature cyst stage, suitable temperature fell above 30°C. Heepchantree (1997) stated that as much as 16 pg/cell of astaxanthin could be induced best at 30°C, 7,400 LUX and 0.2 w/v NaCl. However, cells were easy to die at temperature more than 30°C so the control of temperature of the growth stage of *H. pluvialis* was quite important (Tjahjono et al., 1994). Fan et al., 1994 indicated that cells at 33°C could stimulate most astaxanthin (0.5µg/ml).

D. Carbon dioxide

Literature indicated that carbon dioxide addition facilitated the induction of astaxanthin in *H. pluvialis*. Kang et al. (2006) reported that 5% CO_2 (air flow rate 65 mL/min) could stimulate most astaxanthin which was at 75 mg/g (7.5% astaxanthin by wt). In addition, CO_2 was also used to control pH for astaxanthiun induction. There have been researches which indicated that optimal pH for astaxanthin accumulation was estimated at 7 and significantly lower at pH 6 (Sarada et al., 2001 and Hata et al., 2001).

E. Salt Stress

Induction under salt stress condition is one of the methods for astaxanthin induction from *H. pluvialis*. There have been reports that adding NaCl to culture could promote astaxanthin induction but death rate of cells also increased. Heepchantree (1997) examined the effect of adding NaCl to *H. pluvialis* culture at 7,400LUX light intensity. The results indicated that 0.2% (w/v) NaCl could encourage 16 pg/cell of astaxanthin. However, cell density dropped more than half of the original concentration (Heepchantree, 1997). These results resembled those of Panitchakarn's (2007) who found a decreasing cell density with salt concentration in the medium. Harker et al. (1995) compared sodium chloride (NaCl) potassium chloride (KCl) for astaxanthin induction. The results indicated that using NaCl was more effective in stimulating astaxanthin than potassium chloride.

2.4 Photobioreactors for the induction of astaxanthin from *H. pluvialis*

Reactor types which provide different hydrodynamic behaviors may also have effects on astaxanthin accumulation. There are quite a number of research articles on the comparison of reactor types for astaxanthin induction from *H. pluvialis* (López et al., 2006; Ranjbar et al., 2008; Suh et al., 2006; Li et al., 2011 and Limpanyalert, 2008). Li et al. (2008) reported that oxygen in air could be associated with the induction of astaxanthin. Therefore, hydrodynamics aspect in the reactor may involve astaxanthin induction. Literature illustrates that there have been several reactor types used for stimulating astaxanthin. Tubular reactors were one type used for astaxanthin induction as it provided reasonably good light exposure (López et al., 2006). Airlift photobioreactor was found to give a better performance than bubble columns because it had a better liquid circulation (Ranjbar et al., 2008 and

Limpanyalert, 2008). In 2006, Suh et al. proposed a novel airlift photobioreactor where the inner tube, a riser, was designed for a vegetative growth. The outer area or downcomer with higher light intensity was used to stimulate astaxanthin. This was clamined to be able to cultivate vegetative cell and mature cyst at the same time. The results indicated that final cell density ($5.5x10^5$cell/mL or 25 mg/mL) was obtained at 12 days and astaxanthin content was the highest at 25 days ($3.8x10^5$cell/mL or 357 mg/mL. Recently, 8,000L raceway reactor was used to stimulate astaxanthin in outdoor conditions. The results indicated that this system could stimulate 12 µg/mL astaxanthin and 0.4 g/L dry weight (Li et al., 2011).

From literature review above, the advantages and disadvantages of reactors for the cultivation of *H. pluvialis* and the induction of astaxanthin can be summarized as shown in Tables 2.8 and 2.9. In addition, the conclusion on the characteristics of the reactor for the production of astaxanthin could be summarized as follows:

- cultivate Vegetative cell and Mature cyst in one-step
- provide more area for light intensity
- provide some means for heat transfer to reduce temperature due to radiation
- promote good liquid circulation to ensure effective light utilization of the circulating cells
- To have system protect precipitate of cell due to weight increasing Mature cyst

Table 2.8 Advantages and disadvantages of reactor each type

Reactor types	Advantages	Disadvantages	Reference
Tubular reactor	Area received high light	High heat accumulation	López et al., 2006
Raceway	Good light receiving (outdoor case)	To use more circulation area and cell is easily harmed due to slice force (propeller case)	Li et al., 2011
Vertical airlift photobioreactor	Good liquid circulation and good precipitating protect	Up-scale is rather hard due to be vertical	Limpanyalert, 2008 Ranjbar et al., 2008
Novel airlift photobioreactor	Cultivate vegetative cell and mature cyst at the same time	Light intensity is not stable due to area received light shaded each other	Suh et al., 2006
Bubble column bioreactor	Reduce slice force due to use bubble	Easy precipitation cell	Limpanyalert, 2008 Lopez et al., 2006) Ranjbar et al., 2008

Table 2.9 Literature review for astaxanthin induction in *H. pluvialis*

Ref.	Reactor	Medium	Initial cell	Condition				Final cells	Astaxanthin	% Astaxanthin (by weight)
				Light Intensity (kLUX)	Light source	pH	Temp (°C)			
Qlnglin et al., 2007	Flask 250 mL (30 mL working)	BBM* (N-free)	1 g/L	26.64	Floures-cent lamp	-	23	1.09 g/L	-	3.12 (7 Days)
	Flask 250 mL (30 mL working)	BBM* (N-repletion)	1 g/L	26.64	Floures-cent lamp	-	23	1.64 g/L	-	3.3 (7 Days)
Imamoglu et al., 2009	Flask 1 L (600 mL working)	distilled water + 1.5% CO_2 v/v	0.26 g/L	40.404	Floures cent lamp	< 8	29	-	29.62 mg/g	2.96 (14 Days)
	Flask 1 L (600 mL working)	RM*** (N-free) + 1.5% CO_2 v/v	0.26 g/L	40.404	Floures cent lamp	< 8	29	-	30.07 mg/g	3.07 (14 Days)

Table 2.9 (Continued) Literature review for astaxanthin induction in *H. pluvialis*

Ref.	Reactor	Medium	Initial cell	Condition				Final cells	Astaxanthin	% Astaxanthin (by weight)
				Light Intensity (kLUX)	Light source	pH	Temp (°C)			
Katsuda et al., 2006	Glass vessel (55 mL working)	Kobayashi basal medium	0.02 g/L	0.592 (Flashing light 100 Hz)	Bule LED lamp	6.8	20	0.5 g/L (4.5×10^5 cells/mL)	28 µg/mL	5.6 (12.5 Days)
He et al., 2007	Flask 250 mL	EGJM (free S and P)	2.6×10^5 cells/mL	7.4	Floures cent lamp	7-7.22	23	-	30 pg/cell (4 Days)	-

* BBM = Bold's basal medium

** PTP-2 = Primary-treated piggery wastewater diluted four-fold

*** RM = Rudic's medium

Table 2.9 (Continued) Literature review for astaxanthin induction in *H. pluvialis*

Ref.	Reactor	Medium	Initial cell	Condition				Final cells	Astaxanthin	% Astaxanthin (by weight)
				Light Intensity (kLUX)	Light source	pH	Temp (°C)			
He et al., 2007	Flask 250 mL	EGJM (free S and P)	3.2 x10^5 cells/mL	7.4	Floures cent lamp	7	23	-	25 pg/cell (4 Days)	-
Orosa et al., 2005	Mini bioreactor 400 mL	BBM* add Acetate 2% w/v (20%$NaNO_3$ of full medium)	< 1 x10^4 cells/mL	5.0505 *12 dark:12 light*	Floures cent lamp	7	18	-	49.3 pg/cell	-
Brinda et al., 2004	Flask 250 mL (40 mL working)	BBM* (reduce N and P 10 times	-	2-2.002	Floures cent lamp	8	25	1.26 g/L (12.6 x10^4 cells/mL)	34.39 µg/mL	2.2

* BBM = Bold's basal medium

Table 2.9 (Continued) Literature review for astaxanthin induction in *H. pluvialis*

Ref.	Reactor	Medium	Initial cell	Condition				Final cells	Astaxanthin	% Astaxanthin (by weight)
				Light Intensity (kLUX)	Light source	pH	Temp (°C)			
Tjahjono et al., 1994	Flask 250 mL	BBM*	$3.1x10^4$ cell/mL	3.2	Floures cent lamp		34	$2.1x10^4$ cell/mL	21 pg/cell	-
Fan et al., 1994	Flask 250 mL	BBM*	$1.4x10^4$ cell/mL	4.5	Floures cent lamp		33	$1.3x10^4$ cell/mL	0.5µg/ml	-
Heepchantree, 1997	Flask	BG-11	$2x10^4$ cell/mL	7.4	Floures cent lamp	-	30	$2.2x10^4$ cell/mL	16 pg/cell	-

* BBM = Bold's basal medium

Table 2.9 (Continued) Literature review for astaxanthin induction in *H. pluvialis*

Ref.	Reactor	Medium	Initial cell	Condition				Final cells	Astaxanthin	% Astaxanthin (by weight)
				Light Intensity (kLUX)	Light source	pH	Temp (°C)			
Kaplan and Reinhold, 1999	Flask	NIES-C medium BM (0.25%NaCl in 4.4mM Sodium acetate)	$2x10^4$ cell/mL	1.2	-	- (4% CO_2)	-	-	0.05 µg/mL	- (10 Days)
Sarada et al., 2001	Flask		-	-	-	7 (7% CO_2)	25	-	5.4 µg/mL	- (10 Days)
Hata et al. , 2001	Flask 200 mL	Kobayashi' basal medium	$2x10^4$ cell/mL	0.4	Floures-cent lamp	6.8 (8% CO_2)	20	-	0.08 µg/mL	-
Kang et al., 2006	Flask 200 mL	NIES-C medium	0.33g/L	1.48	-	- (5% CO_2)	23	-	77 mg/g	7.7 (10 Days)

Table 2.9 (Continued) Literature review for astaxanthin induction in *H. pluvialis*

Ref.	Reactor	Medium	Initial cell	Condition				Final cells	Astaxanthin	% Astaxanthin (by weight)
				Light Intensity (kLUX)	Light source	pH	Temp (°C)			
Heepchantree, 1997	Flask	BG-11 (0.2% NaCl w/v)	$2x10^4$	7.4	Floures cent lamp	-	31	$2.1x10^4$	19 pg/cell	-
Harker et al., 1995	Flask 250 ml	BBM (0.5% NaCl w/v)	$1.25x10^4$ cell/mL	1.75	Cool white fluores-cent	-	22	$5x10^4$	25 µg/mL	-
	Flask 250 ml	BBM (0.5% KCl w/v)	$1.25x10^4$ cell/mL	1.75	Cool white fluores-cent	-	22	$5x10^4$	17.5 µg/mL	-
Panitchakarn, 2007	17 L Bubble Column	BG-11 (1% NaCl w/v)	$3 x10^4$ cell/mL (Only Vegetative cell)	5.4	Floures cent lamp	-	27	$1 x10^4$ cell/mL (Only Mature cyst)	-	-

Table 2.9 (Continued) Literature review for astaxanthin induction in *H. pluvialis*

Ref.	Reactor	Medium	Initial cell	Condition				Final cells	Astaxanthin	% Astaxanthin (by weight)
				Light Intensity (kLUX)	Light source	pH	Temp (°C)			
López et al., 2005	Turbular photo bioreactor 55 L	Inorganic medium free acetate	-	9 (Average)	Sunlight	8	20	7 g/L (5×10^6 cells/mL)	-	1.1 (16 days)
	Bubble column 55 L	Inorganic medium free acetate	-	9 (Average)	Sunlight	8	20	1.4 g/L (3.8×10^6 cells/mL)	-	0.2 (16 Days)
Suh et al., 2006	Outner of novel air-lift photobioreactor 500 mL	BBM* (free N) + 5% CO_2 v/v	2×10^5 cells/mL	56.98	Floures cent lamp	-	25	2.5×10^5 cells/mL	-	5.79 (14 Days)
Li et al., 2011	Race way 8000 L	-	6×10^4 cells/mL	-	Sunlight	8 Control by CO_2	< 25	0.4 g/L	12 µg/mL	2.8 (9 Days)

* BBM = Bold's basal medium

Table 2.9 (Continued) Literature review for astaxanthin induction in *H. pluvialis*

Ref.	Reactor	Medium	Initial cell	Condition				Final cells	Astaxanthin	% Astaxanthin (by weight)
				Light Intensity (kLUX)	Light source	pH	Temp (°C)			
Ranjbar et al., 2008	Airlift photobioreactor	Kobayashi basal medium (feed medium for fresh NO_3^- until 8.33 days after pour culture and add distilled water in full)	0.033 g/L	1.591 (0-3.66 days) 3.589 (3.66-8.33 days) 6.978 (8.33-33.33 days)	Floures cent lamp	-	20	-	59 µg/mL (29.1 Days)	-
Limpanyalert (2008)	2.7L Airlift photobioreact	10-fold F1 medium dilution	$1x10^4$ cell/mL	6.5	Floures cent lamp	-	31	$1.7x10^4$ cell/mL	15 µg/mL	3.81 (10Days)
	1.5L Bubble column	10-fold F1 medium dilution	$1x10^4$ cell/mL	6.5	Floures cent lamp	-	31	$1.4x10^4$ cell/mL	13 µg/mL	2.73 (12Days)

CHAPTER III

EXPERIMENTAL

3.1 Algal strain and inoculums culture preparation

H. pluvialis (NIES-144) obtained from the Microbial Culture Collection, National Institute for Environmental Studies, Japan, was used in experiments. Initial inoculums were cultivated in 250 mL Erlenmeyer flasks with the sterilized F1 medium (Fabregas et al., 1998). Table 3.1 shows the composition of the sterilized F1 medium. The temperature in the range of 25±2°C, and light intensity of 2,000 LUX were given to Erlenmeyer flasks. As cells grew in vegetative motile stage, they were scaled up to the 5 and 10 L glass bottles at the same condition. After that, the culture was scaled up again to 17 and 90 L flat panel airlift photobioreactors with the ratio between downcomer per riser cross sectional area (A_d/A_r) of 0.4 and superficial gas velocity (u_{sg}) of 0.4 cm s^{-1} where carbon dioxide rich air (1% vol) was supplied to the system. Temperature of the flat panel airlift photobioreactors was controlled with an evaporator room in the range of 25±4°C. When inoculums entered exponential growth state in the flat panel airlift photobioreactor, they were separated into supernatant and cell pellets using centrifugation at 3,500 rpm (2,054xg), 15°C in 5 min. Thereafter, cell pellets were transferred to reactors for astaxanthin induction. All of these experiments were started with an initial cell density of about 0.16 g L^{-1}.

Table 3.1 Compositions of F1 medium (Fabregas et al., 1998)

Substance	Amount (mg L^{-1})
$CaCl_2 \cdot H_2O$	9.87
KNO_3	410
Na_2HPO_4	300
$C_6H_5FeO_7 \cdot H_2O$	2.22
$MgSO_4 \cdot 7H_2O$	16.41
$CUSO_4 \cdot 5H_2O$	0.008
$Na_2MoO_4 \cdot 2H_2O$	0.08
MoO_3	0.66
Cr_2O_3	0.05
SeO_2	0.036
$CoCl_2 \cdot 6H_2O$	0.0078
$NH_4Fe(C_6H_5O_7)$	6

Part I: Determination of suitable conditions for astaxanthin induction from *H. pluvialis* (NIES-144) in both indoor and outdoor conditions

The works for Part I was performed using 2 L bubble column bioreactor (1.5 L working volume) for testing suitable conditions for astaxanthin induction from *H. pluvialis* (NIES-144). Experiments were studied in indoor system by varying nutrients concentration, light intensity, carbon dioxide, and temperature. The number of cells transformed into motile cells, non-motile cells, immature cyst and mature cyst each day were counted through microscope according to Section 3.8.1 and an amount of astaxanthin was analyzed daily with high-performance liquid chromatography (HPLC) according to Section 3.9. In addition, dry weight was collected on the last day of experiments (14 days) according to Section 3.8.2. A flow chart for the work involved in Part I is displayed in Figure 3.1.

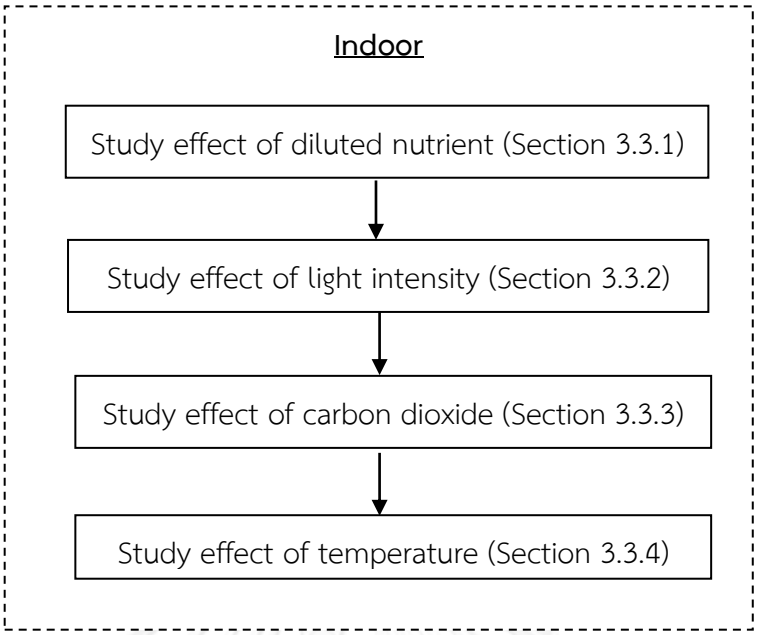


Figure 3.1 Flow chart of works involved in Part I

3.2 Set up of 1.5 L bubble column for studying astaxanthin induction in both indoor and outdoor conditions

The 2 L Duran bottles (diameter 12.5 cm, height 16.3 cm (1.5 L working volume), Figure 3.2) were used for the astaxanthin induction from *H. pluvialis* (NIES-144). Filtered air and carbon dioxide were pumped to the mixing bottle before passing to the flow meter and the porous gas sparger at the central bottom of column. The light source used to supply the required light intensity was the 20 W fluorescent lamps. The spray water and air flow were used to cool the system at the required temperature (to remove the energy from fluorescent lamps). The bioreactor was sterilized prior to use by autoclave at 121^{0}C for 20 min. In addition, the digital LX-5 LUX meter (model LX-50 Digicon) was used to measure the light intensity inside column and average values were reported. A schematic diagram of 1.5 L bubble column in indoor condition is displayed in Figure 3.3.

Figure 3.2 Bottle of 1.5 L for astaxanthin induction

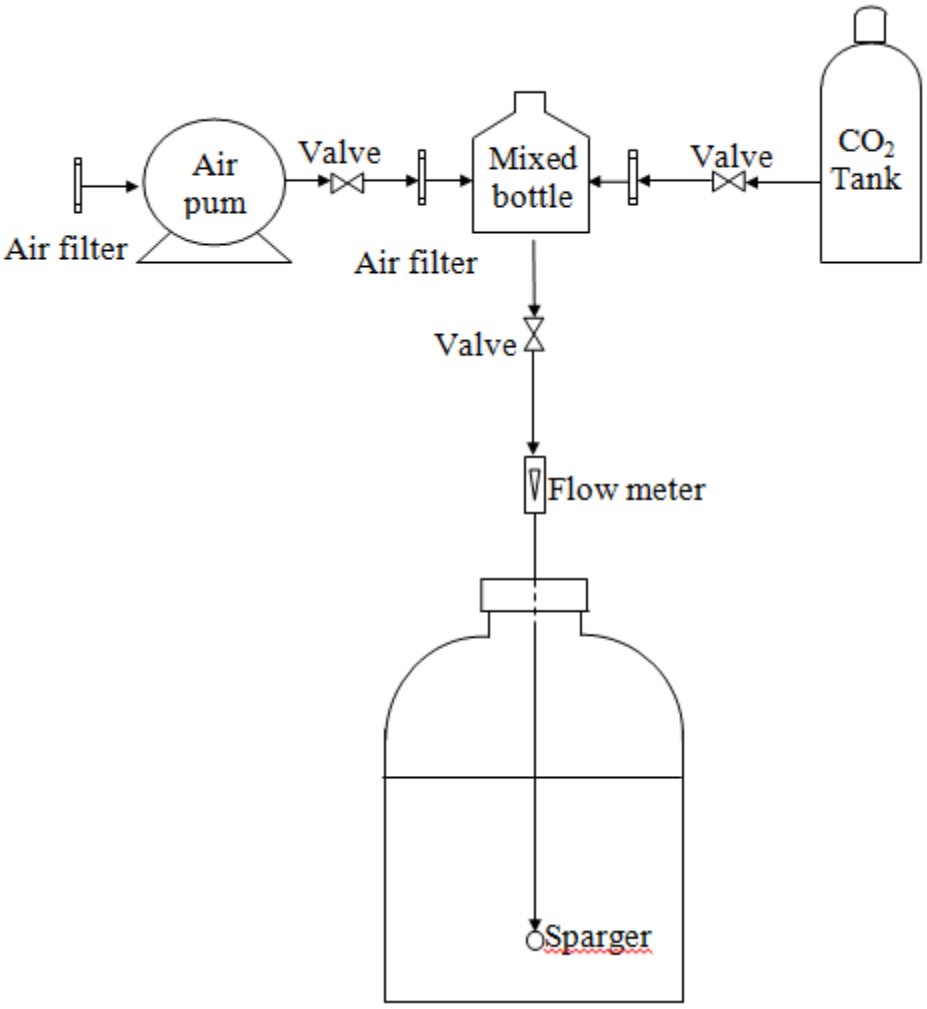


Figure 3.3 Schematic diagram of 1.5 L bubble column

3.3 Experimental procedure for indoor 1.5 L bubble column

3.3.1 Effect of diluted nutrient to astaxanthin induction in 1.5 L bubble column

1) When the cultured algal cells reach exponential growth phase, separate cells from the nutrient by the centrifuge at 3,500 rpm (2,054xg), temperature 15^0C for 5 min
2) Prepare 1.5 L sterilized reverse osmosis water in the bubble column, sterilized by autoclave at 121^0C for 20 min
3) Transfer 0.16 g L^{-1} isolated cells to the sterilized 1.5 L bubble column reactor
4) Dilute isolated nutrient in Step one with distilled water 0-10 times, thereafter mix it to isolated cells in the 1.5 L bubble column reactor
5) Control constant light intensity and superficial gas velocity (u_{sg}) at 35,000 LUX and 0.4 cm s^{-1} throughout the experiment
6) Count the cells (motile cells, non-motile cells, immature cyst, and mature cyst) daily until end of the experiment (14 days)
7) Take 5 mL sample everyday until end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
8) On the last day of the experiment, collect 1 L sample to measure dry weight

3.3.2 Effect of light intensity to astaxanthin induction in 1.5 L bubble column

1) Repeat Steps 1-3 in Section 3.3.1
2) Use the best condition obtained from Section 3.3.1
3) Control superficial gas velocity (u_{sg}) at 0.4 cm s^{-1} throughout the experiment
4) Provide light at 5,000 – 40,000 LUX
5) Repeat Steps 6-8 in Section 3.3.1

3.3.3 Effect of carbon dioxide to astaxanthin induction in 1.5 L bubble column

1) Repeat Steps 1-3 in Section 3.3.1
2) Use the best conditions from Sections 3.3.1 and 3.3.2, respectively
3) Control superficial gas velocity (u_{sg}) at 0.4 cm s^{-1} throughout the experiment
4) Apply of carbon dioxide in the air 0, 1, 2, 3, 4 and, 5%
5) Repeat Steps 6-8 in Section 3.3.1

3.3.4 Effect of temperature to astaxanthin induction in 1.5 L bubble columns

1) Repeat Steps 1-3 in Section 3.3.1
2) Use the best conditions from Sections 3.3.1 - 3.3.3, respectively
3) Vary experiment temperature by flowing cold air and cooling water around the reactor
4) Repeat Steps 6-8 in Section 3.3.1

Part II: Determination of suitable conditions for astaxanthin induction from *H. pluvialis* (NIES-144) in large scale flat panel airlift photobioreactor systems

The works for Part II were performed in flat panel airlift photobioreactor systems as shown in Figure 3.4. Best conditions of part I include diluted nutrient, carbon dioxide, light intensity, temperature and covering shading net were employed in this part. A number of cells that change into motile cells, non-motile cells, immature cyst and mature cyst each day were counted through microscope according to Section 3.8.1 and an amount of astaxanthin which occurs each day was analyzed with high-performance liquid chromatography (HPLC) according to Section 3.9. In addition, dry weight was collected on end days (14 days) of experiments according to Section 3.8.2. A flow diagram of Part II is shown Figure 3.5.

