CHAPTER III
METHODOLOGY

3.1 Research Design

The research purposes were to develop system for algae cultivation with performance and low cost that was suitable for environment in Thailand. It was started with literature reviews in the topics of general characteristics and factors affecting productivity of *Spirulina spp.*, algae cultivation, type of photo-bioreactor and design criteria for photo-bioreactor cultivation. Hence, researcher was divided into two main parts: 1) Study and determine the effects of using fertilizer instead of chemicals for cultivation; find the optimal conditions for biomass production of *Spirulina spp*. in order to design the bioreactors, 2) Design a tubular photo-bioreactor for developing on algae cultivation systems and preliminary performance tests to determine whether the design a photo-bioreactor is suitable for the community usage by comparing the results with an open pond system. All experiments in this study operated under outdoor condition (tropical climate of Bangkok, Thailand).

3.2 Research Process

The process of research conducted was comprised of 5 main steps (Figure 3.1) as detailed in the following:

1) Research Design was the step to determine materials, methods and other details of research in order to set up the research plan.

2) Literature Review was the step to research and review journals, papers and studies related to research's topic in order to compile and integrate the concepts, theories and knowledge into the research fundamental frame and also to strengthen researcher's understanding on carry out research correctly.

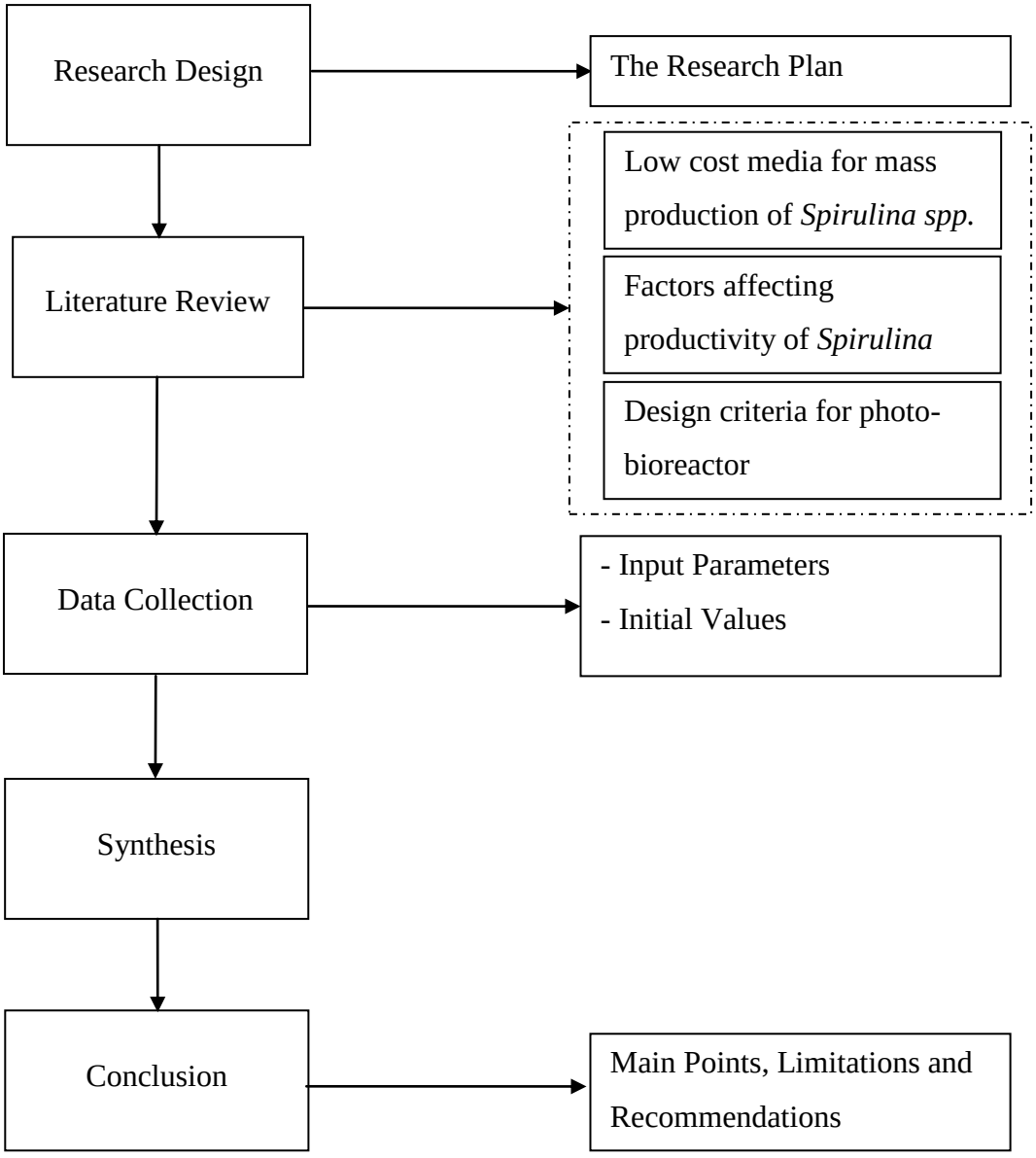

Figure 3.1 Research process.

3) Data Collection was the step to collect secondary data of factors and criteria needed to use as input factors.

4) Synthesis was the step to use results and synthesis the results into 2 forms; tables and charts.

5) Conclusion was the step to summaries the main findings including discussing in the topics of research limitations and recommendations for further studies.

3.3 Organisms and Preparation Inoculums

Spirulina spp. from The Royal Chitralada Projects, Thailand, was used for the study. The stock culture was kept in Zarrouk's media (as described in Appendix A). The inoculums was prepared by transferring the stock culture to Zarrouk's media in 10-liter glass jar under outdoor condition (tropical climate of Bangkok, Thailand) until the optical density (OD) reached to optimal cell concentration about 0.8 (Peerapornpisarn, 2003) at the wavelength of 560 nm or about 1.5 gram wet weight per liter. Therefore be used as inoculums for further study.

