CHAPTER III
RESEARCH METHODOLOGY

3.1 Materials

In this study, Spirulina (*Arthrospira platensis*) strains (H53, Rev, SL, SBL, SB, C005H, and C005L) were obtained from the Suranaree University of Technology (SUT), which was isolated and thoroughly purified.

3.2 Growth medium and culture method

The Spirulina (A. *platensis*) was cultivated with 12 hours light and 12 hours dark photoperiod in Zarrouk's medium at 25 °C, pH 10. They were kept in 500 mL working-volume transparent plastic bottles. The culture bottles were cultured in a white LED fluorescent light shelf with an intensity of 4,500-5,000 lux (Lamptan, Thailand), and an air pump system for the transparent plastic bottles with air tubes. The tube was all set vertically from the top to the to maintain the desired light intensity setting. The BioTek Epoch microplate reader (Winooski, VT, USA) was used to measure the optical density (OD) values at 560 nm in order to calculate the growth rate. The sample was harvested for the FT-IR experiment in the mid-log phase after it had been cultured.

3.3 Photoperiod lengths treatment

The Spirulina cultured in Zarrouk’s medium using distilled water, adjusted to pH 10 with NaOH, under cycles of 8, 12, and 24 hours photoperiod.

3.4 Water Source treatment

The Spirulina was cultured in Zarrouk's medium using groundwater and tap water, adjusted to pH 10 with NaOH, under a cycle of 12 hours light/12 hours dark photoperiod.

3.5 Indoor and Outdoor cultured conditions

The indoor Spirulina was cultured in Zarrouk's medium using distilled water, adjusted to pH 10 with NaOH, under a cycle of 12 hours light/12 hours dark photoperiod. The strain culture in plastic bottles with a working volume of 500 mL. The outdoor Spirulina was culture in in Zarrouk's medium using distilled water, adjusted to pH 10 with NaOH, in green house. Illumination is provided by natural sunlight. The strain culture in 100 L. photobioreactor and an air pump system

3.6 Focal Plane Array (FPA) FT-IR spectroscopy

The Synchrotron Light Research Institute's (SLRI, Nakhon Ratchasima, Thailand) infrared microspectroscopy beamline BL4.1 IR Spectroscopy and Imaging was used to record the samples' infrared spectra. A Vertex 70 FTIR/UV-Vis spectrometer (Bruker Optics, Ettlingen, Germany) connected to an IR microscope (Hypersion 2000, Bruker) with a liquid nitrogen-cooled MCT detector will be used to acquire the spectra.

3.7 FTIR spectroscopy measurements

Mid-log phase Spirulina samples were collected and transferred in 500 µL aliquots into 1.5 mL Eppendorf tubes. The samples were centrifuged at 1200 rpm for 10 minutes. Following centrifugation, the samples were washed three times with a 0.9% (w/v) saline and three times with deionized water (DI water), respectively. Subsequently, 5 µL of each sample was deposited onto a BaF_2 window measuring 13

x 1 mm. Before being analyzed, the windows were kept in a desiccator for a number of hours while being vacuumed. FT-IR microspectroscopy and an FPA detector were used to gather the spectra. The absorbance was measured by rationing the single beam spectrum against the window background. For twelve hours, the samples on the window were dried in a desiccator. 64 scans with a spectral resolution of 6 cm^{-1} were used to record infrared absorption spectra in the 4000-400 cm^{-1} range. Absorbance values were obtained from the measured spectral values.

3.8 Multivariate statistical analysis

The instrument system was controlled and FT-IR spectral data was obtained using OPUS 7.5 software (Bruker Optics Ltd., Ettlingen, Germany). Principal Component Analysis (PCA) was used to identify the samples' spectra using the Unscrambler X 10.5 software's variability (CAMO Software AS, Oslo, Norway) and Quasar. A spectrum is obtained by the FT-IR/UV-Vis Spectrometer for additional analysis.