Figure 3.4 Flat panel airlift photobioreactors

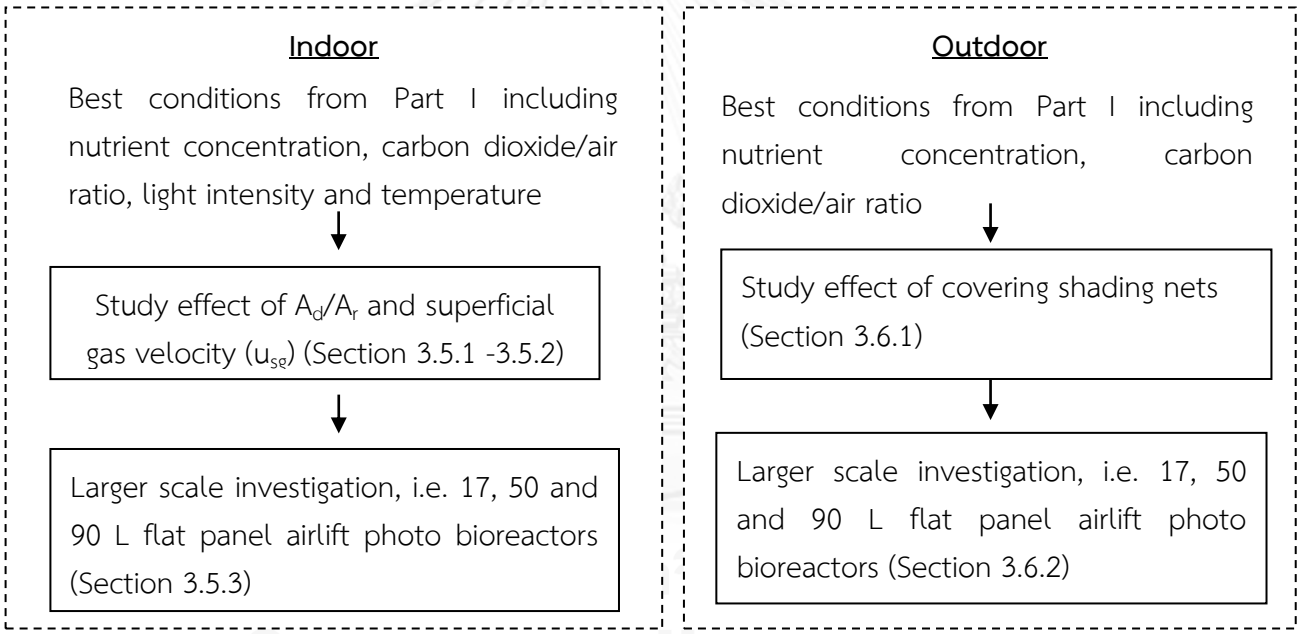


Figure 3.5 Work Flow Diagram of Part II

3.4 Set up of large scale bioreactors for indoor and outdoor experiments

The 17, 50 and 90 L flat panel airlift photobioreactors were used to study astaxanthin induction from *H. pluvialis* (NIES-144). Geometric details and schematic diagram are displayed in Table 3.2 and Figure 3.6, respectively. The acrylic plastic thicknesses which were 5 and 2 mm were used to be column and draft tube, respectively. Air filtered through 0.45×10^{-6} m Gelman filter and carbon dioxide were pumped to mixing bottle before passing to flow meter and the porous gas sparger at

the bottom of the riser section. Superficial gas velocity (u_{sg}) of air and carbon dioxide was 0.4 cm s^{-1}. Light intensity from fluorescent lamps (20 W) was given to both sides of the bioreactors. The bioreactors were sterilized using chlorine (as NaOCl). For the Indoor case, the flat panel airlift photobioreactors were studied in the evaporator room for controlling temperature. The digital LX-5 LUX meter (model LX-50 Digicon) was used to measure the light intensity inside column and average values were reported.

Table 3.2 Geometric details of flat panel airlift photobioreactors

	Symbols	17 L	50 L	90 L
Total volume (L)		19	55	95
Working volume (L)		17	50	90
Column height (cm)	H_F	50	50	50
Column length (cm)	L_F	20	55	100
Column width (cm)	W_F	20	20	20
Draft tube height (cm)	H_D	30	30	30
Bottom clearance (cm)	H_C	10	10	10

For the outdoor cases, a similar set up as the indoor system was employed but with natural light source. The shading net was used to reduce light intensities and temperatures from sunlight. Light sensor was used to measure light intensities from sunlight and record it to recorder (model Vernier LabQuest Instument). The rise temperature from sunlight was reduced by cooling water. In addition, continuous in temperatures were measured by thermocouple and recorded to the recorder (Hanna Instument).

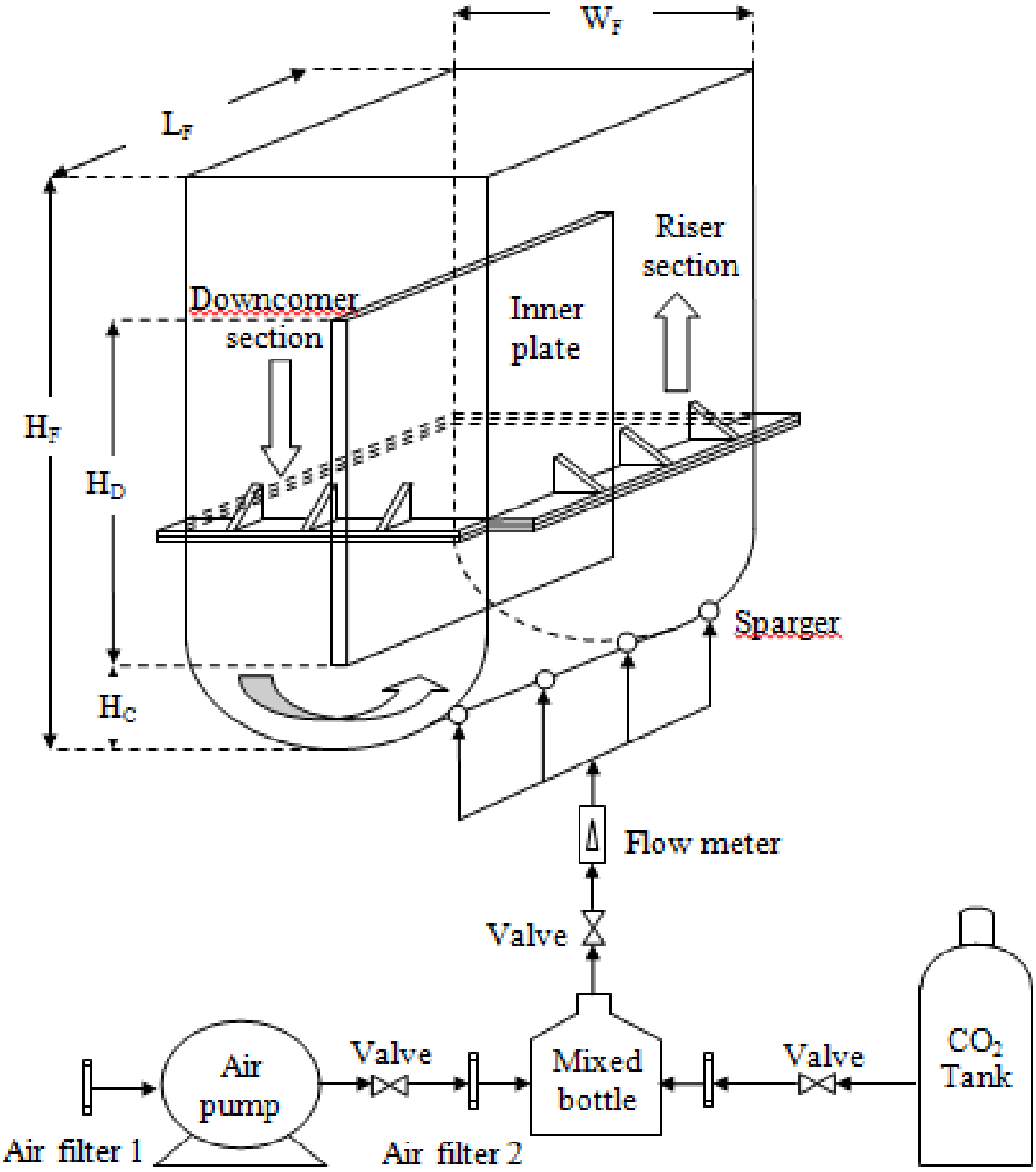


Figure 3.6 Schematic diagram of flat panel airlift photobioreactor

3.5 Experimental procedure in indoor flat panel airlift photobioreactor

3.5.1 Effect of ratio between downcomer per riser cross sectional area (A_d/A_r) of flat panel airlift photobioreactor in indoor system to induction of astaxanthin

1) When the cultured algal cells reach exponential growth phase in 17 L flat panel airlift photobioreactor, separate cells from the nutrient by centrifuge at 3,500 rpm (2,054 x g), 15 ^{0}C for 5 min
2) Chlorinate the tap water in the bioreactor, wait until disintegrates chlorine
3) Transfer the 0.16 g L^{-1} isolated cells to bioreactor
4) Control superficial gas velocity (u_{sg}) at 0.4 cm s^{-1} throughout the experiment
5) Use the best conditions from Sections 3.3.1 - 3.3.4, respectively
6) Apply A_d/A_r of 0.2, 0.4, 0.6, and 0.8
7) Count the cells (motile cells, non-motile cells, immature cyst, and mature cyst) everyday until end of the experiment (14 days)
8) Take 5 mL of sample everyday until end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
9) On the last day of the experiment, collect 1 L sample to measure dry weight

3.5.2 Effect of Superficial gas velocity (u_{sg}) of flat panel airlift photobioreactor in indoor system to induction of astaxanthin

1) When the cultured algal cells reach exponential growth phase in 17 L flat panel airlift photobioreactor, separate cells from the nutrient by centrifuge at 3,500 rpm (2,054 x g), 15 ^{0}C for 5 min
2) Chlorinate the tap water in the bioreactor, wait until disintegrates chlorine
3) Transfer the 0.16 g L^{-1} isolated cells to bioreactor
4) Control 0.4 of A_d/A_r throughout the experiment
5) Use the best conditions from Sections 3.3.1 - 3.3.4, respectively
6) Apply of 0.2, 0.4, 0.6 and, 0.8 cm s^{-1} superficial gas velocity (u_{sg})
7) Count the cells (motile cells, non-motile cells, immature cyst, and mature cyst) everyday until end of the experiment (14 days)

8) Take 5 mL of sample everyday until end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
9) On the last day of the experiment, collect 1 L sample to measure dry weight

3.5.3 Effect of up-scale of flat panel airlift photobioreactor in indoor system to induction of astaxanthin

1) When the cultured algal cells reach exponential growth phase in 17 L flat panel airlift photobioreactor, separate cells from the nutrient by centrifuge at 3,500 rpm (2,054 x g), 15 ^{0}C for 5 min
2) Chlorinate the tap water in the bioreactor, wait until disintegrates chlorine
3) Transfer the 0.16 g L^{-1} isolated cells to bioreactor
4) Use the best conditions from Sections 3.3.1 - 3.3.4, 3.6.1 and, 3.6.2, respectively
5) Count the cells (motile cells, non-motile cells, immature cyst, and mature cyst) everyday until end of the experiment (14 days)
6) Take 5 mL of sample everyday until end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
7) On the last day of the experiment, collect 1 L sample to measure dry weight
8) Repeat Steps 1-9 but experiment in 50 and 90 L flat panel airlift photobioreactor

3.6 Experimental procedure of outdoor flat panel airlift photobioreactor

3.6.1 Effect of reducing light intensity to astaxanthin induction in outdoor flat panel airlift photobioreactor

1) When the cultured algal cells reach exponential growth phase in 17 L flat panel airlift photobioreactor, separate cells from the nutrient by the centrifuge at 3,500 rpm (2,054 x g), 15 ^{0}C for 5 min

2) Chlorinate the tap water in the bioreactor, wait until disintegrates chlorine
3) Transfer the 0.16 g L^{-1} isolated cells to bioreactor
4) Reduce the temperature of bioreactor by spraying cooling water around the reactor
5) Use the best conditions from Sections 3.3.1 - 3.3.3 , 3.6.1 and, 3.6.2, respectively
6) Apply shading net to reduce the light intensity during day ,i.e.1 layer, 2 layers, and 1 layer on the first three days
7) Record light intensity and temperature with a light sensor and thermocouple (with recorder)
8) Count the cells (motile cells, non-motile cells, immature cyst, and mature cyst) every day until end of the experiment (14 days)
9) Take 5 mL of sample every day until the end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
10) On the last day of the experiment, collect 1 L sample to measure dry weight

3.6.2 Effect of up-scale of flat panel airlift photobioreactor in outdoor system

1) Repeat Steps 1-5 in Section 3.7.1
2) Use the best conditions from Sections 3.3.1 - 3.3.3, 3.6.1 - 3.6.2 and, 3.7.1, respectively
3) Record light intensity and temperature with a light sensor and thermocouple (with recorder)
4) Counts the cells (motile cells, non-motile cells, immature cyst, and mature cyst) every day until end of the experiment (14 days)
5) Take 5 mL of sample every day until end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
6) On the last day of the experiment, collect 1 L sample to measure dry weight
7) Repeat Steps 1-6 but experiment in 50 and 90 L flat panel airlift photobioreactor

Part III: Determination of suitable conditions for astaxanthin induction from *H. pluvialis* (NIES-144) with a two-step single reactor process

Generally, astaxanthin induction in *H. pluvialis* (NIES-144) is achieved with a conventional two stage process. The first step is to cultivate cells with suitable conditions to obtain high cell density. After that, received cells from the first step are cultivated to stimulate astaxanthin from *H. pluvialis* (NIES-144) using stress conditions. However, it was proposed here the two-step single reactor process (TSSRP) for both cultivation and induction. The flow diagram of all the works involved in Part III is shown in Figure 3.7.

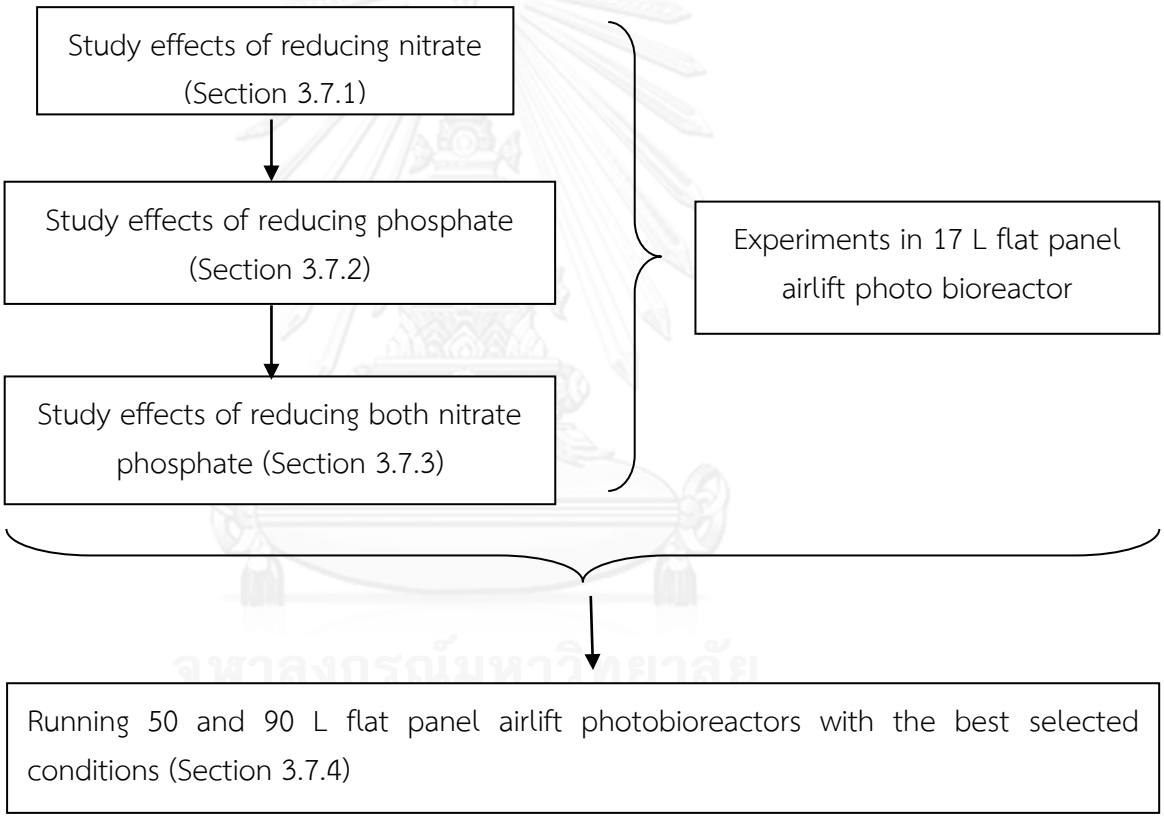


Figure 3.7 Flow Diagram of all the works in Part III

3.7 Experimental procedure by operating of two-step single reactor process

3.7.1 Effect of reducing nitrate of sterilized F1 medium to astaxanthin induction by operating of two-step single reactor process

1) Chlorinate the tap water in the 17 L flat panel airlift photobioreactor, wait until disintegrates chlorine
2) Add sterilized F1 medium with 2 – 10 times lower level of nitrate to the bioreactor
3) Transfer $2x10^{4}$ cell mL^{-1} inoculums to bioreactor
4) Use 2,000 LUX light intensity in cultivation sections, after that wait until 5 day for using light intensity from Section 3.3.2 in stress section
5) Control superficial gas velocity (u_{sg}) at 0.4 cm s^{-1} throughout the experiment
6) Apply 1% carbon dioxide/air ratio in cultivation sections, after that wait until 5 day for using carbon dioxide/air ratio from Section 3.3.3 in stress section
7) Measure light intensity and temperature using a digital LX-5 LUX and thermometer, respectively
8) Counts the cells (motile cells, non-motile cells, immature cyst, and mature cyst) every day until the end of the experiment (14 days)
9) Take 5 mL of sample every day until the end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
10) On the last day of the experiment, collect 1 L of sample to measure dry weight

3.7.2 Effect of reducing phosphate of sterilized F1 medium to astaxanthin induction by operating of two-step single reactor process

1) Chlorinate the tap water in the 17 L flat panel airlift photobioreactor, wait until disintegrates chlorine
2) Use the best cultivating conditions as reported in Sections 3.8.1
3) Add sterilized F1 medium which reduced 2 – 10 times of phosphate to bioreactor

4) Transfer $2x10^4$ cell mL^{-1} inoculums to bioreactor
5) Use 2,000 LUX light intensity in cultivation sections, after that wait until 5 day for using light intensity from Section 3.3.2 in stress section
6) Control superficial gas velocity (u_{sg}) at 0.4 cm s^{-1} throughout the experiment
7) Apply 1% carbon dioxide/air ratio in cultivation sections, wait until 5 day for using carbon dioxide/air ratio from Section 3.3.3 in stress section
8) Measure light intensity and temperature using a digital LX-5 LUX and thermometer, respectively
9) Counts the cells (motile cells, non-motile cells, immature cyst, and mature cyst) every day until the end of the experiment (14 days)
10) Take 5 mL of sample every day until the end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
11) On the last day of the experiment, collect 1 L of sample to measure dry weight

3.7.3 Effect of reducing both nitrate and phosphate of sterilized F1 medium to astaxanthin induction by operating of two-step single reactor process

1) Chlorinate the tap water in the 17 L flat panel airlift photobioreactor, wait until disintegrates chlorine
2) Add sterilized F1 medium with 2 – 10 times lower level of both nitrate and phosphate to the bioreactor
3) Transfer $2x10^4$ cell mL^{-1} inoculums to bioreactor
4) Use 2,000 LUX light intensity in cultivation sections, after that wait until 5 day for using light intensity from Section 3.3.2 in stress section
5) Control superficial gas velocity (u_{sg}) at 0.4 cm s^{-1} throughout the experiment
6) Apply 1% carbon dioxide/air ratio in cultivation sections, after that wait until 5 day for using carbon dioxide/air ratio from Section 3.3.3 in stress section
7) Measure light intensity and temperature using a digital LX-5 LUX and thermometer, respectively

8) Counts the cells (motile cells, non-motile cells, immature cyst, and mature cyst) every day until the end of the experiment (14 days)
9) Take 5 mL of sample every day until the end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
10) On the last day of the experiment, collect 1 L of sample to measure dry weight

3.7.4 Effect of up-scale of flat panel airlift photobioreactor to astaxanthin induction in *H. pluvialis* by operating of two-step single reactor process

1) Chlorinate the tap water in the 50 L flat panel airlift photobioreactor, wait until disintegrates chlorine
2) Use the best conditions from Sections 3.8.1 and 3.8.2
3) Transfer $2x10^4$ cell mL^{-1} inoculums to bioreactor
4) Use 2,000 LUX light intensity in cultivation sections, after that wait until 5 day for using light intensity from Section 3.3.2 in stress section
5) Control superficial gas velocity (u_{sg}) at 0.4 cm s^{-1} throughout the experiment
6) Apply 1% carbon dioxide/air ratio in cultivation sections, wait until 5 day for using carbon dioxide/air ratio from Section 3.3.3 in stress section
7) Measure light intensity and temperature using a digital LX-5 LUX and thermometer, respectively
8) Counts the cells (motile cells, non-motile cells, immature cyst, and mature cyst) every day until the end of the experiment (14 days)
9) Take 5 mL of sample every day until the end of the experiment for the determination of astaxanthin content with high-performance liquid chromatography (HPLC)
10) On the last day of the experiment, collect 1 L of sample to measure dry weight
11) Repeat Steps 1-10 but experiment 90 L flat panel airlift photobioreactor

3.8 Determination of growth

3.8.1 Determination of cell density

Cell density was measured by microscope and the counting of cells was performed using an improved Neubauer haemacytometer (Figures 3.8 and 3.9).

1) Take two 25 µL drops of culture and place them on a clean haemacytometer
2) Place a clean cover slip on the drop so that the drop is evenly dispersed under the cover slip
3) Count cell under a microscope (objective 100x)
4) Calculate the cell density as follows:

$$D = \left[\frac{n_1 + n_2}{18} \right] x10^4 \qquad (3.1)$$

where D = cell density (cell mL^{-1})

n_1 and n_2 = number of cells count in upper and lower grid (cells)

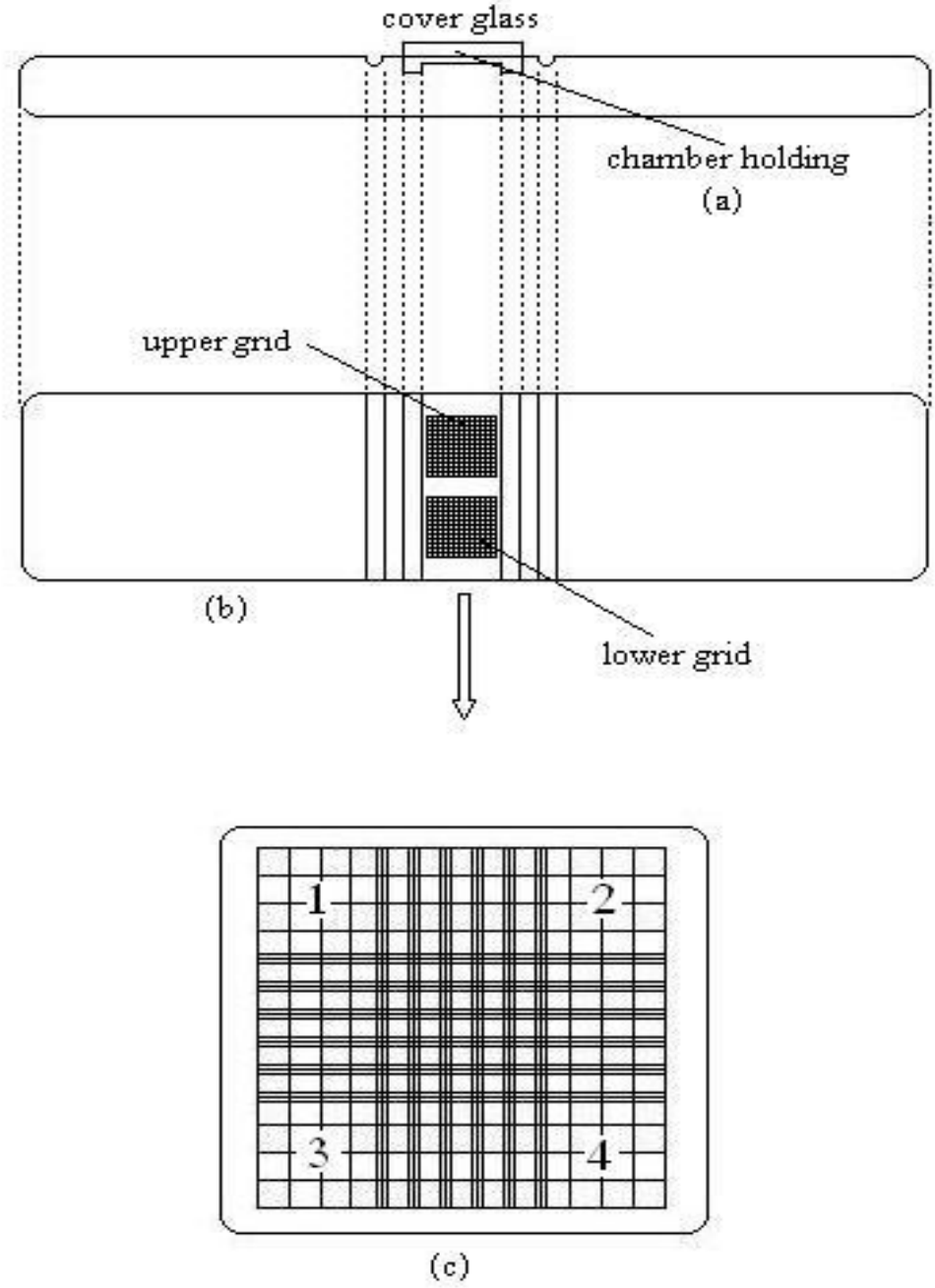


Figure 3.8 (a) Side view of the cell counting chamber showing the cover glass and the space beneath it that holds a microalgae suspension. (b) Top view of the chamber. The chamber had two grids located in the center of the side. (c) An enlarged view of the grid. The microalgae in the squares 1, 2, 3 and 4 were used for cell count. (Limpanyalert, 2008)

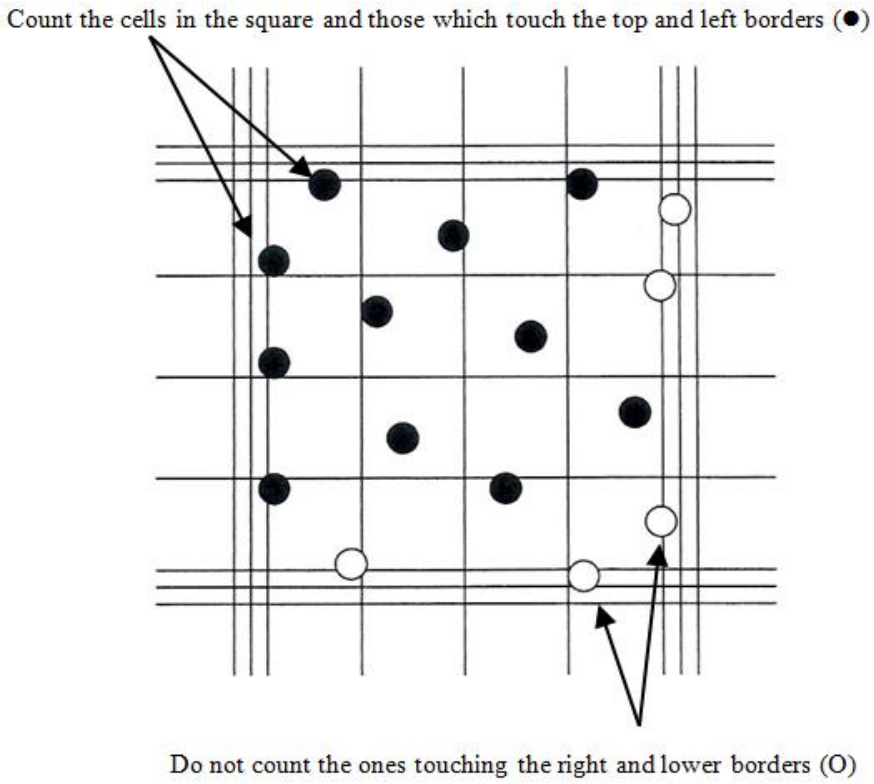


Figure 3.9 Counting cell densities

Count the cells in the square and those which touch the top and left border (●), do not count the ones touching the right and lower border (○) (Limpanyalert, 2008)

3.8.2 Determination of cell dry weight

1) Take 1 L culture sample on the last day of experiment (14 days of experiment) where most cells accumulate astaxanthin
2) Separate supernatant and cell pellets at 3,500 rpm (2,054 x g), 15 $^{\circ}$C for 5 min
3) Braze the cell pellets at -18 ^{0}C, freeze-dry at -42^{0}C and 1.58 x 10^{5} bar, for dry weight calculation

3.9 Determination of astaxanthin

1) Separate cell pellets from the 1 mL culture sample at 2,500 rpm (1,048 x g) for 15 min
2) Add 1 mL methanol used as solvent into cell pellets
3) Break cells by manual homogenizer to extract the total pigment from the cells
4) Repeat the extraction procedure until the cell pellets becomes white
5) After that, separate the white cell pellets from supernatant at 2,500 rpm (1,048xg) for 15 min
6) Analyze astaxanthin concentration with high-performance liquid chromatography (HPLC) (with the C-18 column 4.6x150 mm)

Note: Steps 1-6 must be conducted in dark condition, where the analyzing condition of the high performance liquid chromatography (HPLC) for analyzing astaxanthin content is given in Table 3.3.