3.4 Cultivation Equipment and Condition

3.4.1 Effect of low cost media on growth of *Spirulina spp.* in outdoor culture

3.4.1.1 The first preliminary test for the growth of *Spirulina spp.* in low cost media

To find the optimal concentration on the growth, this experiment was designed 4 different concentration of urea fertilizer (0, 0.3, 0.6 and 1.2 g/L). Compare the growth of *Spirulina spp.* when cultivating at 4 different concentration of urea fertilizer with standard media (Zarrouk's media). The media content in this experiment was shown in Table 3.1

Table 3.1 Media content in the first preliminary test

Experiment	Urea (g/L)	$NaHCO_3$ (g/L)
1	0	8.5
2	0.3	8.5
3	0.6	8.5
4	1.2	8.5

3.4.1.2 The second preliminary test for the growth of *Spirulina spp.* in low cost media

From the first preliminary test, researcher conducted experiment by using experiments from different studies as reference. The media content in this experiment was shown in Table 3.2

Table 3.2 Media content in the second preliminary test

Components	Concentration (g/L)			
	Zarrouk's media	**Phetmani's media (Formular 3)**	**Tri-Panji's media**	**Raoof's media**
$NaNO_3$	2.50	0.15	-	2.50
K_2HPO_4	0.50	0.50	0.15	-
K_2SO_4	1.00	0.30	-	-
NaCl	1.00	0.30	-	0.50
$MgSO_4.7H_2O$	0.20	0.06	0.18	0.15
$CaCl_2.2H_2O$	0.04	0.012	-	0.04
$FeSO_4.7H_2O$	0.01	0.003	-	-
EDTA	0.08	0.024	-	-
$NaHCO_3$	16.80	3.00	0.6	8.00
A_5 Solution*	1 ml/l	-	-	-
B_6 Solution**	1 ml/l	-	-	-
SSP	-	-	-	1.25
MOP	-	-	-	0.898
Urea	-	-	0.6	-

*A_5 Solution: H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.22; $CuSO_4.5H_2O$, 0.08; MoO_3, 0.01 (g/L)

**B_6 Solution: NH_4VO_3, 22.9; $NiSO_4.7H_2O$, 47.8; $Na_2WO_4.2H_2O$, 17.9; $Ti(SO_4)_3$, 40.0; $CO(NO_3)_2.6H_2O$,4.4 (g/L)

3.4.1.3 Effect of low cost media on the growth of *Spirulina spp.*

This study, used the 2×4 factorial design in randomized experiments as showed in Table 3.3. The education factor was divided into two factors: Factor 1, types of nutrients, and Factor 2, concentration of nitrogen. Factor 1 was used as a nitrogen source, including urea ($Co(NH_2)_2$) and potassium nitrate (KNO_3). The concentration of nitrogen occurred at four levels: 10%, 20%, 30% and 40% of the nitrogen concentration in the Zarrouk's media.