3.10 Determination of astaxanthin productivity

Astaxanthin productivity is calculated

$$\text{Astaxanthin productivity (g m}^{-3}\text{ day}^{-1}\text{)} = \frac{C_f - C_i}{\left(t_f - t_i\right)} \qquad (3.2)$$

where C_i, = initial and final astaxanthin concentrations (g m^{-3})

C_f,= final astaxanthin concentrations (g m^{-3})

t_i = initial times (day)

t_f = final times (day)

Table 3.3 Analyzing conditions of high performance liquid chromatography (HPLC) for astaxanthin content

Conditions	Details
Detector	Waters 2487 Dual λ Absorbance Detector
Column	C-18 column 4.6 x 150 mm (inside column made from Octadecyl Dimethyl Silane)
Standard	Astaxanthin crystalline from Alexis biochemical company
Mobile phase (%)	Acetone nitrite 79.9% Metanal 10 % Dichloromethane 10 % Water 0.1 % (HPLC grade for all chemicals)
Wavelength (nm)	480 nm
Flow rate of mobile phase (mL/min)	1 mL/min

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Determination of suitable indoor conditions for astaxanthin induction from *H. pluvialis* (NIES-144)

In this research, *H. pluvialis* (NIES-144) in vegetative cell growth stage was cultivated in 5, 10 L Bubble Column-Photobioreactors (BC-PBRs) and 17 L Flat panel airlift photobioreactor (FP-ALPBR). Growth behavior of *H. pluvialis* (NIES-144) of all reactors, as illustrated in Figure 4.1, indicates that the 17 L airlift exhibited a better cultivation with the highest cell density of 3.24 $x10^5$ cell mL^{-1} (in 7 days).The bubble columns, on the other hand, could only show an inferior performance with the 5 L provided the maximum cell density of only 2.2 $x10^5$ cell mL^{-1} (in 8 days) and the worst performance was obtained from the larger 10 L bubble column where the cell density could only reach 1.6 $x10^5$ cell mL^{-1} after 11 days of cultivation. The vegetative *H. pluvialis* used in this investigation was therefore, unless stated otherwise, prepared using the 17 L FP-ALPBR.

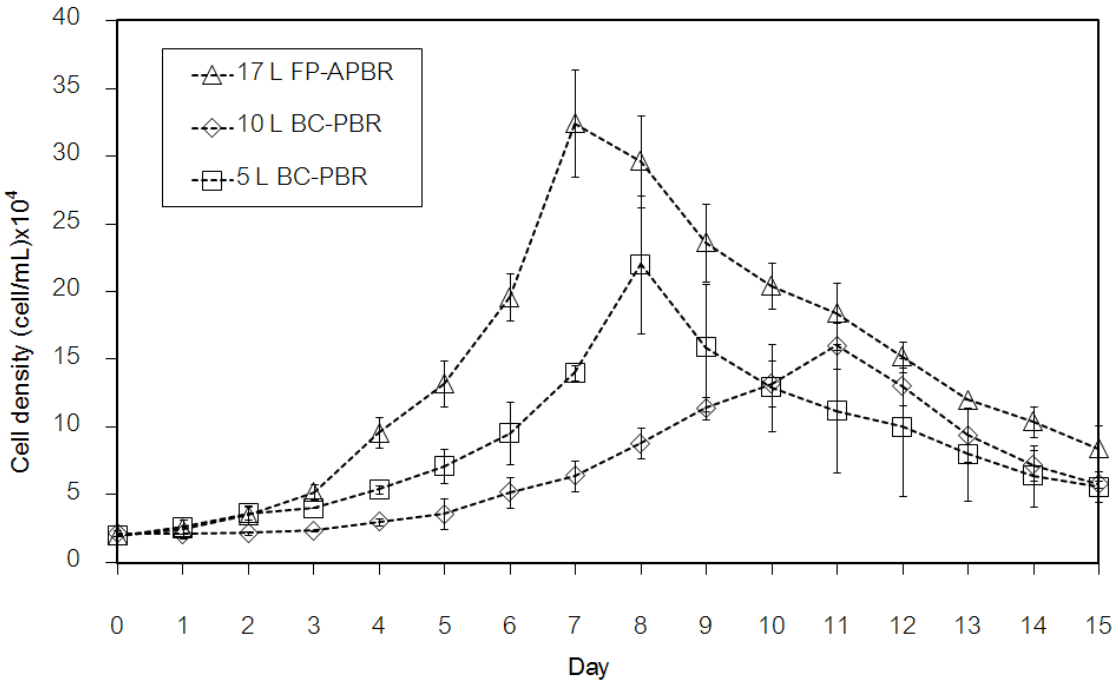


Figure 4.1 Growth behavior of *H. pluvialis* (NIES-144) in 5 and 10 L BC-PBRs and 17 L FP-ALPBR

4.1.1 Effect of diluted nutrient on astaxanthin induction in 1.5 L bubble columns

The active cell of *H. pluvialis* was further induced for the accumulation of astaxanthin in 1.5 L bubble columns, and the induction was carried out with the mediums of various concentrations. The F1-medium was further used as an induction medium but with diluted concentration of nutrients (0.5x, 0.25x and 0.1x F1-medium dilutions), and the effect of diluted nutrient to astaxanthin induction was then investigated. The sterilized water without adding any nutrients was also employed for this investigation. Growth behavior of *H. pluvialis* (NIES-144) and time profiles of astaxanthin concentrations under 0.5x, 0.25x and 0.1x F1-medium dilutions and sterilized water conditions are shown in Figures 4.2 and 4.3, respectively, whereas Table 4.1 displays the percent astaxanthin (by weight), dry weight of astaxanthin after 14 days of induction and astaxanthin productivity. The results indicate that, in all diluted nutrient cases, astaxanthin concentration quickly increased in the earliest period of induction followed by a slower induction period. The induction of astaxanthin seemed to stop after 5-6 days regardless of the nutrient concentration, but the level of accumulated astaxanthin varied with the initial nutrient dilution. The astaxanthin after six days of induction experiment and astaxanthin productivity of 0.5x, 0.25x and 0.1x F1-medium dilution conditions were 1.92, 3.03 and 5.05 mg L^{-1} (0.92, 1.21 and 1.87% by weight), and 0.50, 0.62 and 0.96 mg L^{-1} day^{-1}, respectively. The clean pure water, on the other hand, provided a much better astaxanthin inducing environment. The results indicate that the pure water could continuously stimulate astaxanthin at a virtually constant rate throughout the experiment, and the astaxanthin in the last day of experiment and astaxanthin productivity were as high as 18.21 mg L^{-1} (3.64% by weight) and 1.29 mg L^{-1} day^{-1}. This is almost twice the figure obtained from the experiment with diluted nutrient, not to mention that the experiments with diluted nutrients were much more prone to other microorganism contamination than the induction with sterilized reverse osmosis (RO) water which so far has not seen a single evidence of foreign species in our non-aseptic inducing system.

The results indicate that cell density was quickly reduced in the first 1-3 days of experiment, particularly for those experiments with diluted mediums. After that, cell density began to stabilize throughout the experiment. Cell density in the last day of the experiments were 2.2 $x10^4$, 3.2 $x10^4$ and 3.5 $x10^4$ cell mL^{-1} or 0.21, 0.25 and 0.27 g L^{-1} of dry weights for the 0.5x, 0.25x and 0.1x F1-medium dilution conditions, respectively. Meanwhile, the cell density obtained from the pure water condition

was stable throughout the experiment. This could be because, in the first few days of experiment with diluted nutrients, *H. pluvialis* still grew but with a lower nutrient concentration and therefore some part of the cells died as they did not have enough nutrients for their growth. In addition, the culture became highly vulnerable to contaminations such as *Scenedesmus* and *Vorticella* which are shown in Figure 4.4 which then harmed the algal cells. After that, *H. pluvialis* began to transform to the mature cyst with a thickening cell wall to protect themselves from the danger induced from the external environment. In the case of sterilized RO water, as there was no nutrient right from the beginning of the experiment, cells started to enter their cyst stage straightway after the induction and no cell loss was observed. In addition, since there was no nutrient available to other microorganisms, no contamination was allowed in the system. And due to no cell loss, a higher astaxanthin concentration could be obtained from this condition.

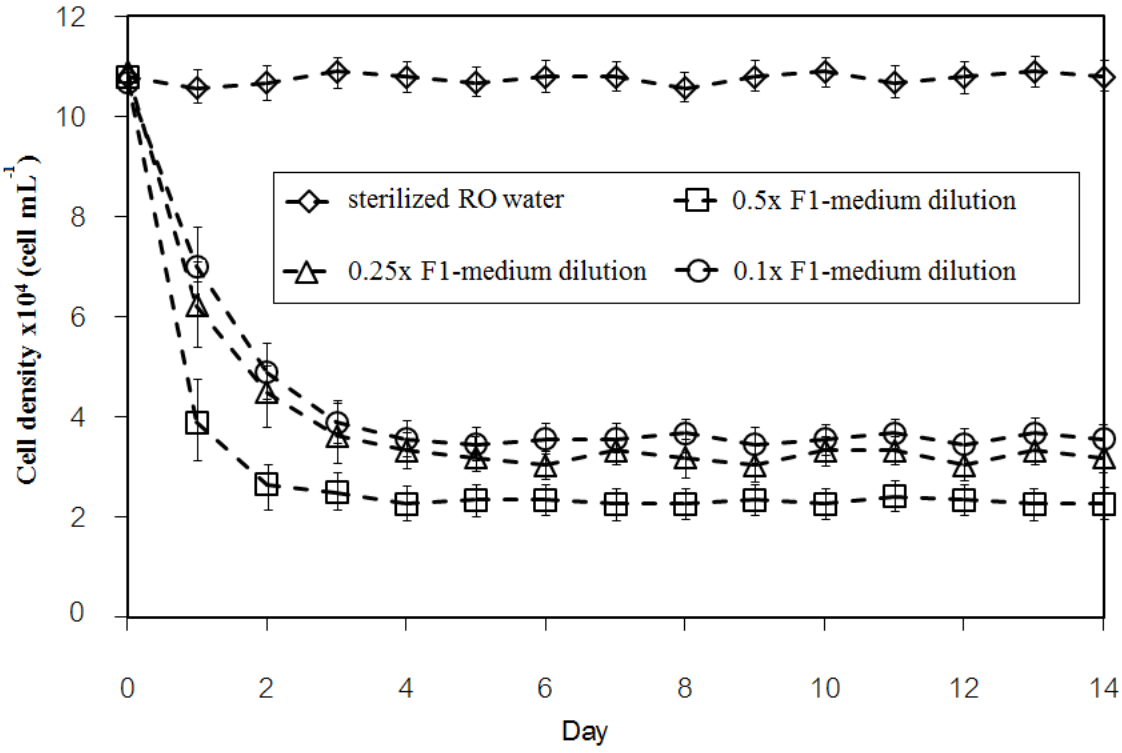


Figure 4.2 Growth behavior of *H. pluvialis* (NIES-144) induced with sterilized RO water, 0.5x, 0.25x, and 0.1x F1-medium dilution conditions

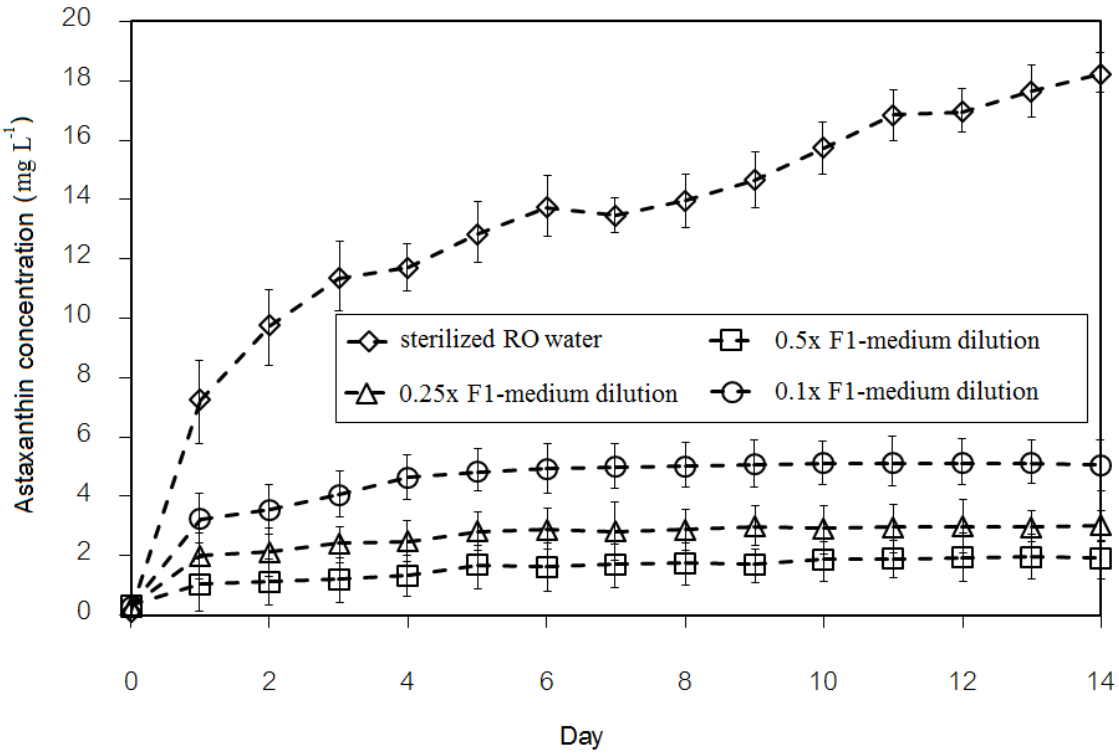
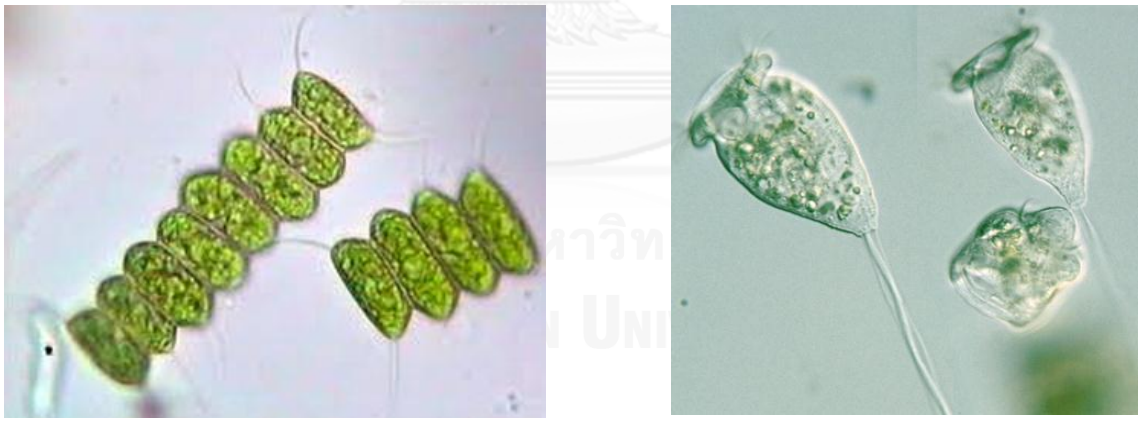


Figure 4.3 Astaxanthin concentrations from culture induced with sterilized RO water, 0.5x, 0.25x, and 0.1x F1-medium dilution conditions



Scenedesmus *Vorticella*

Figure 4.4 Contaminations happened under F1-medium dilution conditions

Table 4.1 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction of the culture with sterilized RO water, 0.5x, 0.25x, and 0.1x F1-medium dilution conditions

Conditions	Dry weight (g L^{-1})	%Astaxanthin (by weight)	Astaxanthin productivity (mg L^{-1} day^{-1})
sterilized RO water	0.50	3.64	1.29
0.5x F1-medium dilution	0.21	0.92	0.50
0.25x F1-medium dilution	0.25	1.21	0.62
0.1x F1-medium dilution	0.27	1.87	0.96

4.1.2 Effect of light intensity on astaxanthin induction in 1.5 L bubble columns

The effect of light intensity on astaxanthin induction was studied by setting the light intensity at 12,000, 30,000, 35,000, 40,000 and 45,000 LUX. The growth behavior of *H. pluvialis* (NIES-144) and time profiles of astaxanthin concentrations under these conditions are summarized in Figures 4.5 and 4.6, respectively, whilst the percent astaxanthin (by weight) astaxanthin productivity and its dry weight are given in Table 4.2. At 12,000, 30,000 and 35,000 LUX (low intensity range), astaxanthin seemed to increase with light intensity, and the final astaxanthin concentrations obtained from each experiment were: 10.55, 14.23 and 18.21 mg L^{-1} (2.76, 3.16 and 3.64% by weight, and dry weight of 0.38, 0.45, and 0.5 g L^{-1}), respectively. In addition, astaxanthin productivity at 12,000, 30,000 and 35,000 LUX were 0.74, 0.92 and 1.29 mg L^{-1} day^{-1}, respectively. Above 35,000 LUX (high light intensity), the effect of light seemed to be insignificant and the final astaxanthin concentrations were maintained at 17.25 and 17.59 mg L^{-1} (3.52 and 3.59% by weight, or both at 0.49 g L^{-1}), for the induction at 40,000 and 45,000 LUX, respectively. In addition, astaxanthin productivity at 40,000 and 45,000 LUX were 1.27 and 1.24 mg L^{-1} day^{-1}, respectively. Note that the light intensity of 35,000 LUX was 17.5 times that of the recommended growth intensity of 2,000 LUX (Kaewpintong et al., 2006).

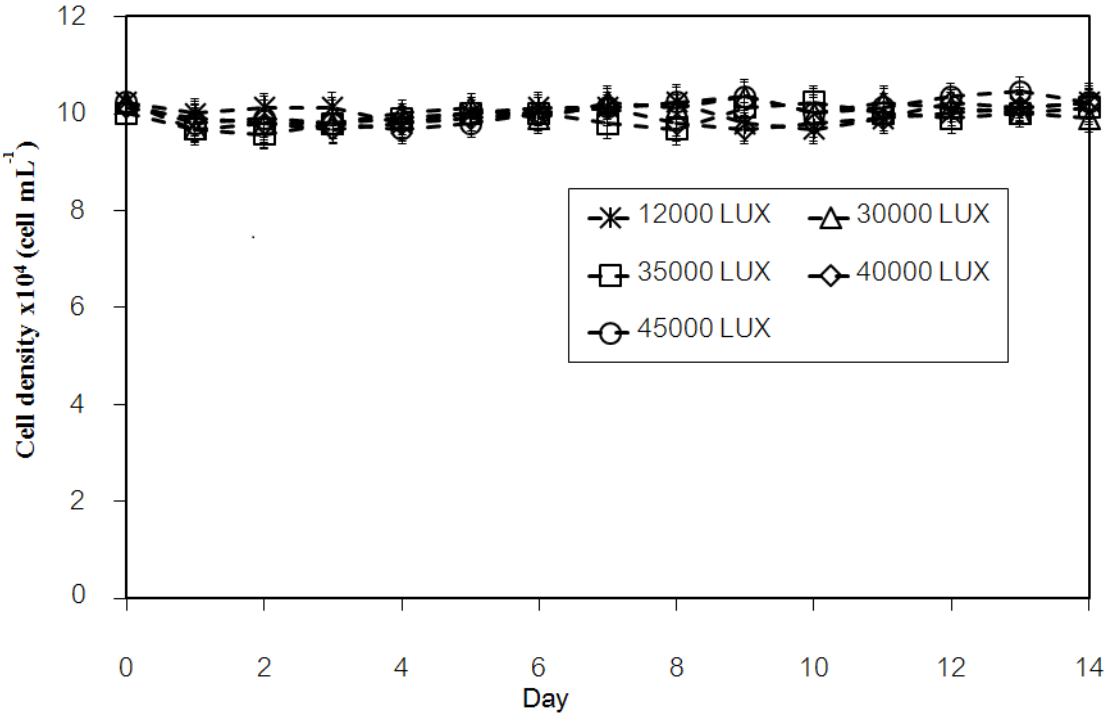


Figure 4.5 Growth behavior of *H. pluvialis* (NIES-144) under various light intensity conditions

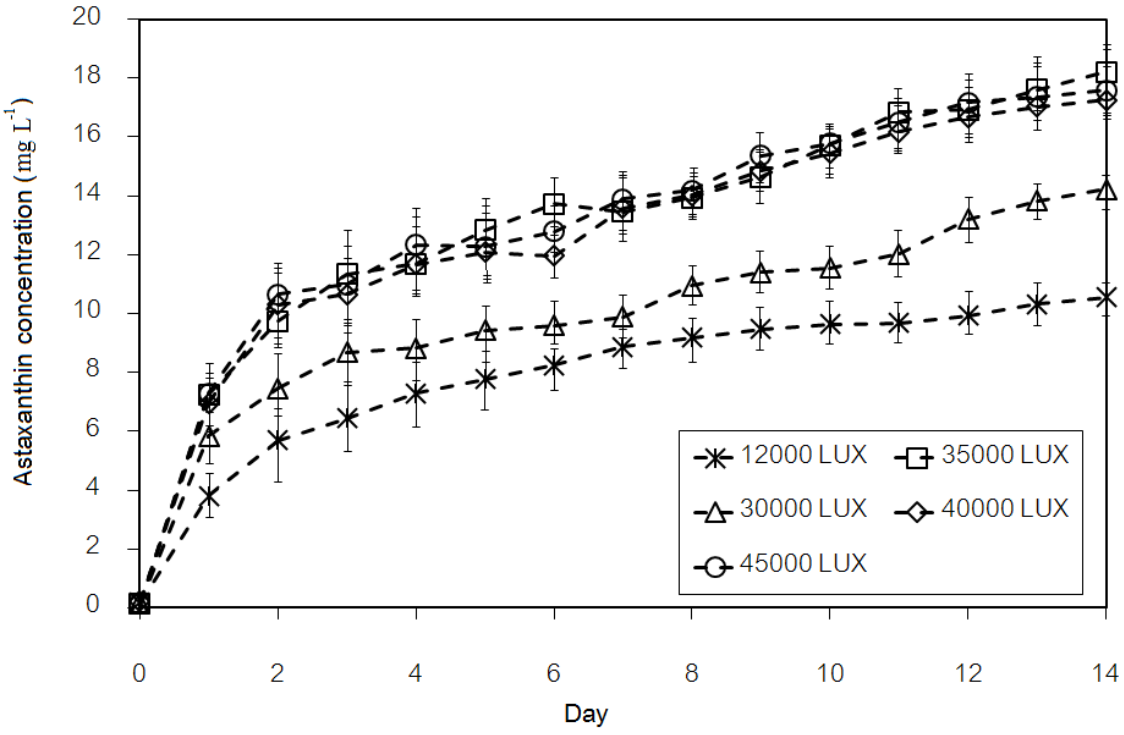


Figure 4.6 Astaxanthin concentrations under various light intensity conditions

Table 4.2 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration under various light intensity conditions

Conditions	Dry weight (g L^{-1})	%Astaxanthin (by weight)	Astaxanthin productivity (mg L^{-1} day^{-1})
12,000 LUX	0.38	2.76	0.74
30,000 LUX	0.45	3.16	0.92
35,000 LUX	0.50	3.64	1.29
40,000 LUX	0.49	3.52	1.27
45,000 LUX	0.49	3.59	1.24

4.1.3 Effect of carbon dioxide on astaxanthin induction in 1.5 L bubble columns

The effect of carbon dioxide on astaxanthin induction in *H. pluvialis* was examined at the carbon dioxide concentrations of 0, 1, 2, 3, 4 and 5% by volume of the supplied air (at superficial velocity of 0.4 cm s^{-1}) or equivalent to 0, 0.49, 0.98, 1.47, 1.96 and 2.45 cm^3 s^{-1} of CO_2 loading. The growth behavior of *H. pluvialis* (NIES-144) and time profiles of astaxanthin concentrations with this induction condition are demonstrated in Figures 4.7 and 4.8, respectively, and percent astaxanthin (by weight) and dry weight of astaxanthin at the harvesting day (14 days of induction) are given in Table 4.3. Increasing CO_2 in the range from 0–3% seemed to give positive effect to the accumulation of astaxanthin in *H. pluvialis* as the concentration of astaxanthin went up step by step from the supply of 0, 1, 2 and 3% CO_2 to 10.12, 13.45, 15.67 and 18.21 mg L^{-1} or 2.47, 3.06, 3.33 and 3.64% by weight, respectively. In addition, astaxanthin productivity of 0, 1, 2 and 3% CO_2 were 0.71, 0.95, 1.11 and 1.29 mg L^{-1} day^{-1}, respectively. This indicates that the addition of inorganic carbon source could promote the synthesis of astaxanthin in the cell.

Cell density was quite stable with no loss of cells though these experiments. However, when increasing CO_2 loading further to 4 and 5% CO_2 in the air supply, astaxanthin concentration and cell density slightly dropped. This could be attributed to the drop in pH of the nutrient which could harm the cell (see Table 4.3). Therefore a decrease in cell density at 4 and 5% CO_2 was observed where astaxanthin concentration of 17.96 and 17.26 mg L^{-1} (3.59 and 3.58% by weight) and

astaxanthin productivity of 1.27 and 1.22 mg L^{-1} day^{-1} were obtained, respectively. In addition, a more or less the same astaxanthin concentration was found in the last day of experiments with 4 and 5% CO_2.

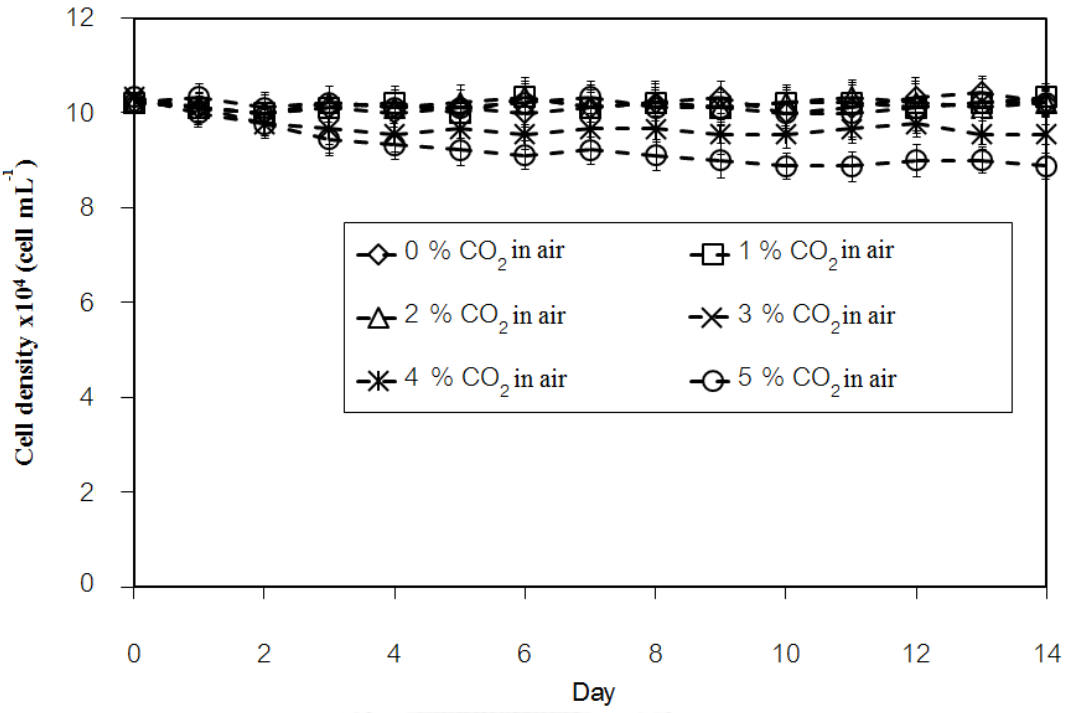


Figure 4.7 Growth behavior of *H. pluvialis* (NIES-144) under carbon dioxide induction conditions

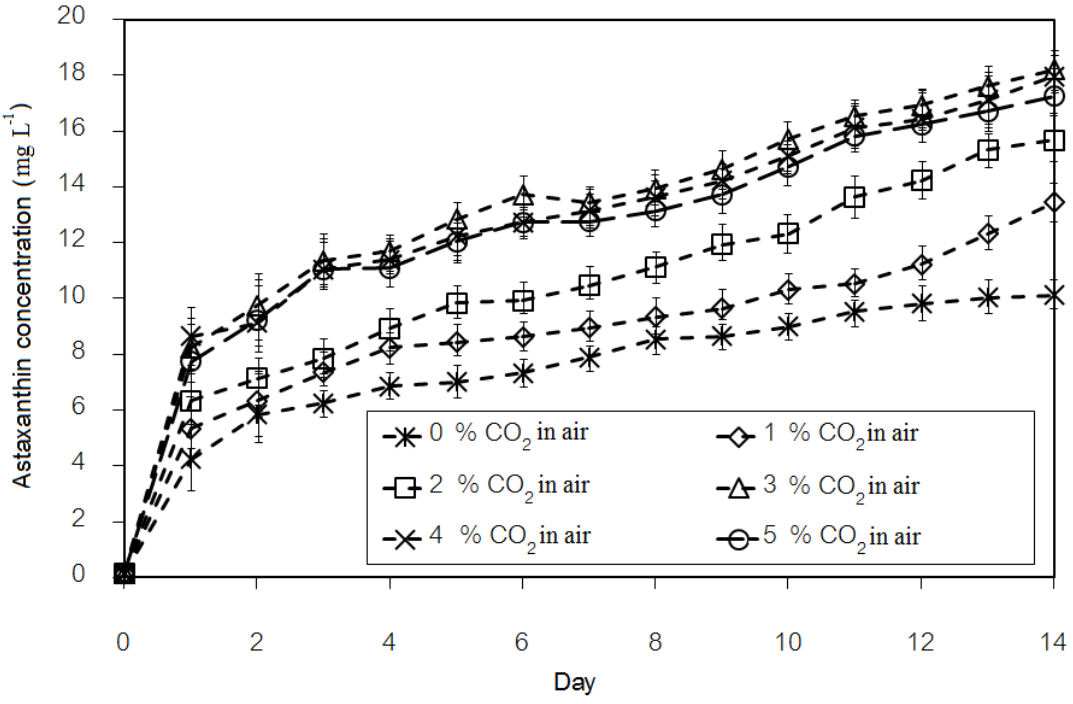


Figure 4.8 Astaxanthin concentrations under carbon dioxide induction conditions

Table 4.3 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under carbon dioxide supplemental conditions (aeration rate = 49.1 cm^3 s^{-1})

Conditions	CO_2 loading (cm^3 s^{-1})	pH	Dry weight (g L^{-1})	%Astaxanthin (by weight)	Astaxanthin productivity (mg L^{-1} day^{-1})
0% CO_2 in air	0	7.4-7.9	0.41	2.47	0.71
1% CO_2 in air	0.49	6.6-7.7	0.44	3.06	0.95
2% CO_2 in air	0.98	6.4-7.5	0.47	3.33	1.11
3% CO_2 in air	1.47	6.3-7.4	0.50	3.64	1.29
4% CO_2 in air	1.96	6.1-7.2	0.50	3.59	1.27
5% CO_2 in air	2.45	5.9-7.3	0.48	3.58	1.22

4.1.4 Effect of temperature on astaxanthin induction in 1.5 L bubble columns

The effect of temperature on astaxanthin induction in *H. pluvialis* was studied by controlling temperature from 30, 31, 33, 35 and 37^{0}C. The growth behavior of *H. pluvialis* (NIES-144) and time profiles of astaxanthin concentrations under this varying temperature condition are given in Figures 4.9 and 4.10, and the percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction are shown in Table 4.4. Similarly, there seems to occur the most suitable temperature range where astaxanthin could best accumulate, and in this experiment, this was found to be around 33^{0}C. Increasing temperature from 30 to 33^{0}C demonstrated an increase in astaxanthin induction (from 12.55 to 18.21 mg L^{-1} or 3.14 to 3.64% by weight). However, *H. pluvialis* began to lose its integrity and died at 35^{0}C where cell density and astaxanthin concentration at this temperature dropped to 6 x10^4 cell mL^{-1} and 6.62 mg L^{-1} by weight). *H. pluvialis* died completely at 37^{0}C which indicates that the induction with temperature must be well controlled under 35^{0}C. It should be noted that the suitable temperature condition for the growth of *H. pluvialis* was 23-25^{0}C (Kaewpintong et al., 2006) which is much lower than the induction temperature.

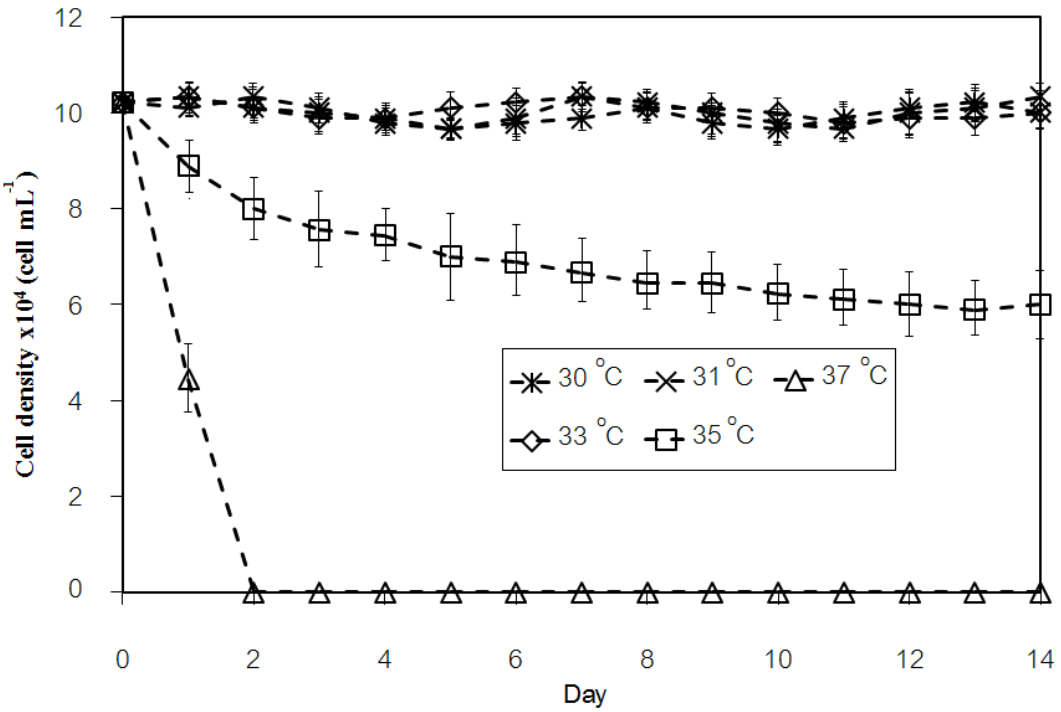


Figure 4.9 Growth behavior of *H. pluvialis* (NIES-144) under temperature induction conditions

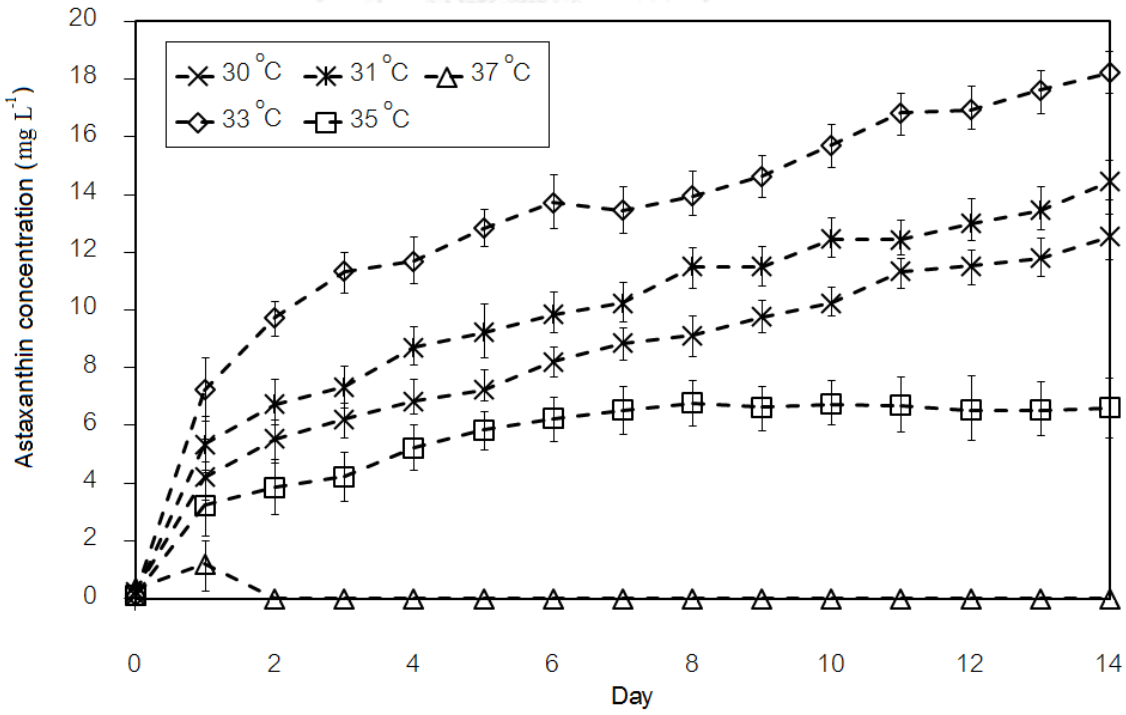


Figure 4.10 Astaxanthin concentrations under various temperature induction conditions

Table 4.4 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under various temperature conditions

Conditions	Dry weight (g L^{-1})	%Astaxanthin (by weight)	Astaxanthin productivity (mg L^{-1} day^{-1})
30^0C	0.40	3.14	0.87
31^0C	0.42	3.44	1.02
33^0C	0.50	3.64	1.29
35^0C	0.19	3.49	0.46
37^0C	0	0	0

4.2 Determination of suitable conditions for astaxanthin induction from *H. pluvialis* (NIES-144) in flat panel airlift photobioreactor systems

4.2.1 Effect of ratio between downcomer and riser cross sectional area (A_d/A_r) of flat panel airlift photobioreactor on induction of astaxanthin

The effect of the different ratios between downcomer and riser cross sectional area (A_d/A_r) in flat panel airlift photobioreactors was studied by varying A_d/A_r at 0.2, 0.4, 0.6 and 0.8. The growth behavior of *H. pluvialis* (NIES-144) and time profiles of astaxanthin concentrations under these varying A_d/A_r range are given in Figures 4.11 and 4.12, and the percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction are shown in Table 4.5. The results indicated that reducing A_d/A_r ratio at 0.8, 0.6 and 0.4 enhanced astaxanthin accumulation. Astaxanthin concentration after 14 days from the reactors with A_d/A_r of 0.8, 0.6 and 0.4 were 10.53, 16.35 and 26.63 mg L^{-1} (or 3.29, 4.19 and 5.32% by weight) and astaxanthin productivity from the reactors with A_d/A_r of 0.8, 0.6 and 0.4 were 0.73, 1.15 and 1.88 mg L^{-1} day^{-1}, respectively. This finding could be explained as follows.

Reducing A_d/A_r ratio means that the riser cross sectional area (A_r) of the system increased. This resulted in two major effects. First, a large riser enhanced the gas-liquid mass transfer rate and this allowed a faster transfer of CO_2 from gas to liquid. This greater inorganic carbon then allowed the cells to uptake and to use it to produce cellular components including astaxanthin. In addition, reducing A_d/A_r ratio

also enhanced the downcomer liquid velocity which exerted the up-force at the bottom of the reactor. As a result, lesser cell precipitated at the reactor bottom, or in other words, cell dispersed more thoroughly in the reactor. This allows a better mass transfer (of carbon and nutrient sources) from the medium to the cell. A much too large riser, i.e. at A_d/A_r of 0.2, resulted in a poor circulation of cells and this led to cell death and low astaxanthin productivity. Astaxanthin concentration after 14 days from the reactors with A_d/A_r of 0.2 was 18.53 mg L^{-1} (or 4.41% by weight) and astaxanthin productivity from the reactors with A_d/A_r of 0.2 was 1.30 mg L^{-1} day^{-1}, respectively. In addition, cell density from the reactors with A_d/A_r ratio of 0.2 was 8 $x10^4$ cell mL^{-1}.

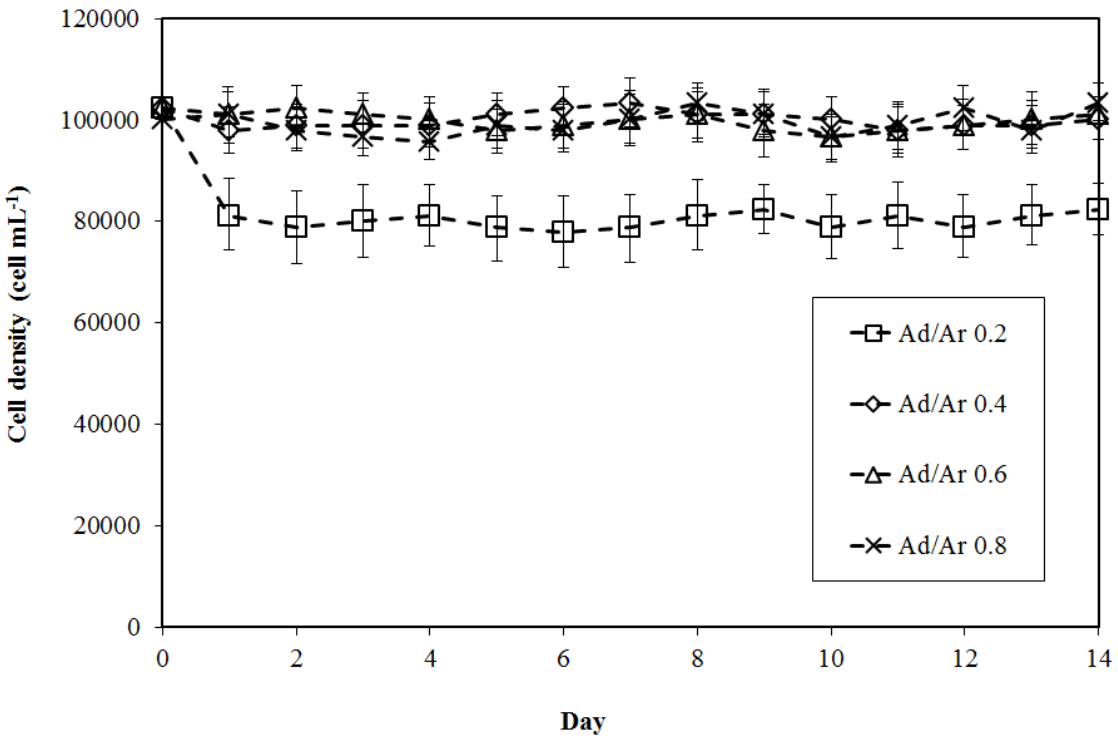


Figure 4.11 Growth behavior of *H. pluvialis* (NIES-144) under various ratios between downcomer per riser cross sectional area

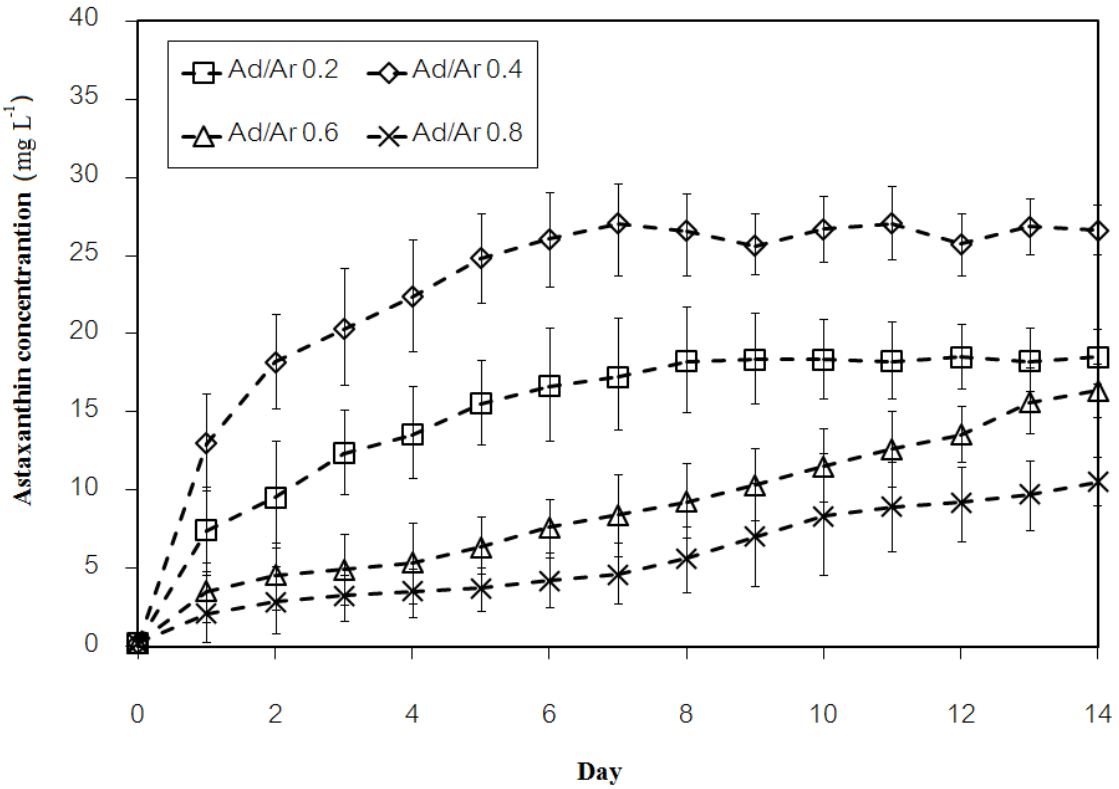


Figure 4.12 Astaxanthin concentrations under various ratios between downcomer per riser cross sectional area

Table 4.5 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration various ratios between downcomer per riser cross sectional area

Conditions (A_d/A_r)	Dry weight (g L^{-1})	%Astaxanthin (by weight)	Astaxanthin productivity (mg L^{-1} day^{-1})
0.2	0.42	4.41	1.30
0.4	0.50	5.32	1.88
0.6	0.39	4.19	1.15
0.8	0.32	3.29	0.73

4.2.2 Effect of superficial gas velocity (u_{sg}) of flat panel airlift photobioreactor on induction of astaxanthin

Superficial gas velocity (u_{sg}) in flat panel airlift photobioreactors was studied by varying it at 0.2, 0.4, 0.6 and 0.8 cm s^{-1}. The growth behavior of *H. pluvialis* (NIES-144) and time profiles of astaxanthin concentrations under a varying superficial gas velocity (u_{sg}) range are given in Figures 4.13 and 4.14, where the percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction are shown in Table 4.6.The results indicate that an increase in superficial gas velocity from 0.2 to 0.4 cm s^{-1} enhanced astaxanthin production. Astaxanthin concentration obtained from the systems with u_{sg} of 0.2 and 0.4 cm s^{-1} were 14.43 and 26.63 mg L^{-1} (or 4.98 and 5.32% by weight), and astaxanthin productivity obtained from the systems with u_{sg} of 0.2 and 0.4 cm s^{-1} were 1.29 and 1.88 mg L^{-1} day^{-1}, respectively. No cell death was observed from both experiments. Similar explanation with that in the previous section could be applied here, i.e. increasing u_{sg} led to a better gas-liquid mass transfer and also a better circulation of cell suspension. However, a further increase in u_{sg} to 0.6 and 0.8 cm s^{-1} resulted in cell death in 7 and 3 days of induction, respectively. This was attributed to the associate shear force at this high u_{sg} range which had negative impact on cell viability.