Table 3.3 The experimental design for this study

Nitrogen Sources	Treatment			
	Nitrogen concentration			
	10%	20%	30%	40%
$Co(NH_2)_2$	3	4	5	6
KNO_3	7	8	9	10

Cultivated *Spirulina spp.* in 10-liter glass jars. The alga was grown for 18 days and growth measured every 3 days. Algal were washed by 0.9% NaCl solution for removal of residual nitrate (Danesi et al., 2011). Growth rate of algae was measured in terms of dried weight (g/L). This growth rate was compared with standard media, including Zarrouk's media (Treatment 1) and Raoof's media (Treatment 2). Type and concentration of nutrients used in this experiments was showed in Table 3.4

Table 3.4 Type and concentration of nutrients used in the experiments.

Zarrouk's media			**New media**			
Component	**Concentration**		**Component**		**Concentration**	
	g/L	**(mM) N**		**Treatment**	**g/L**	**(mM) N**
$NaNO_3$	2.50	29.42	**N-sources**			
K_2HPO_4	0.50		$Co(NH_2)_2$	3	0.088	2.94
K_2SO_4	1.00			4	0.176	5.88
NaCl	1.00			5	0.264	8.83
$MgSO_4.7H_2O$	0.20			6	0.352	11.77
$CaCl_2.2H_2O$	0.04		KNO_3	7	0.297	2.94
$FeSO_4.7H_2O$	0.01			8	0.595	5.88
EDTA	0.08			9	0.892	8.83
$NaHCO_3$	16.80			10	1.189	11.77
A_5 Solution*	1 ml/l		$NaNO_3$	2	2.5	29.42
B_6 Solution**	1 ml/l		**Non-N sources**			
			Single super phosphate		1.250	
			Muriate of potash		0.898	
			$NaHCO_3$ (commercial)		8.00	

*A_5 Solution: H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.22; $CuSO_4.5H_2O$, 0.08; MoO_3, 0.01 (g/L)

**B_6 Solution: NH_4VO_3, 22.9; $NiSO_4.7H_2O$, 47.8; $Na_2WO_4.2H_2O$, 17.9; $Ti(SO_4)_3$, 40.0; $CO(NO_3)_2.6H_2O$, 4.4 (g/L)

3.4.2 Design a tubular photo-bioreactor for the community level

3.4.2.1 Laboratory Scale Tubular Photo-bioreactor

A tubular photo-bioreactor was shown in Figure 3.2, It consists of transparent rubber tube (150 cm in length and 3 cm in inner diameter). It was made up of 2 tubes and connected by joints to a single loop form. The material thickness was 5 mm. This material had transparency about 85%. The top end of loop reactor was left open for deoxygenating. Culture can be drained from the bottom opening which was capped with a stopper.

Light, Temperature, pH and Dissolved oxygen was not controlled. Dissolved oxygen was stripped at the top end of reactor to the air. The experiments were cultured under outdoor condition (tropical climate of Bangkok, Thailand)

Mixing or liquid circulation was done using a reciprocating pump. The velocity was adjusted by ex-controlling valve.

The height was 150 cm from the bottom. The design based on the laboratory results in which the Renold's number to prevent wall growth.

Figure 3.2 A tubular photo-bioreactor in this study

3.4.2.2 Effect of media flow rate and substrate feeding rate in photo-bioreactor

This experiment was study the relationship between *Spirulina spp.* growth and nutrient content. The alga was grown for 15 days and the culture was harvested and analyzed for dry weight every 3 days. The solution was also checked for HCO_3^- alkalinity.

3.4.3 Preliminary performance tests tubular photo-bioreactor

Cultivation system in tubular photo-bioreactor was conducted in semi-continuous system at media flow rate about 2.5 liter per minute. Substrate feeding rate was defined at 0.0404 liter per min. Substrate concentration for feeding was 0.6 gram per liter which using drip system at 10 drops per minute. At steady state, it was equal to the dilution rate at 0.0058 hr^{-1}. To estimate efficiency of constructed photo-bioreactor for *Spirulina spp*. cultivation, the growth rate from this photo-bioreactor was compared with the open pond system (glass jar).

3.5 Analytical Methods

In the experiments were monitored:

- **Growth Measurement:**

Growth was measured by dry weight determination as described in Appendix C.

- **Chemical composition of cell:**

Chlorophyll concentration as described in Appendix D.

- **Chemical composition of media:**

After cell was separated, the liquid was analyzed for HCO_3^- alkalinity by titration with H_2SO_4 acid as described in Appendix E.