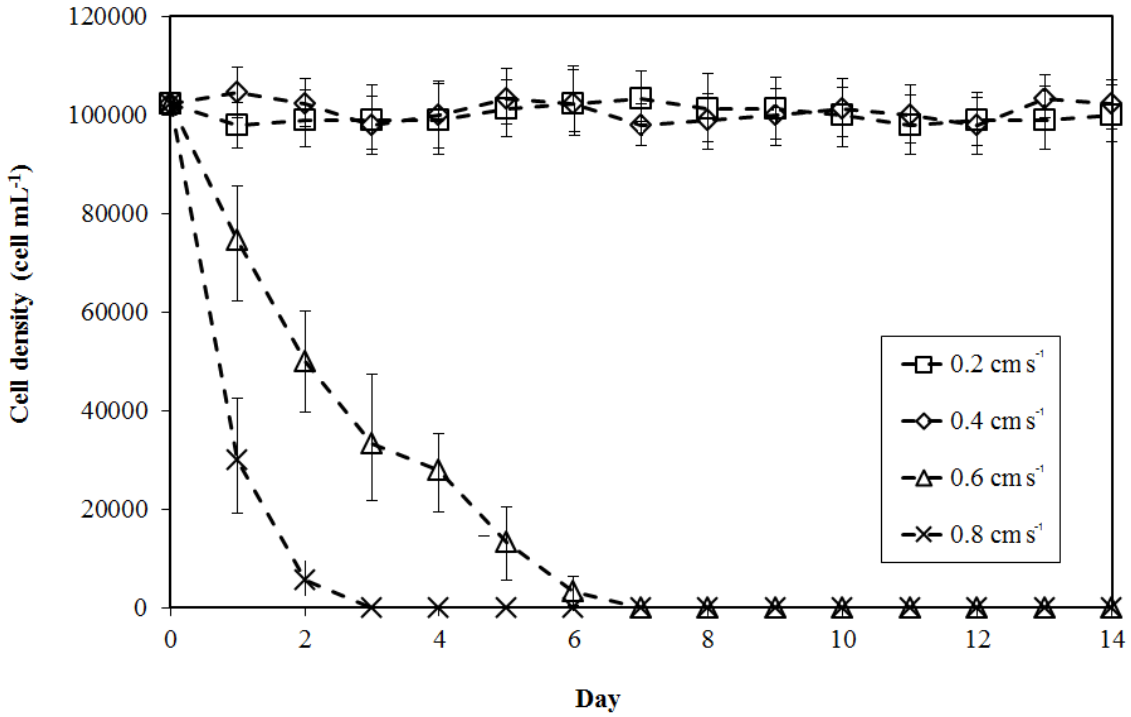


Figure 4.13 Growth behavior of *H. pluvialis* (NIES-144) at various superficial gas velocities (u_{sg})

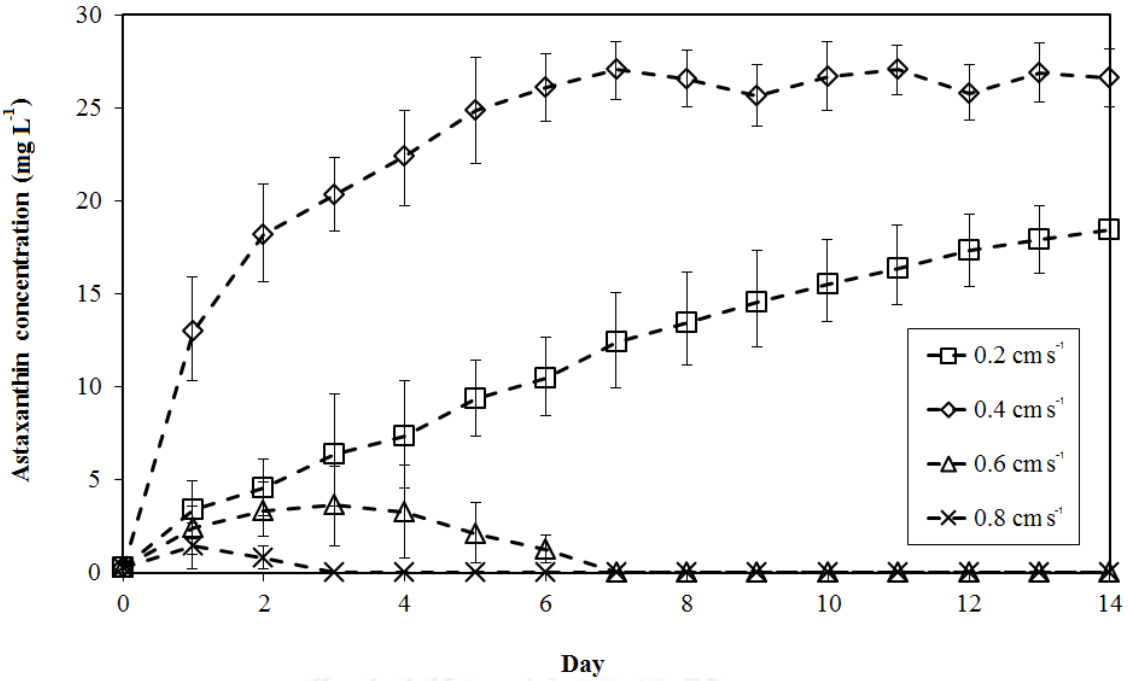


Figure 4.14 Astaxanthin concentrations at various superficial gas velocities (u_{sg})

Table 4.6 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration of superficial gas velocities (u_{sg})

Conditions	Dry weight ($g\ L^{-1}$)	%Astaxanthin (by weight)	Astaxanthin productivity ($mg\ L^{-1}\ day^{-1}$)
0.2	0.37	4.98	1.29
0.4	0.50	5.32	1.88
0.6	0	0	0
0.8	0	0	0

4.2.3 Effect of up-scale of indoor flat panel airlift photobioreactor on induction of astaxanthin

Flat panel airlift photobioreactor systems of different sizes, i.e. 17, 50 and 90 L, under indoor conditions were examined. Time profiles of astaxanthin concentration from the induction in FP-APBRs, astaxanthin productivity and % astaxanthin (by weight), dry weight after 14 days inductions are shown in Figures 4.15,

4.16 and Table 4.7, respectively. The results indicate that an increase in system size could only slightly negatively influence the final astaxanthin concentrations in the reactor, and the 17, 50 and 90 L systems gave a similar growth pattern with the final concentrations of 26.63 (5.32% by weight), 25.84 (5.27% by weight) and 24.94 mg L^{-1} (5.20% by weight), respectively. This corresponded to the dried weight of 0.50, 0.49 and 0.48 g L^{-1}, respectively, and the astaxanthin productivity of 1.88, 1.83 and 1.77 m^{-3} day^{-1}, respectively. This demonstrates that the flat panel airlift systems could be up-scaled without significantly losing its growth performance. In addition, it was noticed that astaxanthin concentration often was the highest during the mid-second week (or about 10 days after induction). Hence, it becomes quite appropriate to employ this induction conditions for further experiments.

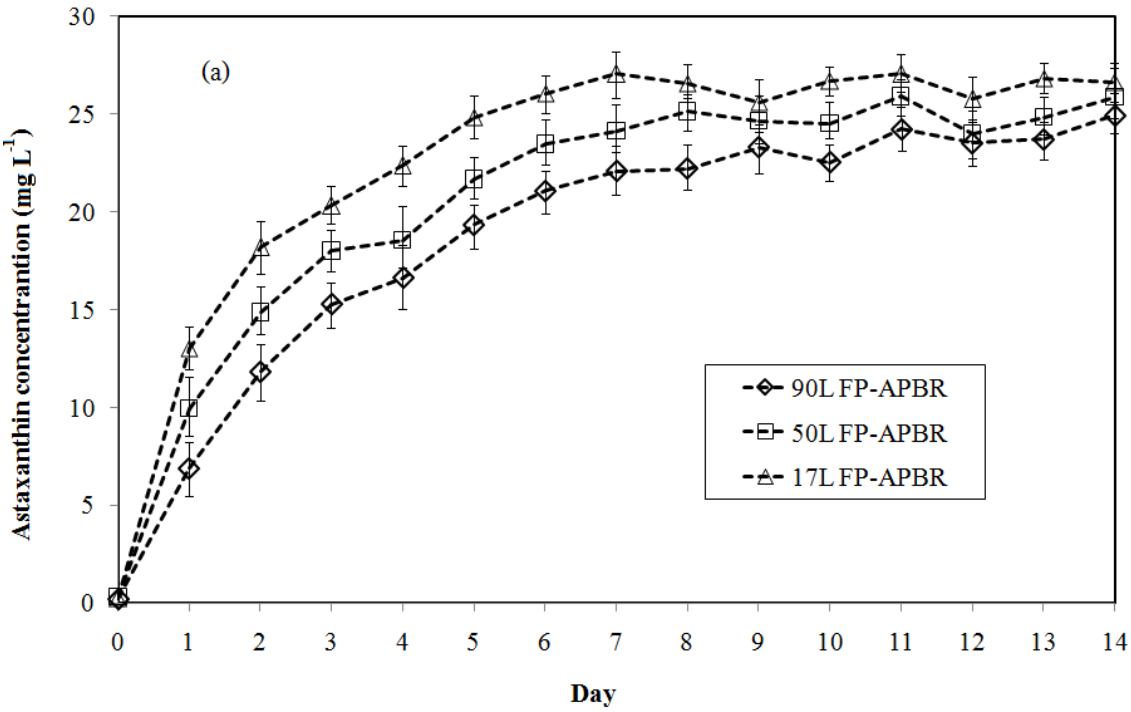


Figure 4.15 Astaxanthin concentrations in 17, 50 and 90 L FP-APBRs under indoor conditions

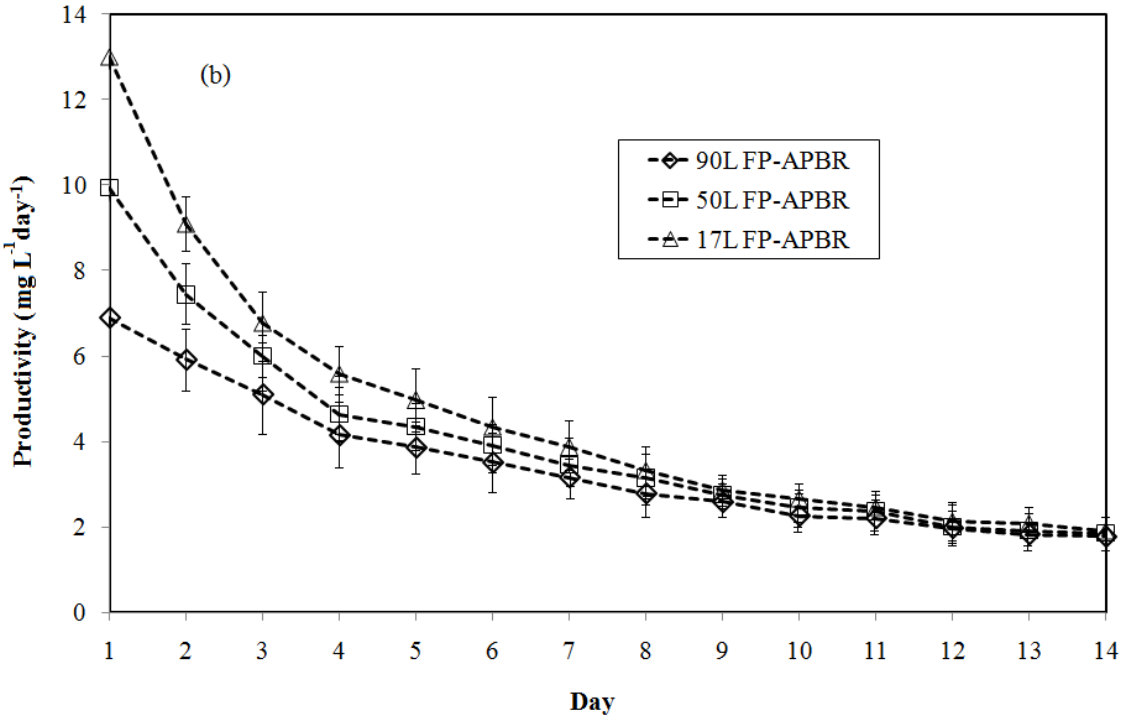


Figure 4.16 Productivity of astaxanthinin 17, 50 and 90 L FP-APBRs under indoor conditions

Table 4.7 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration in 17, 50 and 90 L FP-APBRs under indoor conditions

Conditions	Dry weight ($g\ L^{-1}$)	% Astaxanthin (by weight)	Astaxanthin productivity ($mg\ L^{-1}\ day^{-1}$)
17 L FP-APBR	0.50	5.32	1.88
50 L FP-APBR	0.49	5.27	1.83
90 L FP-APBR	0.48	5.20	1.77

4.2.4 Effect of up-scale of flat panel airlift photobioreactor in outdoor system

The culture was tested with actual climate condition in Thailand. Four experiments were conducted in the 17 L FP-ALPBR: (i) with direct sunlight, (ii) with 1-layer shading net, (iii) with 2-layer shading net, and (iv) with 1-layer shading net on the first three days. Light intensities and temperatures data of these three experiments were measured at every 15 minutes and the results are shown in

Figures 4.17-4.20, respectively. The results demonstrate that the reactor with direct sunlight was subject to very high light intensity at certain time periods of the day and this could negatively affect the cell viability particularly during the first few days when cells were in an adapting period of vegetative motile cell to mature cyst cell. In this period, cells were still not fully protected and high light intensity could lead to cell death. However, cell death stopped when cells entered its cyst stage where they were fully protected from environmental condition. This cell decay was not found when the reactor was covered with shading nets as it helped lower light intensity by 48%. The 2-layer shading net condition, however, filtered out too much light intensity (77%), and as a result, it stimulated the least amount of astaxanthin at 14.92 mg L^{-1} in 14 days (3.82% by weight). The experiment with 1-layer shading net seemed to provide the most proper light intensity as it could encourage a rather high astaxanthin accumulation at 20.11 mg L^{-1} in 14 days (4.37% by weight). An extra experiment was performed where the 1-layer shading net was only applied for the first three days (the net was removed after the third day) during which the cells seemed to be most vulnerable. The results indicate that this could stimulate the most astaxanthin accumulation 20.9 mg L^{-1} in 14 days (4.45% by weight). This was because during the first three days, cells needed some shelter to protect themselves against the high light intensity. However, cells began to build up astaxanthin which helped protect themselves and the external shelter was therefore not further required. At this point, it was better to remove the shelter as cells would be exposed to a stronger light intensity which induced a larger quantity of astaxanthin.

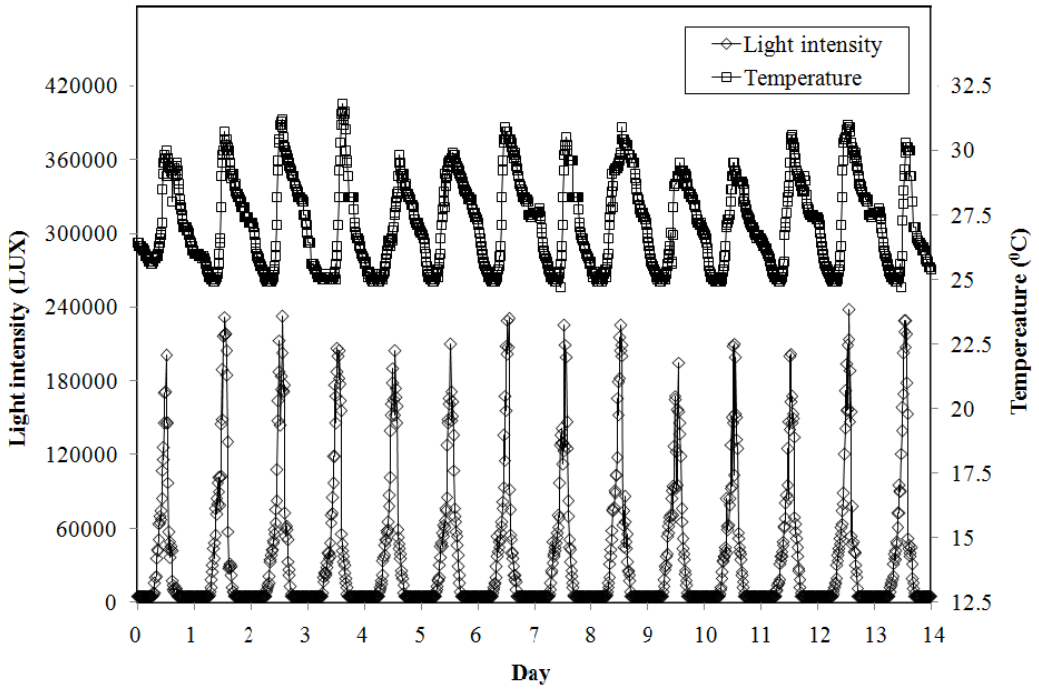


Figure 4.17 Time courses of temperatures and light intensities during the induction under outdoor condition with no shading net (sunlight) in 17 L FP-APBR

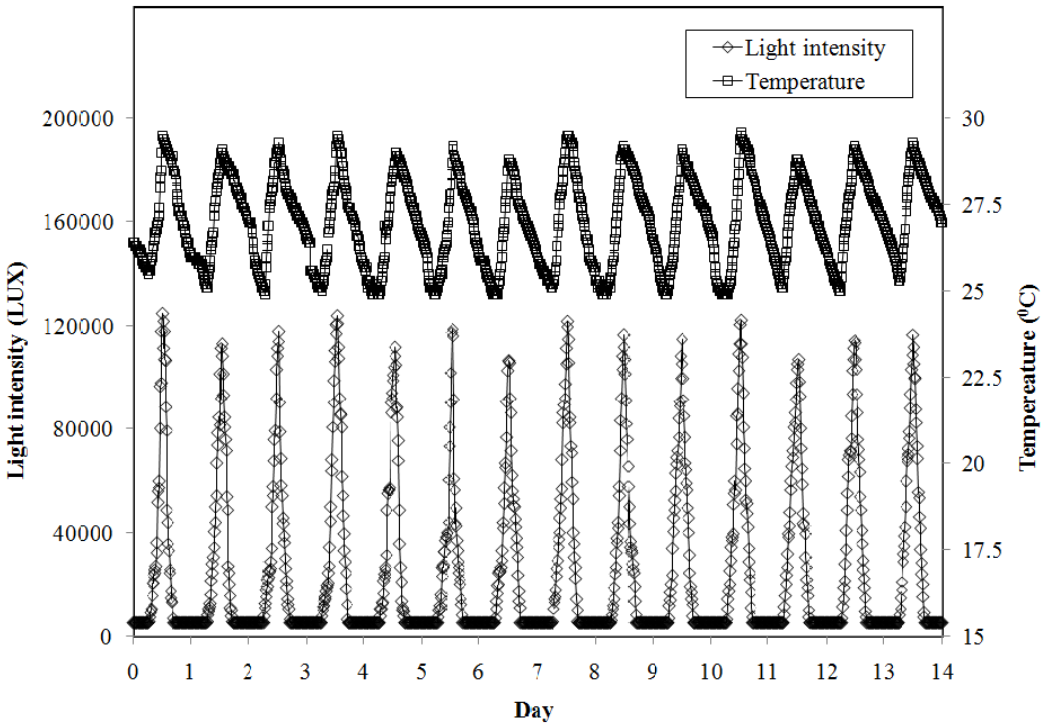


Figure 4.18 Time courses of temperatures and light intensities during the induction under outdoor condition with 1-layer shading net in 17 L FP-APBR

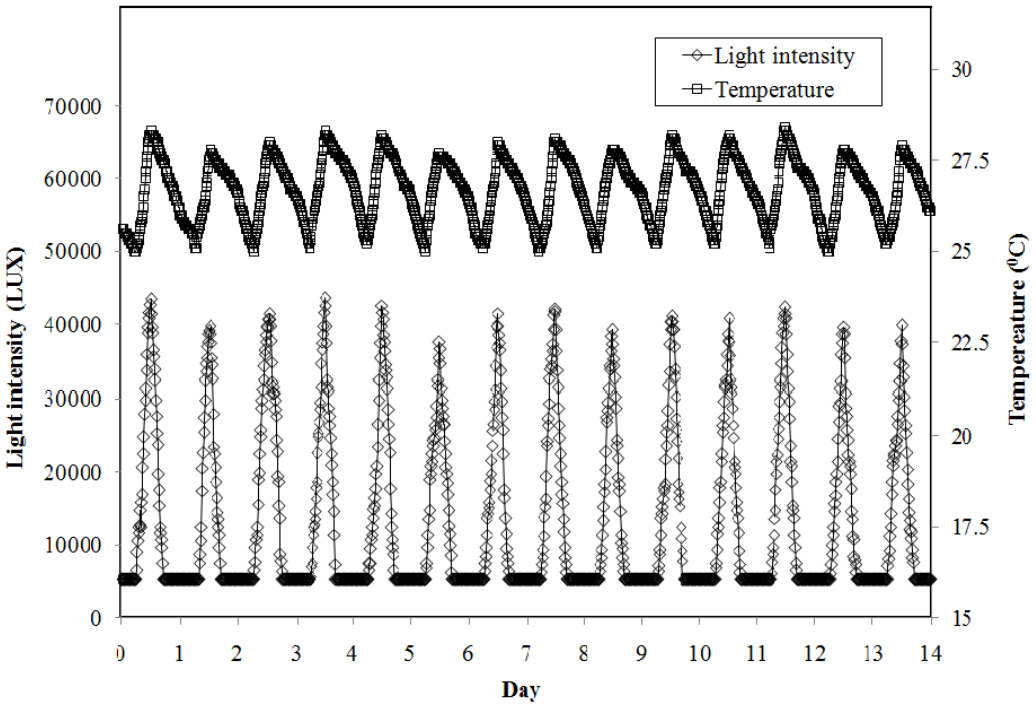


Figure 4.19 Time courses of temperatures and light intensities during the induction under outdoor condition with 2-layer shading net in 17 L FP-APBR

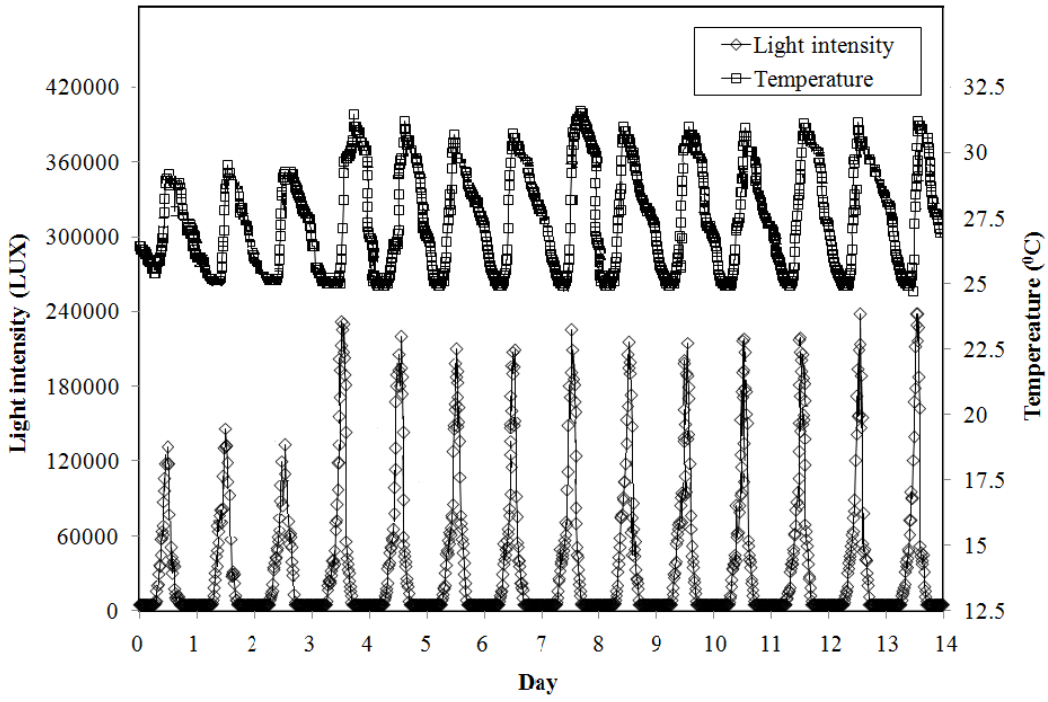


Figure 4.20 Time courses of temperatures and light intensities during the induction under outdoor condition with 1-layer shading net on 1-3 day in 17 L FP-APBR

In terms of astaxanthin production, experimental results indicate that the 1-layer shading net on the first three days condition gave the highest accumulation of astaxanthin followed by the experiment with ,1-layer shading net, direct sunlight, where the 2-layer provided the worst results, i.e. astaxanthin productivities at Day 14 from reactors with 1-layer shading net on the first three days, 1-layer shading net, direct sunlight and 2-layer shading net are 1.47, 1.42, 1.21 and 1.05 mg L^{-1} day^{-1} respectively. Cell dry weights after 14 days for the same order of experiments were 0.47, 0.45, 0.4 and 0.39 g L^{-1}. Time profiles of astaxanthin concentration, astaxanthin productivity, astaxanthin productivity and dry weights, % astaxanthins by weight are shown in Figures 4.21, 4.22 and Table 4.8, respectively.

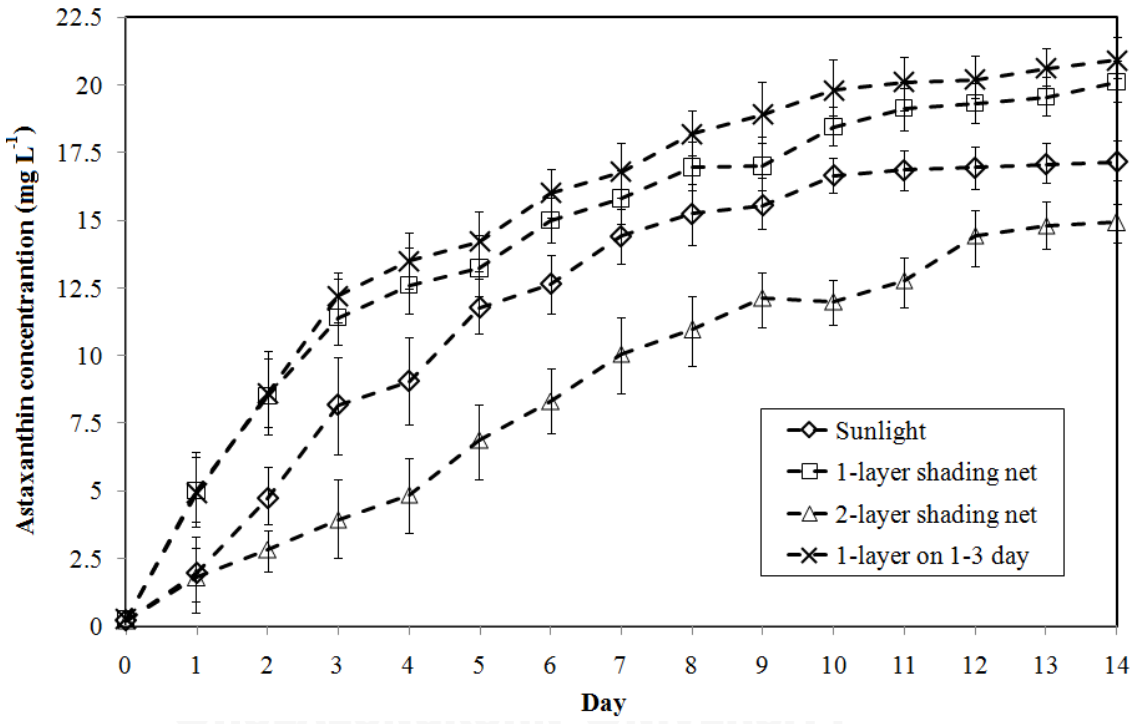


Figure 4.21 Astaxanthin induction in 17 L FP-APBR under outdoor conditions without shadings

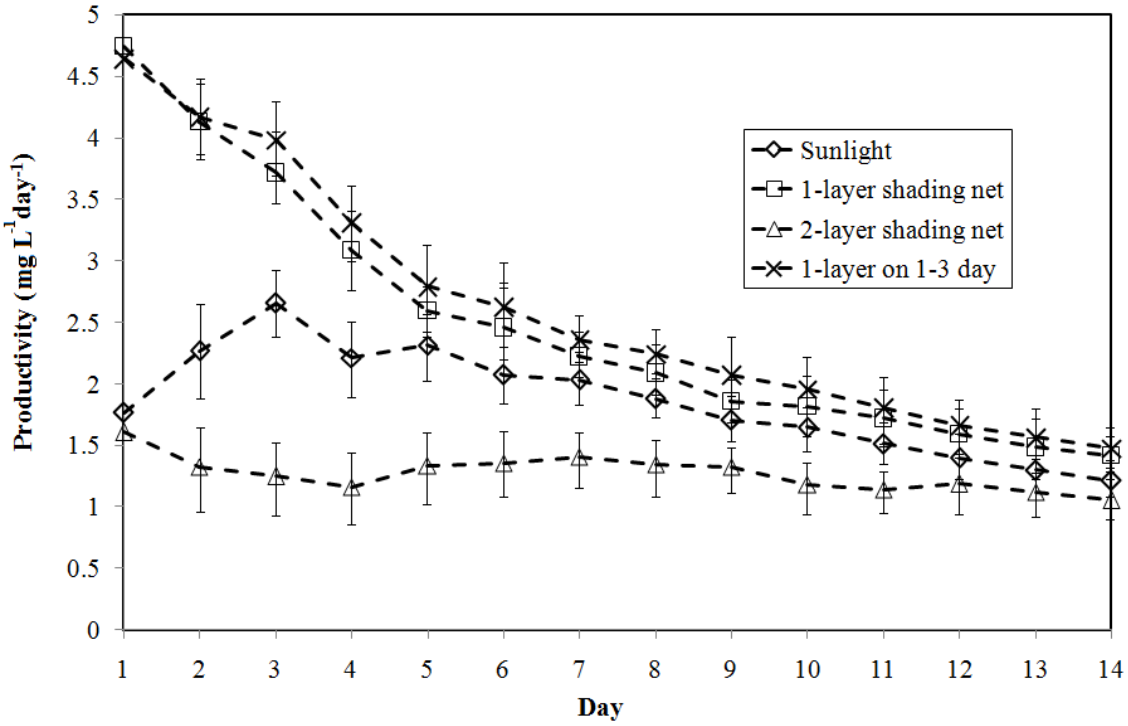


Figure 4.22 Productivity of astaxanthin in 17 L FP-APBR under outdoor conditions without shadings

Table 4.8 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction concentration in 17 L FP-APBR under outdoor conditions

Conditions	Dry weight (g L^{-1})	% Astaxanthin (by weight)	Astaxanthin productivity (mg L^{-1} day^{-1})
Sunlight	0.40	4.29	1.21
1-layer shading net	0.45	4.37	1.42
2-layer shading net	0.39	3.82	1.05
1-layer shading net on the first three days	0.47	4.45	1.47

Flat panel airlift photobioreactor systems of 50 and 90 L with 1-layer shading net on the first three days were tested under light intensity and temperature profiles as displayed in Figures 4.23 and 4.24. Time profiles of astaxanthin concentration from

the induction in 17, 50 and 90 FP-APBRs are shown in Figure 4.25. The results indicate that the smaller size system was actually able to stimulate more astaxanthin than the large one, but only slightly. In other words, the 17 L system yielded 20.9 g astaxanthin m^{-3} in 14 days (4.45% by weight) whereas the 50 and 90 L yielded 19.6 (4.35% by weight) and 18.6 g astaxanthin m^{-3} (4.32% by weight) of astaxanthin, respectively. Dry weights on the last day (14 days) from the 50 and 90 L are 0.45, and 0.43 g L^{-1}, and astaxanthin productivities are 1.38 and 1.31 mg L^{-1} day^{-1}, respectively (see Table 4.9 and Figure 4.26). These were only slightly lower than those obtained from the 17 L-airlift as mentioned in the previous paragraph.

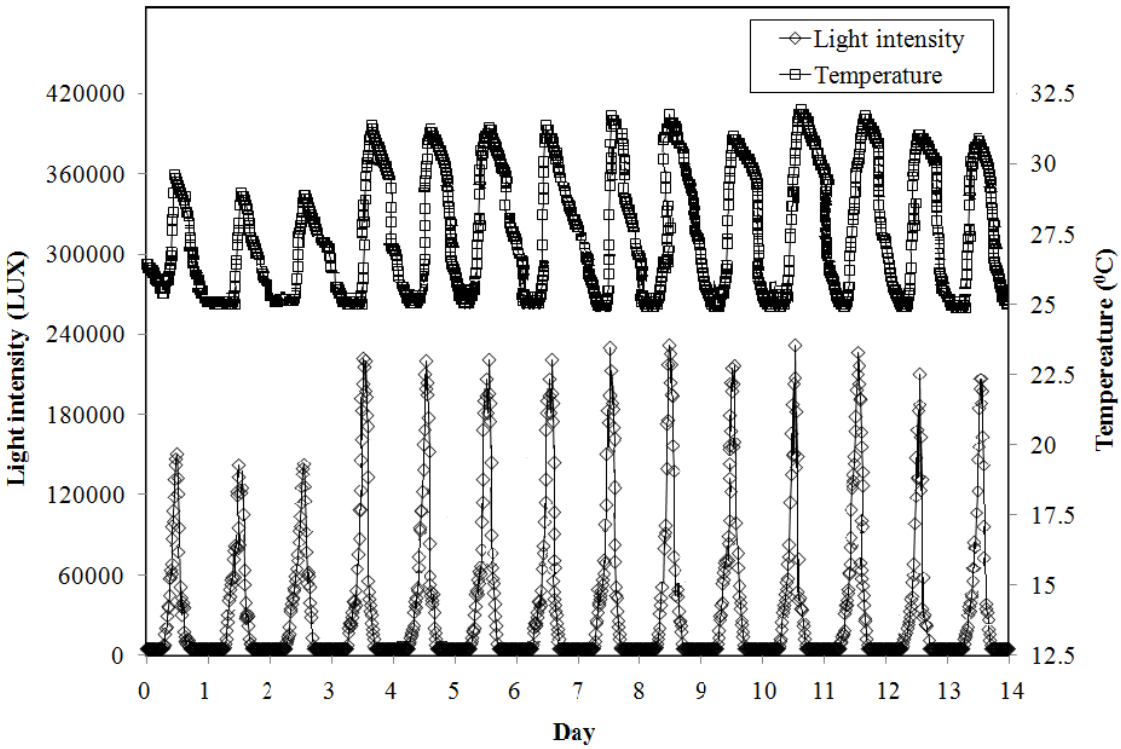


Figure 4.23 Time courses of temperatures and light intensities during the induction under outdoor condition with 1-layer shading net on 1-3 days in 50 L FP-APBR

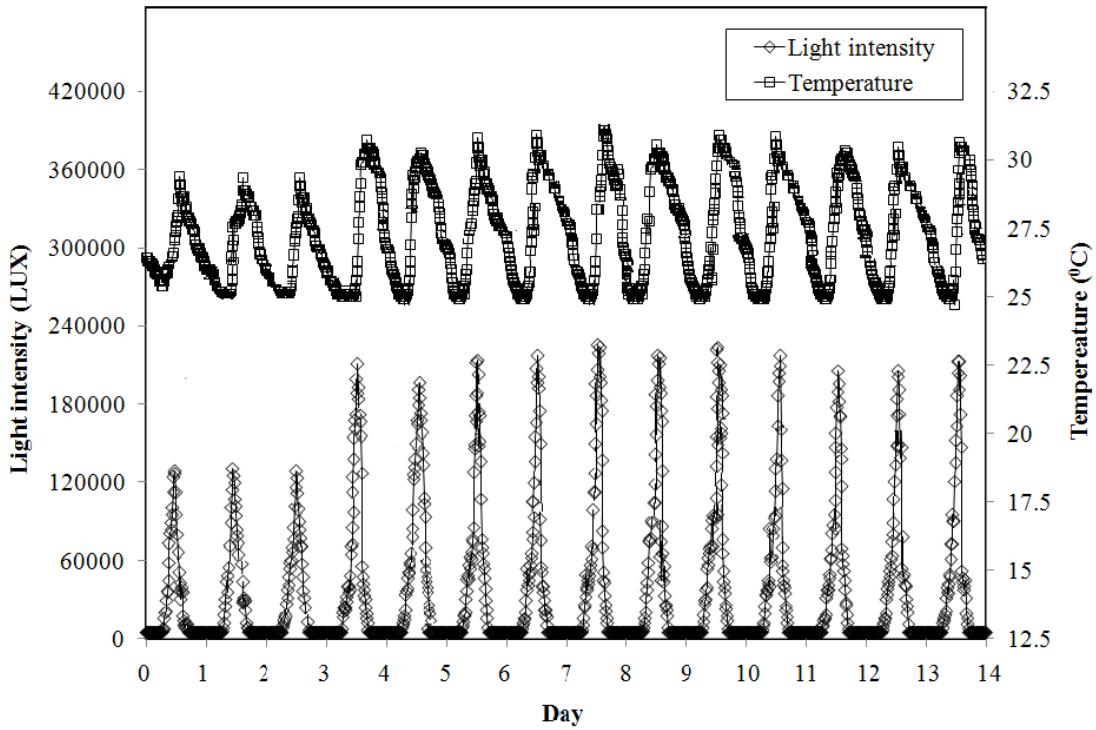


Figure 4.24 Time courses of temperatures and light intensities during the induction under outdoor condition with 1-layer shading net on 1-3 days in 90 L FP-APBR

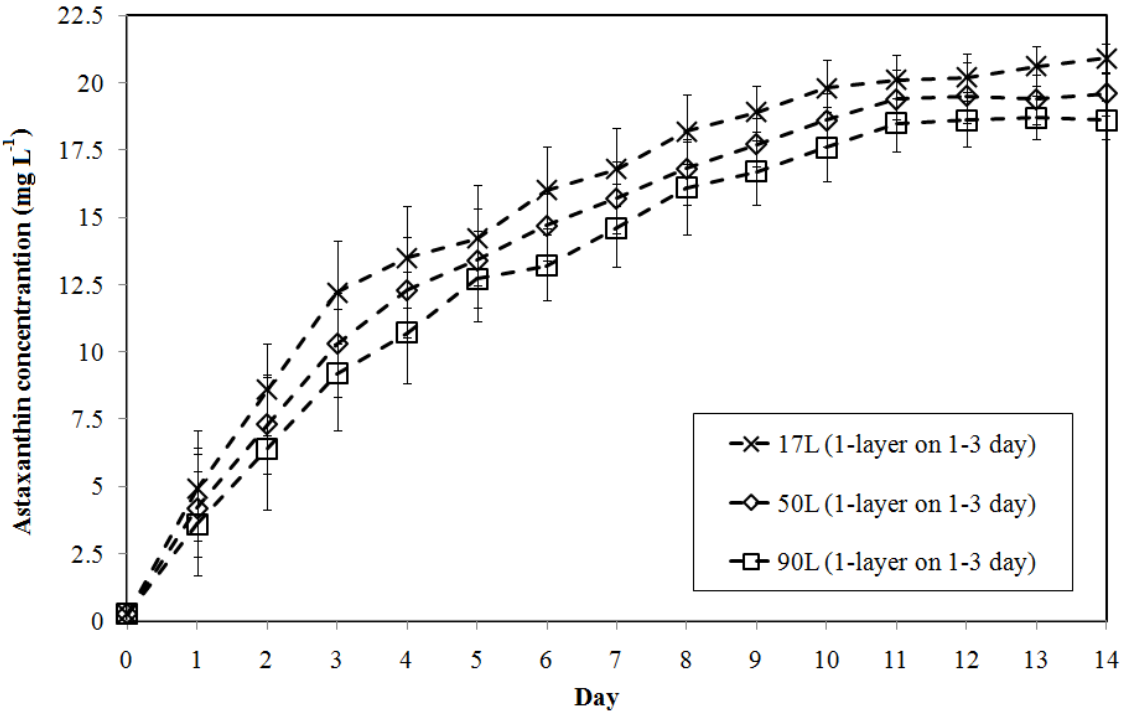


Figure 4.25 Astaxanthin inductions in 17, 50 and 90 L FP-APBRs under outdoor conditions with 1-layer shading net on 1-3 days

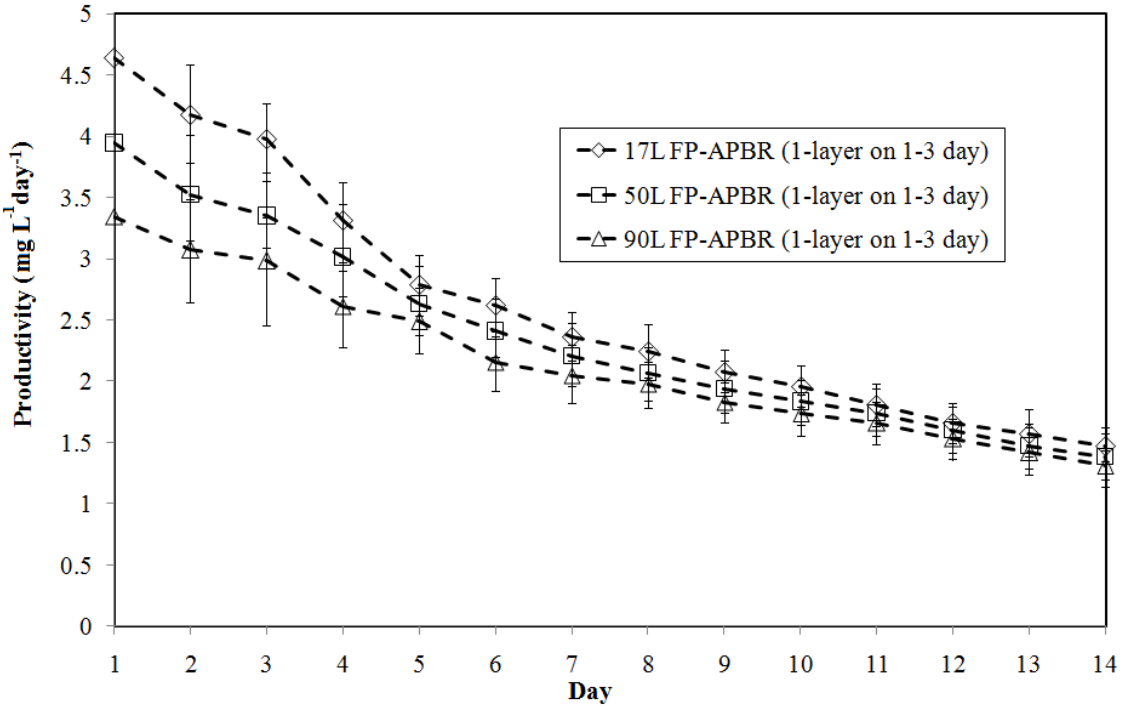


Figure 4.26 Astaxanthin inductions in 17, 50 and 90 L FP-APBRs under outdoor conditions with 1-layer shading net on 1-3 days

Table 4.9 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction in 17, 50 and 90 L FP-APBRs under outdoor conditions with 1-layer shading net on 1-3 days

Conditions	Dry weight ($g\ L^{-1}$)	% Astaxanthin (by weight)	Astaxanthin productivity ($mg\ L^{-1}\ day^{-1}$)
17 L (1-layer shading net on 1-3 day)	0.47	4.45	1.47
50 L (1-layer shading net on 1-3 day)	0.45	4.35	1.38
90 L (1-layer shading net on 1-3 day)	0.43	4.32	1.31

4.3 Determination of suitable conditions for astaxanthin induction from *H. pluvialis* (NIES-144) with a two-step single reactor process

The astaxanthin production with two-step single reactor process (TSSRP) means that the active vegetative cell culture is further induced without the need for a pre-harvest. However, the cultivation still needed to be separated into two periods. The first period was the cultivation of the green vegetative cells where the cultivation conditions were 2,000 LUX, 25^0C, 1% CO_2 in the air supply (or 1.6, 4.4 and 8 cm^3 s^{-1} of CO_2 loading in 17, 50 and 90 L FP-APBRs, respectively), and this was maintained for 5 days. After that, the stress conditions at 35,000 LUX, 33^0C, 3% CO_2 per air (or 4.8, 13.2 and 24 cm^3 s^{-1} of CO_2 loading in 17, 50 and 90 L FP-APBRs, respectively) was applied for another 5 days for the induction of astaxanthin from *H. pluvialis*.

4.3.1 Effect of reducing nitrate in sterilized F1 medium on astaxanthin induction with two-step single reactor process

This experiment was examined using 17 L FP-APBR. The growth behavior of *H. pluvialis* (NIES-144) and time profiles of astaxanthin concentration under reducing nitrate are given in Figures 4.27 and 4.28, respectively, and percent astaxanthin (by weight), dry weight of astaxanthin and astaxanthin productivity after 14 days of induction are provided in Table 4.10. When reducing nitrate by 2-fold of that in the F1 medium (0.5xNO_3^-), the cultivation behavior was almost unaltered, which indicates that there were enough nutrients even in 0.5xNO_3^- for the growth of *H. pluvialis* and therefore the results were not much different than that when cultivated with the typical F1. The final astaxanthin concentration of the normal F1 medium and 0.5xNO_3^- were 0.54 (0.24% by weight) and 0.85 mg L^{-1} (0.35% by weight), respectively, and the corresponding astaxanthin productivities were 0.03 and 0.05 mg L^{-1} day^{-1}, respectively. In addition, the maximum cell density (after 7 days) of the normal F1 medium and 0.5xNO_3^- were 29.1 x10^4 and 27 x10^4 cell mL^{-1}, respectively and the final cell density of the normal F1 medium and N2&P1 were 9.5 x10^4 and 8.5 x10^4 cell mL^{-1} (or 0.24 and 0.23 g L^{-1} of dry weight), respectively. When reducing nitrate further to 3, 4 and 5-fold of that in the F1 medium (0.33xNO_3^-, 0.25xNO_3^- and 0.2xNO_3^-), more astaxanthin could be stimulated indicating a better stressing condition with 0.25xNO_3^- being the most effective option. The final astaxanthin concentration, percent astaxanthin (by weight), astaxanthin productivity, dry weight of astaxanthin after 14 days of induction and the maximum cell density obtained from the cultivation with

0.25xNO_3^- were 25.1 mg L^{-1}, 4.52% by weight, 1.63 mg L^{-1} day^{-1},0.56 g $L^{-1}$and 1.7 x10^5 cell mL^{-1}, respectively. 0.33xNO_3^-, on the other hand, could stimulate the least amount of astaxanthin, i.e. with the following production detail: 16 mg L^{-1} final astaxanthin concentrations, 2.76% astaxanthin by weight, 1.11 mg L^{-1} day^{-1} astaxanthin productivity, 0.54 g L^{-1}, dry weight of astaxanthin after 14 days of induction and 2.3 x10^5 cell mL^{-1}maximum cell density, respectively. Therefore it could mean that reducing nitrogen source by 3-fold from the standard F1 medium (0.33xNO_3^-) still provided sufficient nutrient for the alga and therefore there was no nutrient stress at the beginning of the cultivation, and the alga therefore did not accumulate astaxanthin as much as it should. This is why 0.33xNO_3^- could only produce less astaxanthin than that from 0.25xNO_3^- and 0.2xNO_3^-. When considered 0.2xNO_3^-, this could be the most unsuitable condition for cell growth, therefore *H. pluvialis* exhibited the worst growth and therefore there was only a small amount of cell for the accumulation of astaxanthin. The final astaxanthin concentrations, percent astaxanthin (by weight), astaxanthin productivity, dry weight of astaxanthin after 14 days of induction and the maximum cell density obtained from the culture with 0.2xNO_3^- were 11.3 mg L^{-1}, 4.40 % by weight, 0.77 mg L^{-1} day^{-1}, 0.25 g L^{-1} and 5 x10^4 cell mL^{-1}, respectively. Therefore, this experiment suggested that reducing nitrate in F1 medium could induce high astaxanthin accumulation whilst reducing growth. The maximum productivity is a compromise between the increasing astaxanthin accumulation and the reducing growth, and in this case, this occurred at 0.25xNO_3^-.

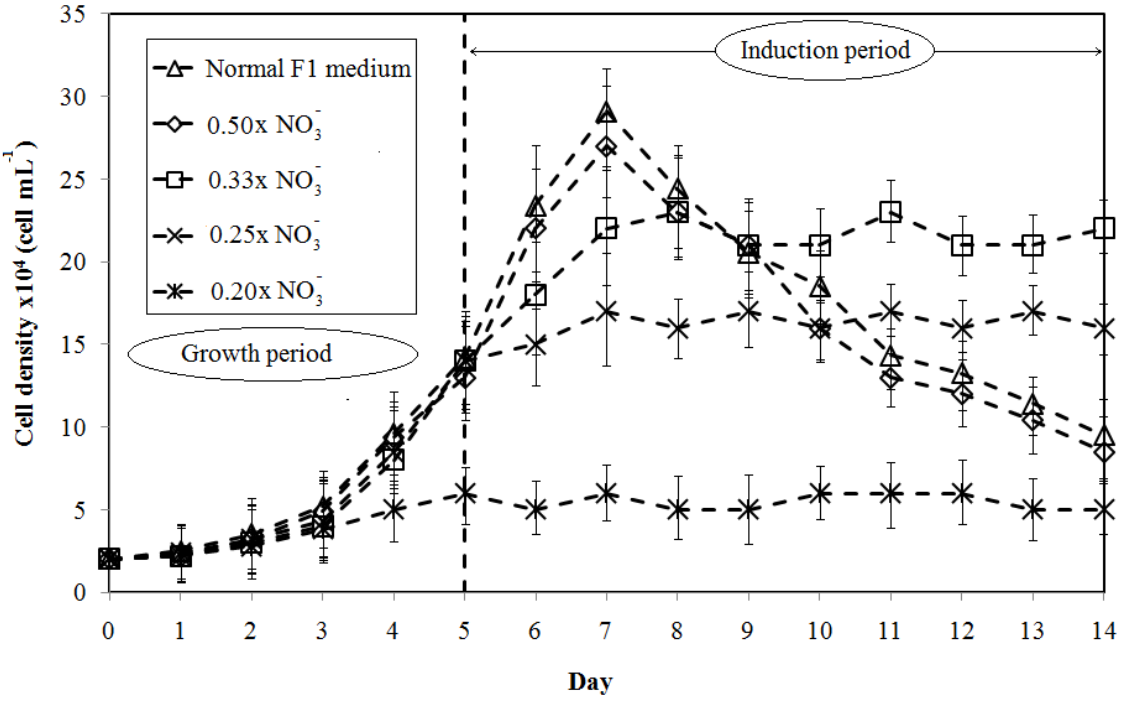


Figure 4.27 Growth behavior of *H. pluvialis* (NIES-144) under reducing nitrate for TSSRP in 17 L FP-APBR

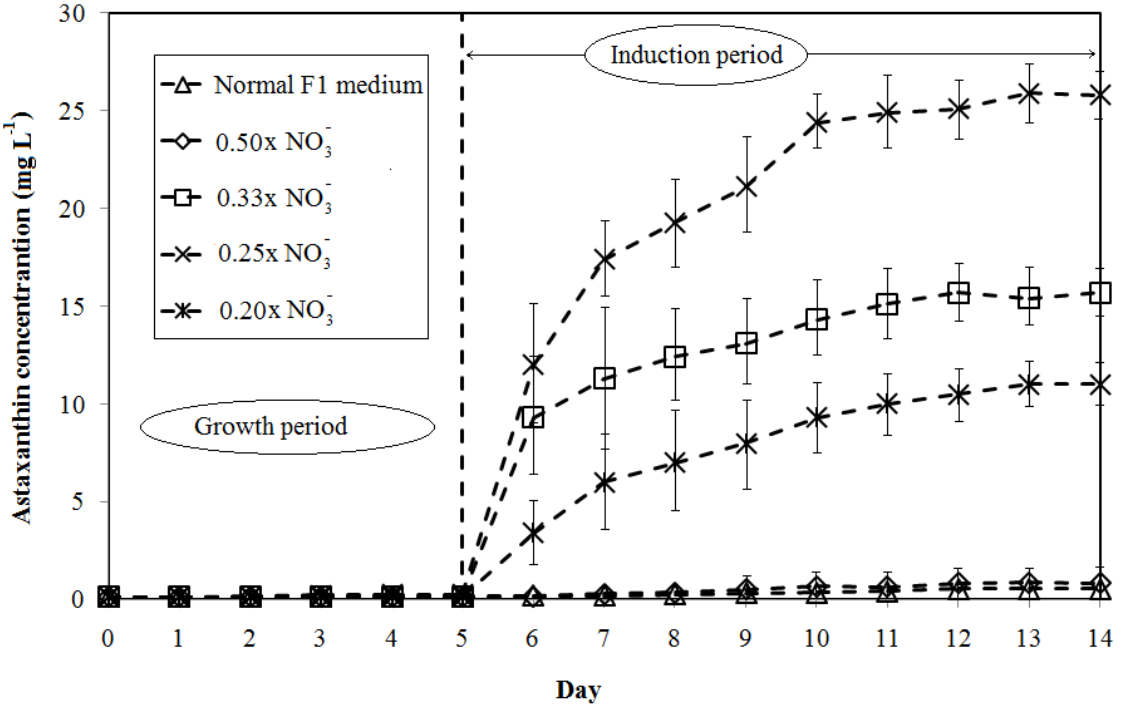


Figure 4.28 Astaxanthin induction under reducing nitrate for TSSRP in 17 L FP-APBR

Table 4.10 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under reducing nitrate in TSSRP in 17 L FP-APBR

Conditions	Dry weight ($g\ L^{-1}$)	% Astaxanthin (by weight)	Astaxanthin productivity ($mg\ L^{-1}\ day^{-1}$)
Normal F1 medium	0.23	0.24	0.03
$0.50xNO_3^-$	0.24	0.35	0.05
$0.33xNO_3^-$	0.54	2.70	1.11
$0.25xNO_3^-$	0.56	4.52	1.63
$0.20xNO_3^-$	0.25	4.40	0.77

4.3.2 Effect of reducing phosphate in F1 medium on astaxanthin induction with two-step single reactor process

This section focuses on the examination of the effect of reducing phosphate in the F1 medium on astaxanthin induction using the TSSRP in 17 L FP-APBR. Figures 4.29–4.30 and Table 4.11 illustrate the growth behavior of *H. pluvialis* (NIES-144) and time profiles of astaxanthin concentration under such condition. With the standard F1 medium and a 2-fold reduction in phosphate concentration ($0.5xPO_4^{3-}$), the cells could still grow normally in vegetative stage which indicates that there was still sufficient nutrient for *H. pluvialis*. The final astaxanthin concentrations, percent astaxanthin (by weight), astaxanthin productivity, dry weight of astaxanthin after 14 days of induction and maximum cell density (7 day) of normal F1 medium condition were 0.54 $mg\ L^{-1}$, 0.24% by weight, 0.03 $mg\ L^{-1}\ day^{-1}$, 0.23 $g\ L^{-1}$ and 2.91 $x10^5$ cell mL^{-1}, respectively, and those from $0.5xPO_4^{3-}$ were 0.66 $mg\ L^{-1}$, 0.35% by weight, 0.04 $mg\ L^{-1}\ day^{-1}$,0.19 $g\ L^{-1}$ and 2.73 $x10^5$ cell mL^{-1}. Reducing phosphate further to 6 and 8-fold ($0.16xPO_4^{3-}$ and $0.12xPO_4^{3-}$) revealed some sign of the transformation to mature cyst which could be due to phosphate stressing condition where the final astaxanthin concentrations were 14.7 and 5.32 $mg\ L^{-1}$, the percent astaxanthin (by weight) were 2.88 and 2.87% by weight, and astaxanthin productivity were1.04 and 0.37 $mg\ L^{-1}\ day^{-1}$for $0.16xPO_4^{3-}$ and $0.12xPO_4^{3-}$, respectively. However, a drought of phosphate (P) below a certain threshold value could cease the cell division activity leading to a drop in cell number as can be seen from the case of $0.12xPO_4^{3-}$

.Therefore, cell density and dry weight obtained from $0.12xPO_4^{3-}$ were lower than that from N1&P6, i.e. cell density and dry weight of $0.12xPO_4^{3-}$ were 3 $x10^4$ cell mL^{-1} and 0.19 g L^{-1}, respectively, whereas cell density and dry weight of $0.16xPO_4^{3-}$ were 1.7 $x10^5$ cell mL^{-1} and 0.52 g L^{-1}, respectively. The cultivation with a 4-fold reducing phosphate condition ($0.25xPO_4^{3-}$) could stimulate less astaxanthin than those from $0.16xPO_4^{3-}$ and $0.12xPO_4^{3-}$, where the cell density decreased after 7 days of cultivation in a similar fashion to those from $0.5xPO_4^{3-}$. Visual observation revealed that this condition resulted in a mixture of vegetative cells and cysts. Vegetative cells after some time died instead of entering their cyst which could be due to unsuitable environment, and the final astaxanthin concentrations, percent astaxanthin (by weight), astaxanthin productivity, dry weight of astaxanthin, and the maximum cell density from this condition were 10.8 mg L^{-1}, 2.61% by weight, 0.76 mg L^{-1} day^{-1}, 0.42 g L^{-1} and 2.4 $x10^5$ cell mL^{-1}, respectively. Therefore, this experiment demonstrates that reducing phosphate in F1 medium could not significantly induce the astaxanthin accumulation when compared with the case of reducing nitrate.

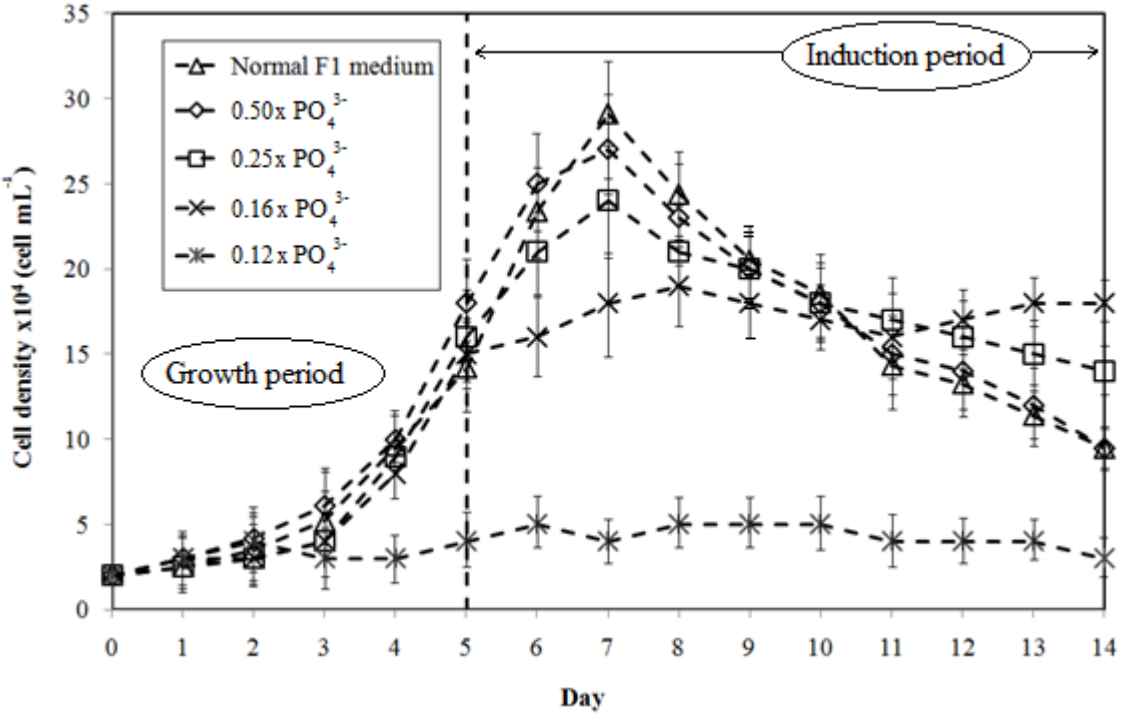


Figure 4.29 Growth behavior of *H. pluvialis* (NIES-144) under reducing phosphate for TSSRP in 17 L FP-APBR

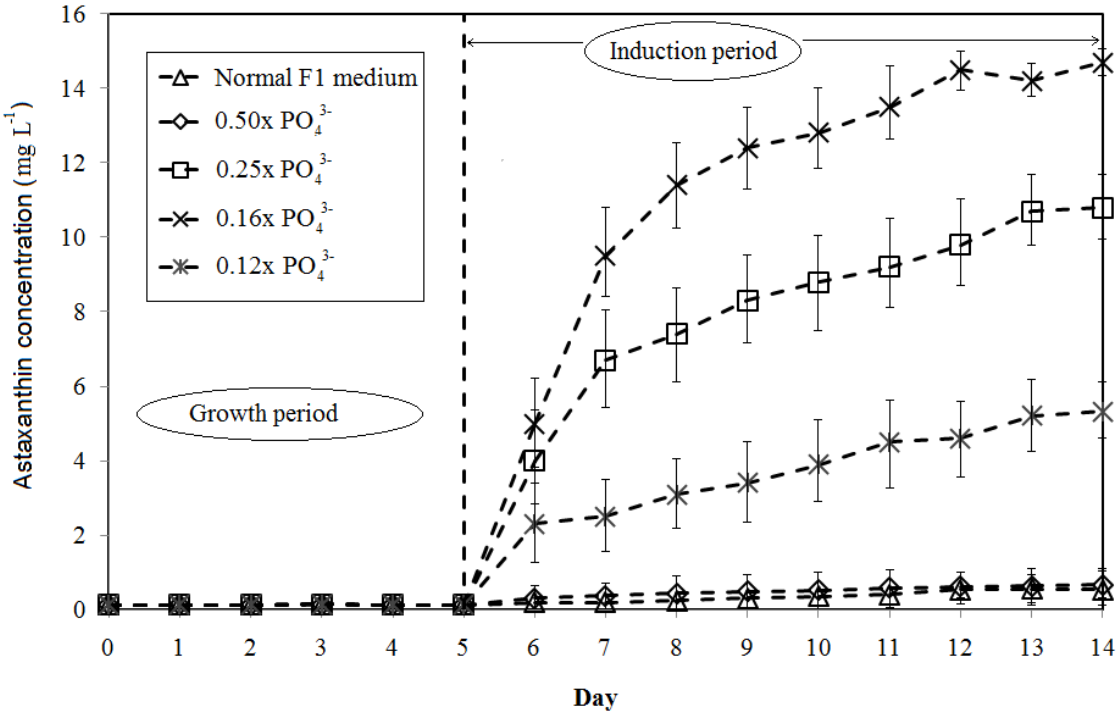


Figure 4.30 Astaxanthin inductions under reducing phosphate for TSSRP in 17 L FP-APBR

Table 4.11 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under reducing phosphate in TSSRP in 17 L FP-APBR

Conditions	Dry weight ($g\ L^{-1}$)	% Astaxanthin (by weight)	Astaxanthin productivity ($mg\ L^{-1}\ day^{-1}$)
Normal F1 medium	0.23	0.24	0.03
$0.50xPO_4^{3-}$	0.19	0.35	0.04
$0.25xPO_4^{3-}$	0.42	2.61	0.76
$0.16xPO_4^{3-}$	0.52	2.88	1.04
$0.12xPO_4^{3-}$	0.19	2.87	0.37

4.3.3 Effect of reducing nitrate and phosphate in sterilized F1 medium on astaxanthin induction with two-step single reactor process

The previous two sections suggest that there are effects of nutrient stress on astaxanthin induction from *H. pluvialis*. This section explores this stress further by having both nitrate and phosphate limitations at the same time, and the results are demonstrated in Figures 4.31-4.32 and Table 4.12. When considered 4-fold of reducing nitrate with 2, 4 and 6-fold of reducing phosphate conditions ($0.25xNO_3^-$ &$0.50xPO_4^{3-}$, $0.25xNO_3^-$&$0.25xPO_4^{3-}$ and $0.25xNO_3^-$&$0.16xPO_4^{3-}$), the results indicate that when phosphate was reduced, astaxanthin induction increased. Astaxanthin concentration after 14 days of induction of N4&P2, N4&P4 and N4&P6 were 22.6, 23.9 and 28 mg L^{-1} (or 3.74, 4.19 and 5.24 % by weight), respectively andastaxanthin productivity of N4&P2, N4&P4 and N4&P6 were 1.60, 1.67 and 1.82 mg L^{-1} day^{-1}, respectively. In addition, cell density after 14 days of induction under $0.25xNO_3^-$ &$0.50xPO_4^{3-}$, $0.25xNO_3^-$&$0.25xPO_4^{3-}$ and $0.25xNO_3^-$&$0.16xPO_4^{3-}$ conditions were 17 $x10^4$, 15 $x10^4$ and 11.9 $x10^4$ cell mL^{-1} (or equivalent to 0.60, 0.59 and 0.59 g L^{-1} dry weight), respectively. In case of 4-fold of reducing nitrate with 8 and 10-fold of reducing phosphate ($0.25xNO_3^-$&$0.12xPO_4^{3-}$ and $0.25xNO_3^-$&$0.10xPO_4^{3-}$) conditions, the result indicates that those conditions have efficiency of astaxanthin induction to be similar with that obtained from N4&P6. Percent astaxanthin (by weight) of $0.25xNO_3^-$ &$0.12xPO_4^{3-}$ and $0.25xNO_3^-$&$0.10xPO_4^{3-}$ conditions were 5.02 and 4.95% by weight (or calculating to be 20.5 and 9.4 mg L^{-1}), respectively and astaxanthin productivity of $0.25xNO_3^-$&$0.12xPO_4^{3-}$ and $0.25xNO_3^-$&$0.10xPO_4^{3-}$ conditions were 1.45 and 0.66 mg L^{-1} day^{-1}, respectively. However, cell density in the first period of cultivation only increased slightly. This could be due to the fact that phosphate is an important component in the cell wall and reducing too much phosphate negatively affected the multiplication of algal cells. Cell density after 14 days of induction under $0.25xNO_3^-$&$0.12xPO_4^{3-}$ and $0.25xNO_3^-$&$0.10xPO_4^{3-}$ conditions were 8 $x10^4$ and 4 $x10^4$ cell mL^{-1} (or calculating to be 0.41 and 0.19 g L^{-1} of dry weight), respectively. Therefore, the all results received from this section led to a conclusion that $0.25xNO_3^-$&$0.16xPO_4^{3-}$ condition could stimulate the best astaxanthin. Consequently, $0.25xNO_3^-$&$0.16xPO_4^{3-}$ condition was used in next section.

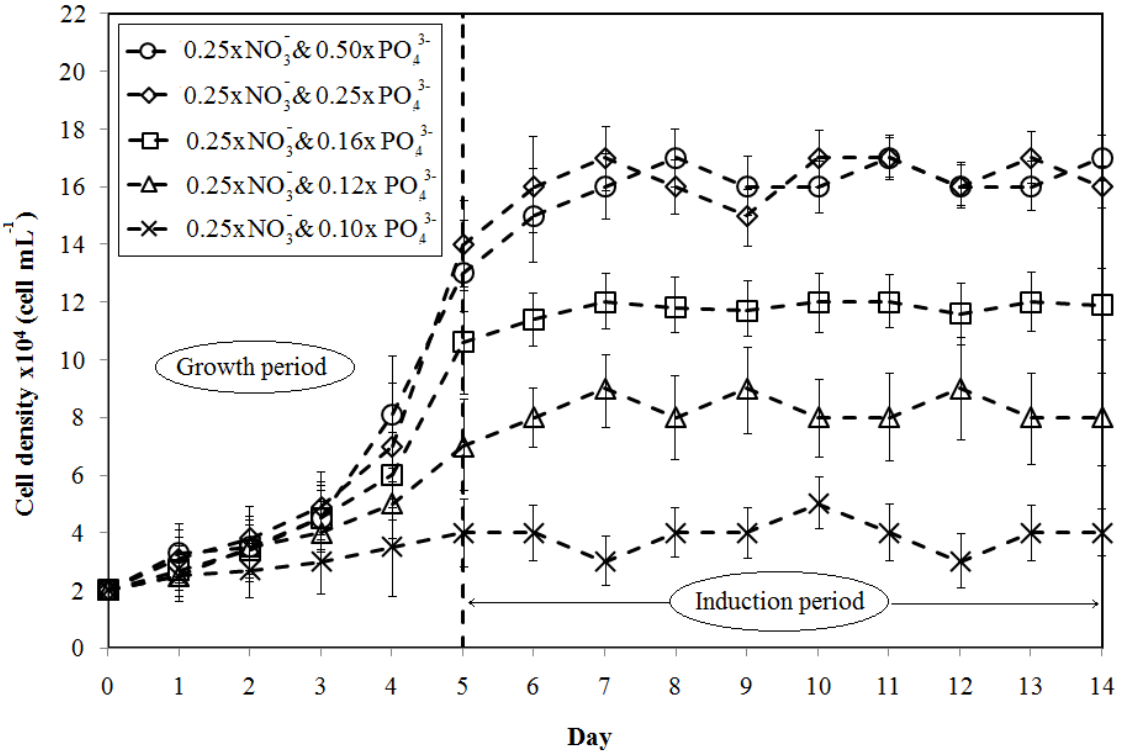


Figure 4.31 Growth behavior of *H. pluvialis* (NIES-144) under reducing nitrate and phosphate for TSSRP in 17 L FP-APBR

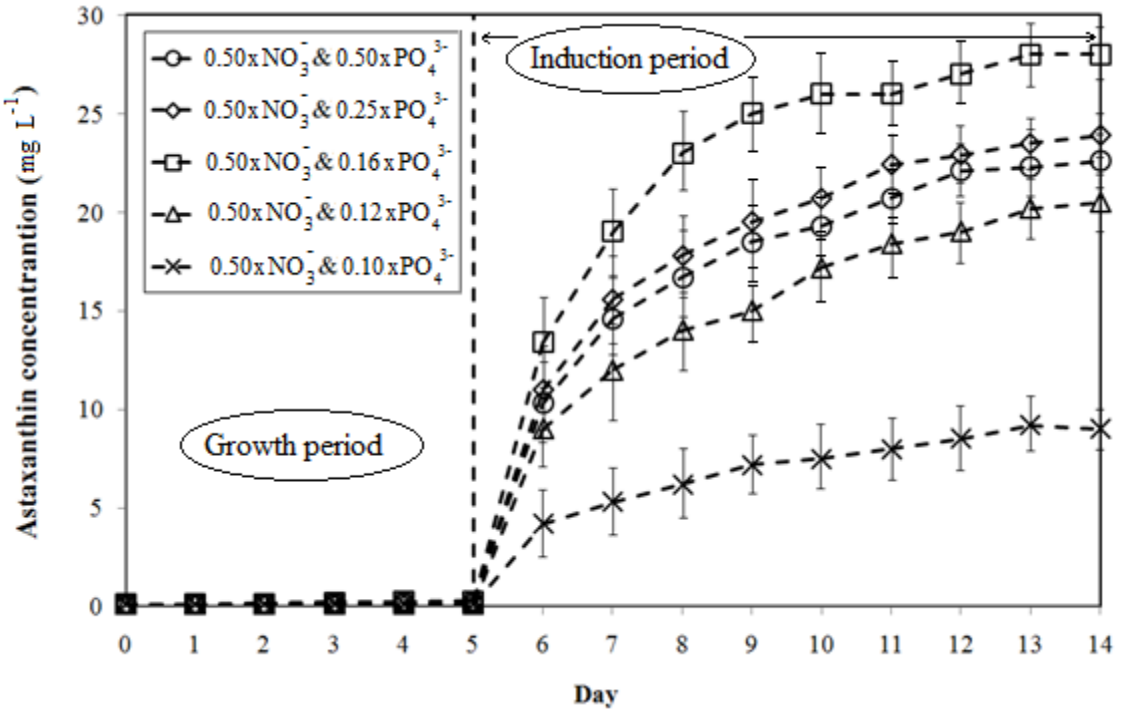


Figure 4.32 Astaxanthin induction under reducing nitrate and phosphate for TSSRP in 17 L FP-APBR

Table 4.12 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under reducing nitrate and phosphate in TSSRP in 17 L FP-APBR

Conditions	Dry weight ($g\ L^{-1}$)	%Astaxanthin (by weight)	Astaxanthin productivity ($mg\ L^{-1}\ day^{-1}$)
$0.25xNO_3^-\&0.5xPO_4^{3-}$	0.60	3.74	1.60
$0.25xNO_3^-\&0.25xPO_4^{3-}$	0.59	4.19	1.67
$0.25xNO_3^-\&0.16xPO_4^{3-}$	0.59	5.24	1.82
$0.25xNO_3^-\&0.12xPO_4^{3-}$	0.41	5.02	1.45
$0.25xNO_3^-\&0.10xPO_4^{3-}$	0.19	4.95	0.66

4.3.4 Effect of up-scale in flat panel airlift photobioreactor on astaxanthin induction in *H. pluvialis* with two-step single reactor process

The effect of up-scale of FP-APBR for TSSRP was examined in 17, 50 and 90 L. The best condition obtained from Section 4.3.3 ($0.25xNO_3^-\&0.16xPO_4^{3-}$) was used in all experiments in this section. Growth behavior of *H. pluvialis* (NIES-144) and time profiles of astaxanthin concentration under a large scale of FP-APBRs for TSSRP are given in Figures 4.33 and 4.34, respectively and percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction and astaxanthin productivity in Table 4.13. The results indicate that when scale up, astaxanthin concentration decreased slightly. In addition, dry weight was decreased slightly. The astaxanthin at the last day of experiment from the 17, 50 and 90 L reactors were 28, 26.51 and 24.67 $mg\ L^{-1}$ (or equivalent to 5.24, 4.92 and 4.90 % by weight), respectively and astaxanthin productivity of 17, 50 and 90 L were 1.82, 1.80 and 1.75 $mg\ L^{-1}\ day^{-1}$. In addition, cell density after 14 days of induction under 17, 50 and 90 L were 11.9 $x10^4$, 11 $x10^4$ and 10.7 $x10^4$ cell mL^{-1} (0.51, 0.49 and 0.48 $g\ L^{-1}$ of dry weight), respectively. This could be due to the inevitable irregularity of fluid flows in the large system which might allow some dead zone or poor local circulation in some part of the system. In this regard, the small system stimulated a better astaxanthin than the large system.

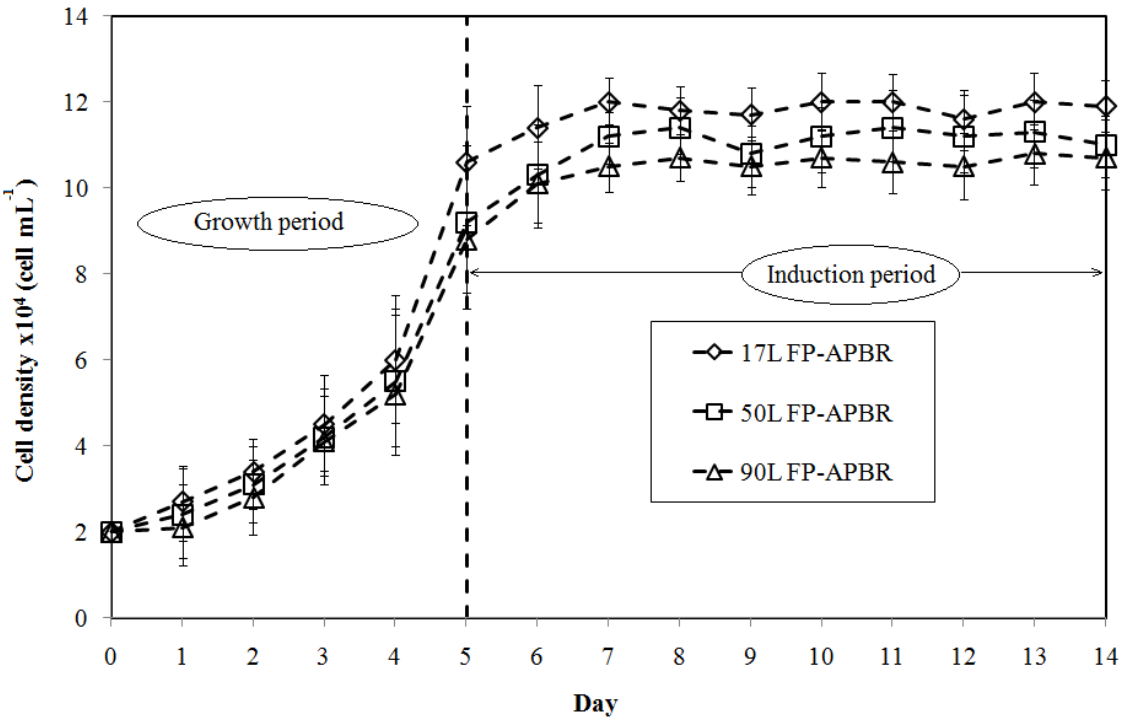


Figure 4.33 Growth behavior of *H. pluvialis* (NIES-144) under reducing nitrate and phosphate for TSSRP with 17, 50 and 90 L FP-APBRs

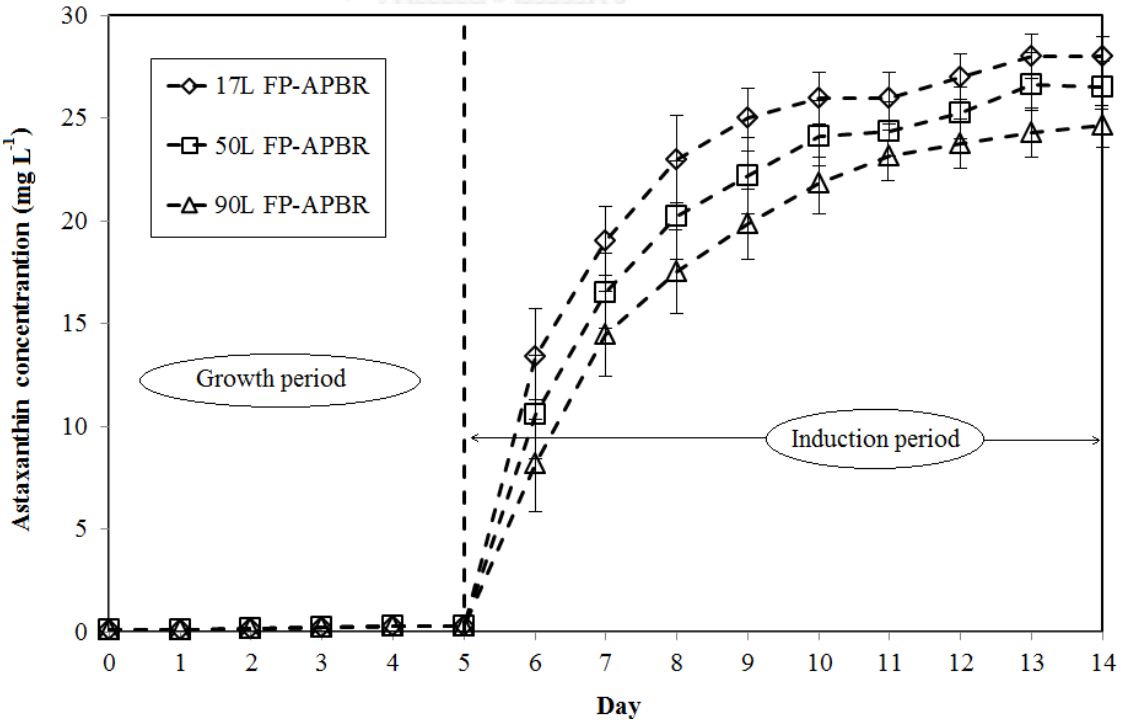


Figure 4.34 Astaxanthin induction under reducing nitrate and phosphate for TSSRP with17, 50 and 90 L FP-APBRs

Table 4.13 Percent astaxanthin (by weight) and dry weight of astaxanthin after 14 days of induction under reducing nitrate and phosphate in TSSRP with 17, 50 and 90 L FP-APBRs

Conditions	Dry weight (g L^{-1})	% Astaxanthin (by weight)	Astaxanthin productivity (mg L^{-1} day^{-1})
17 L FP-APBR	0.59	5.24	1.82
50 L FP-APBR	0.49	4.95	1.80
90 L FP-APBR	0.48	4.87	1.75

4.4 Annual costs estimation for astaxanthin induction from *H. pluvialis*

Annual cost estimations of astaxanthin induction from *H. pluvialis* with flat panel airlift photobioreactor using 17, 50 and 90 L FP-APBRs in indoor, outdoor and TSSRP systems were conducted based on the best operating condition obtained from Sections 4.2.3, 4.2.4 and 4.3.4, respectively, and are given in Tables 4.14-4.16, respectively. These evaluations were based on the target productivity of 1 kg astaxanthin. These results indicate that total operating charges of each system depended mostly on total electricity charges and affected only slightly by carbon dioxide, water and nutrient charges, respectively. When compared the total electricity charges of the 17, 50 and 90 L FP-APBRs both indoor and outdoor systems, results indicated that indoor system charges were the most expensive with the three system running at 6,553, 6,367 and 6,227 \$ $year^{-1}$ (or 213,824, 207,755 and 203,187 Bath), respectively, whilst outdoor system charges were cheaper, i.e. 759, 578 and 554 \$ $year^{-1}$ (or 24,766, 18,860 and 18,077 Bath), respectively. This was because indoor systems required the use of fluorescent lamps as a light source for astaxanthin induction which consumed a large quantity of electrical power. On the other hand, the outdoor systems only used sunlight. Consequently, the total operating charges of indoor systems was much higher than the outdoors, i.e. the 17, 50 and 90 L indoor systems required 6,811, 6,636 and 6,494 \$ $year^{-1}$ (or 222,243, 216,532 and 211,899 Bath) for electricity per year, respectively, and the outdoor systems required only 1,113, 950 and 936 \$ $year^{-1}$ (or 36,317, 30,998 and 30,541 Bath), respectively. In case of the one-step indoor system, this system still suffered electricity charges from the use of artificial light, however, no electricity charge was

incurred from the centrifugation of cells while switching from growth to induction stages. This helped reducing electricity charge slightly. The merit of this system was the risk reduction from contamination which might have occurred from the separation of cells from the nutrient. The total electricity charges of this systems were 6,213, 5,996 and 5,912 $ $year^{-1}$ (or 202,730, 195,649 and 192,908 Bath) for the 17, 50 and 90L reactors, respectively, and the total operating charges were 6,541, 6,348 and 6,270 $ $year^{-1}$ (or 213,432, 207,135 and 204,590 Bath), respectively. Nevertheless, an outdoor vegetative culture could provide a significantly cheaper production of astaxanthin as reported by Li et al. (2011) (718 $ kg^{-1} $year^{-1}$), but, of course, with a higher risk of contamination. When compared with the annual worldwide aquaculture market where a 3% by wt astaxanthin costs around 2,500-3,000 $ kg^{-1} (Milledge, 2011), the 4.47% by weight astaxanthin from the outdoor systems presents an attractive preliminary feasibility.

Table 4.14 Annual costs estimation for astaxanthin production from H. pluvialis using 17, 50 and 90 L FP-APBRs with indoor system

Details	Symbols	Unit	17 L		50 L		90 L	
			C	I	C	I	C	I
Volume	A	m^3 batch^{-1}	0.017	0.017	0.05	0.05	0.09	0.09
Cultivation period (per crop)	B	day batch^{-1}	7	7	7	7	8	7
Number of cycle	C=300/B	batch year^{-1}	42.86	30	42.86	30	38	30
Astaxanthin concentration	D	g astaxanthin m^{-3}	-	26.58	-	25.85	-	25
Astaxanthin productivity	E=D/B	g astaxanthin m^{-3} day^{-1}	-	2.66	-	2.58	-	2.55
	F=E*A*300	g astaxanthin year^{-1}	-	13.56	-	38.77	-	68.85
Dry weight cell (at Maximum cell density)	G	g dry cell L^{-1}	0.15	-	0.14	-	0.13	-
Productivity	H=G*1000/B	g dry cell m^{-3} day^{-1}	21	-	20	-	16	-
	I=H*A*300	g dry cell year^{-1}	109	-	300	-	439	-
Target productivity	J	g astaxanthin year^{-1}	1,000	1,000	1,000	1,000	1,000	1000
Reactor requirements								
(Cultivation case)	K=J/I	Unit	9	-	3	-	2	-
(Astaxanthin induction case)	K=J/F	Unit	-	73.77	-	25.79	-	14.52
Maximum reactors in chamber room	L	Unit	18	18	9	9	5	5
Requirements								
1.Lighting	M	W	60	120	120	300	160	500
	N=(M/1000)*300*24*K	KWh year^{-1}	3,952	63,736	2,880	55,710	2,626	53,333
2.Fan	O	W	350	350	350	350	350	350
	P=(O/1000)*300*12*(K/L)	KWh year^{-1}	640	5,163	467	3,610	574	3,660
3.Controlling system	Q	W	20	20	20	20	20	20
	R=(Q/1000)*300*24*(K/L)	KWh year^{-1}	73	590	53	412	66	410
4.Number of air pumps	S	unit	5	5	6	6	7	7
5.Air pump	T	W	10	10	10	10	10	10
	U=(T/1000)*300*24*S	KWh year^{-1}	360	360	432	432	504	504
6.Number of water pumps	V	unit	-	-	-	-	0	-
7.Water pump	W	W	-	-	-	-	0	-
	X=(W/1000)*300*24*V	KWh year^{-1}	-	-	-	-	0	-
8.Centrifuge	Y	W	1,000	1,000	1,000	1,000	1,000	1000
(Base on 1 hr/ 0.25 m^3)	Z=(Y/1000)*(1/0.25)*(A*C*K)	KWh year^{-1}	27	150	28.57	155	31	160
9.Freeze-dry cell	a	W	-	800	-	800	0	800
(Base on 8 hr/ 0.5 m^3)	b=(a/1000)*(8/0.5)*(A*C*K)	KWh year^{-1}	-	481	-	495	0	414
Electricity charge (0.1$US per kWh)	c=(N+P+R+U+X+Z+b)*0.1	$US year^{-1}	505	6,048	386	5,981	380	5,847
Total Electricity charge	1*	$US year^{-1}	**6,553**		**6,367**		**6,227**	
Nutrient charge ($0.003/dm^3)	d=(A*K*1000*0.003)*C	$US year^{-1}	20	-	21.43	-	23	-
Total nutrient charge	2*	$US year^{-1}	**20**		**21.43**		**23**	
Carbon dioxide charge ($0.005/dm^3)	e=(A*K*1000*0.005)*C	$US year^{-1}	33.2	188	35.71	193	37	192
Total carbon dioxide charge	3*	$US year^{-1}	**221.2**		**228.71**		**229**	
1.Nutrient solution	f=A*K*1000	L	156	1,254	167	1,289	205	1,307
2.Washing activity	g	L	39	313.52	42	322	51	326
3.Cooling water	h	L	-	-	-	-	0	0
Water charge (0.31$US per cum)	i=(f+g+h)*0.31*C/1000	$US year^{-1}	2.58	14.58	2.77	15	2.98	15.2
Total water charge	4*		**17.16**		**17.77**		**18.18**	
Total charge	j=c+d+e+i	$US year^{-1}	561	6,250	446	6,190	443	6,051
Overall production charge	1*+2*+3*+4*	$US year^{-1}	**6,811**		**6,636**		**6,494**	
		Baht year^{-1}	**222,243**		**216,532**		**211,899**	

* Exchange rate on March 31, 2014: 1 $US = 32.63 Baht

* Based on 24 hours per day, 300 days per year operation

* C =Cultivation, I = Astaxanthin induction

Table 4.15 Annual costs estimation for astaxanthin production from *H. pluvialis* using 17, 50 and 90 L FP-APBRs with outdoor system

Details	Symbols	Unit	17 L		50 L		90 L	
			C	I	C	I	C	I
Volume	A	m^3 batch^{-1}	0.017	0.017	0.05	0.05	0.09	0.09
Cultivation period (per crop)	B	day batch^{-1}	7	11	7	11	8	11
Number of cycle	C=300/B	batch year^{-1}	42.86	30	42.86	30	37.50	30
Astaxanthin concentration	D	g astaxanthin m^{-3}	-	19.8	-	18.6	-	17.60
Astaxanthin productivity	E=D/B	g astaxanthin m^{-3} day^{-1}	-	1.98	-	1.86	-	1.76
	F=E*A*300	g astaxanthin year^{-1}	-	10.10	-	27.9	-	47.52
Dry weight cell (at Maximum cell density)	G	g dry cell L^{-1}	0.15	-	0.14	-	0.13	-
Productivity	H=G*1000/B	g dry cell m^{-3} day^{-1}	21.43	-	20	-	16.25	-
	I=H*A*300	g dry cell year^{-1}	109.29	-	300	-	438.75	-
Target productivity	J	g astaxanthin year^{-1}	1,000	1,000	1,000	1,000	1,000	1,000
Reactor requirements								
(Cultivation case)	K=J/I	Unit	9	-	3	-	2	-
(Astaxanthin induction case)	K=J/F	Unit	-	99	-	36	-	21
Maximum reactors in chamber room	L	Unit	18	-	9	-	5	-
Requirements								
1.Lighting	M	W	60	-	120	-	160	-
	N=(M/1000)*300*24*K	KWh year^{-1}	3,953	-	2,880	-	2,625	-
2.Fan	O	W	350	-	350	-	350	-
	P=(O/1000)*300*12*(K/L)	KWh year^{-1}	640	-	467	-	574	-
3.Controlling system	Q	W	20	-	20	-	20	-
	R=(Q/1000)*300*24*(K/L)	KWh year^{-1}	73.20	-	53.33	-	65.64	-
4.Number of air pumps	S	unit	5	6	6	6	7	7
5.Air pump	T	W	10	10	10	10	10	10
	U=(T/1000)*300*24*S	KWh year^{-1}	360	432	432	432	504	504
6.Number of water pumps	V	unit	-	10	-	5	-	4
7.Water pump	W	W	-	20	-	20	-	20
	X=(W/1000)*300*24*V	KWh year^{-1}	-	1,440	-	720	-	576
8.Centrifuge	Y	W	1,000	1,000	1,000	1,000	1,000	1,000
(Base on 1 hr/ 0.25 m^3)	Z=(Y/1000)*(1/0.25)*(A*C*K)	KWh year^{-1}	26.67	185	28.57	200	30.77	192
9.Freeze-dry cell	a	W	-	800	-	800	-	800
(Base on 8 hr/ 0.5 m^3)	b=(a/1000)*(8/0.5)*(A*C*K)	KWh year^{-1}	-	602	-	632	-	532
Electricity charge (0.1$US per kWh)	c=(N+P+R+U+X+Z+b)*0.1	$US year^{-1}	505.33	254	386.06	192	380.04	174
Total Electricity charge	1*	$US year^{-1}	**759**		**578**		**554**	
Nutrient charge ($0.003/dm^3)	d=(A*K*1000*0.003)*C	$US year^{-1}	20	-	21.43	-	23.08	-
Total nutrient charge	2*	$US year^{-1}	**20**		**21.43**		**23.08**	
Carbon dioxide charge ($0.005/dm^3)	e=(A*K*1000*0.005)*C	$US year^{-1}	33.33	252.53	35.71	268.82	36.92	272
Total carbon dioxide charge	3*	$US year^{-1}	**272.56**		**304.53**		**309.65**	
1.Nutrient solution	f=A*K*1000	L	156	1,683	167	1,792	205	1,893
2.Washing activity	g	L	38.89	420	41.67	448	51.28	473.48
3.Cooling water	h	L	-	841	-	896	-	946.97
Water charge (0.31$US per cum)	i=(f+g+h)*0.31*C/1000	$US year^{-1}	2.58	27.4	2.77	29.17	2.98	30.82
Total water charge	4*		**30**		**31.94**		**33.8**	
Total charge	j=c+d+e+i	$US year^{-1}	561.25	551.75	445.97	504.03	443.02	492.98
Overall production charge	1*+2*+3*+4*	$US year^{-1}	**1,113**		**950**		**936**	
		Baht year^{-1}	**36,317**		**30,998**		**30,541**	

* Exchange rate on March 31, 2014: 1 $US = 32.63 Baht

* Based on 24 hours per day, 300 days per year operation

* C =Cultivation, I = Astaxanthin induction

Table 4.16 Annual costs estimation for astaxanthin production from *H. pluvialis* using 17, 50 and 90 L FP-APBRs with TSSRP

Details	**Symbols**	**Unit**	**17 L**	**50 L**	**90 L**
Volume	A	m^3 $batch^{-1}$	0.017	0.05	0.09
Cultivation period (per crop)	B	day $batch^{-1}$	10	10	10
Number of cycle	C=300/B	batch $year^{-1}$	30	30	30
Astaxanthin concentration	D	g astaxanthin m^{-3}	25.6	23.8	23.4
Astaxanthin productivity	E=D/B	g astaxanthin m^{-3} day^{-1}	2.56	2.38	2.34
	F=E*A*300	g astaxanthin $year^{-1}$	13.06	35.7	63.18
Target productivity	J	g astaxanthin $year^{-1}$	1,000	1,000	1,000
Reactor requirements	K=J/F	Unit	76	28	16
Maximum reactors in chamber room	L	Unit	18	9	5
Requirements					
1.Lighting	M	W	120	270	470
	N=(M/1000)*300*24*K	KWh $year^{-1}$	55,147	54,453	53,561
2.Fan	O	W	350	350	350
	P=(O/1000)*300*12*(K/L)	KWh $year^{-1}$	5,361	3,921	3,988
3.Controlling system	Q	W	20	20	20
	R=(Q/1000)*300*24*(K/L)	KWh $year^{-1}$	612	448	455
4.Number of air pumps	S	unit	5	5	5
5.Air pump	T	W	10	10	10
	U=(T/1000)*300*24*S	KWh $year^{-1}$	360	360	360
6.Number of water pumps	V	unit	-	-	-
7.Water pump	W	W	-	-	-
	X=(W/1000)*300*24*V	KWh $year^{-1}$	-	-	-
8.Centrifuge	Y	W	1,000	1,000	1,000
(Base on 1 hr/ 0.25 m^3)	Z=(Y/1000)*(1/0.25)*(A*C*K)	KWh $year^{-1}$	156	168	170
9.Freeze-dry cell	a	W	800	800	800
(Base on 8 hr/ 0.5 m^3)	b=(a/1000)*(8/0.5)*(A*C*K)	KWh $year^{-1}$	500	537	441
Total electricity charge (0.1$US per kWh)	c=(N+P+R+U+X+Z+b)*0.1	$US $year^{-1}$	**6,213**	**5,996**	**5,912**
Total nutrient charge (0.003$/$dm^3$)	d=(A*K*1000*0.003)*C	$US $year^{-1}$	**117**	**126**	**128**
Total carbon dioxide charge (0.005$/$dm^3$)	e=(A*K*1000*0.005)*C	$US $year^{-1}$	**195**	**210**	**213**
1.Nutrient solution	f=A*K*1000	L	1,302	1,400	1,424
2.Washing activity	g	L	325	350	356
3.Cooling water	h	L	0	0	0
Total water charge (0.31$US per cum)	i=(f+g+h)*0.31*C/1000	$US $year^{-1}$	**15.13**	**16.28**	**16.55**
Overall production charge	j=c+d+e+i	$US $year^{-1}$	**6,541**	**6,348**	**6,270**
		Baht $year^{-1}$	**213,432**	**207,135**	**204,590**

* Exchange rate on March 31, 2014: 1 $US = 32.63 Baht

* Based on 24 hours per day, 300 days per year operation

* C =Cultivation, I = Astaxanthin induction

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. In studying suitable induction conditions, results indicated that conditions of medium, light intensity, carbon dioxide, superficial gas velocity (u_{sg}), ratio between downcomer and riser cross section area (A_d/A_r) of the airleft reactor and temperature could have significant effects on astaxanthin induction in *H. pluvialis*. The best induction conditions can be summarized as belows:
    - clean water as nutrient
    - 35,000 LUX
    - $33^{O}C$
    - 3% carbon dioxide concentration, with the aeration rate of 0.4 cm s^{-1} (superficial) (or CO_2 volume flow rate of 1.47 cm^3 s^{-1} and aeration rate of 49.1 cm^3 s^{-1} at the operating temperature)
    - A_d/A_r of 0.4
2. In studying astaxanthin induction in indoor and outdoor conditions, the findings suggested that cells needed protection from the strong sunlight during the first period of induction to withstand the harsh environmental condition. Hence, providing adequate shading seemed appropriate, and from the results, applying one layer shading net condition on the first three days of the induction period provided the best result for the accumulation of astaxanthin.
3. In studying astaxanthin induction using two-step single reactor process system, results indicated that reducing nitrate and phosphate in F1 medium affected astaxanthin induction and growth of *H. pluvialis*. This can be summarized as belows:
    - Reducing nitrate could stimulate more astaxanthin than reducing phosphate. However, reducing nitrate also negatively affected cell growth more than reducing phosphate.
    - The best condition of reducing nitrate and phosphate were $0.25xNO_3^-$ and $0.16x\ PO_4^{3-}$. In addition, the best condition of reducing both nitrate and phosphate was $0.25xNO_3^-$&$0.16xPO_4^{3-}$.

4. In comparing the growth performances in Bubble Column-Photobioreactors (BC-PBR) and Flat Panel airlift Photobioreactor (FP-ALPBR), FP-ALPBR could encourage more astaxanthin than BC-PBR. This could be the result of better mixing and less cell precipitation in the airlift system which could lower the induction of astaxanthin.
5. In studying annual costs for astaxanthin induction, results indicated that total operating charges of each system depended mostly on total electricity charges and was affected only slightly by carbon dioxide, water and nutrient charges, respectively. This can be summarized as below:
    - Indoor systems required the use of fluorescent lamps as a light source for astaxanthin induction which consumed a large quantity of electrical power. On the other hand, the outdoor systems only used sunlight. Consequently, the total operating charges of indoor systems was much higher than the outdoors.
    - The one-step process system also suffered electricity charges from the use of artificial light, however, no electricity charge was incurred from the centrifugation of cells while switching from growth to induction stages. This helped reducing electricity charge slightly.

5.2 Contributions

1. Previous study (Issarapayup et. al., 2009) has proven the possibility and feasibility of cultivating vegetative cells of *Haematococcus pluvialis* in the flat panel airlift photobioreactors operated under controlled environment. This work extended the work to cover the astaxanthin induction stage and it was proven to be possible and also economically attractive, but with some water cooling facility to dissipate the solar heat which could harm the cell particularly during the first period of induction.
2. It was demonstrated in this work that the flat panel airlift photobioreactors could be effectively employed for both the growth of *Haematococcus pluvialis* and the induction of astaxanthin, where as much as 26.63 g astaxanthin m^{-3} (5.34% by weight) could be achieved. This is considerably high when compared with typical outdoor astaxanthin content reported elsewhere.
3. This work also revealed that the one-step process system could be employed to combine both growth and induction stages of *Haematococcus pluvialis*.

The merit of this system was the risk reduction from contamination which might have occurred from the separation of cells from the nutrient. In addition, this system helps to economize energy consumption within the production system.

5.3 Recommendations

1. Result of annual cost estimations indicated that total operating charges of indoor systems depended mostly on total electricity charges of lighting. Future research is needed to minimize this cost which might be achieved by re-designing or re-structure of the system such that a better light utilization could be achieved. For instance, the configuration of reactors as presented in Figure 5.1 might help where induction reactors (with high light intensity demand) are located near the light source to filter some light for the vegetative reactor (low light intensity stage) installed at the middle.

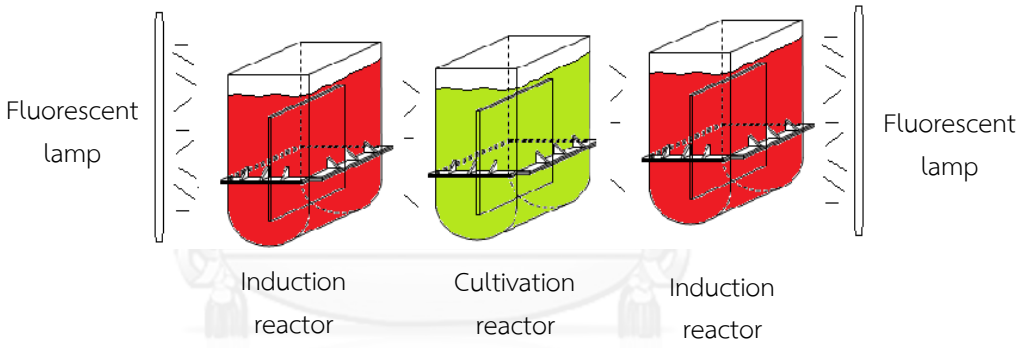


Figure 5.1 Possible reactor configuration for a more effective use of light

2. Results of this work indicated that using carbon dioxide could encourage more astaxanthin. However, adding overabundant carbon dioxide might raise the acidity and cause cell death. Some might consider the use of buffer to stabilize pH at neutral range to enable a greater induction with CO_2.

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APPENDIX

LIST OF PUBLICATION

1. Poonkum W., Powtongsook S. and Pavasant P. 2013. Astaxanthin Induction in Microalga *H. pluvialis* with Flat Panel Airlift Photobioreactors under Indoor and Outdoor Conditions. Preparative Biochemistry and Biotechnology IN PRESS

2. Poonkum W., Powtongsook S. and Pavasant P. 2012. Astaxanthin Induction in *H.Pluvialis* Using Flat Panel Airlift Photobioreactor. Engineering Journal 4: 19-36 (In Thai)

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Mr. Woradej Poonkum was born on 24th May, 1983 in Bangkok. He finished his higher secondary course from Watsraket school in March, 2002. After that, he studied in the major of Chemical Engineering in Faculty of Engineering at King mongkut's university of technology thonburi. He continued his further study for Master's degree in Chemical Engineering at Chulalongkorn University in 2006. He participated in the Environmental Chemical Engineering research group in 2009 and achieved his Doctoral degree in March, 2013.