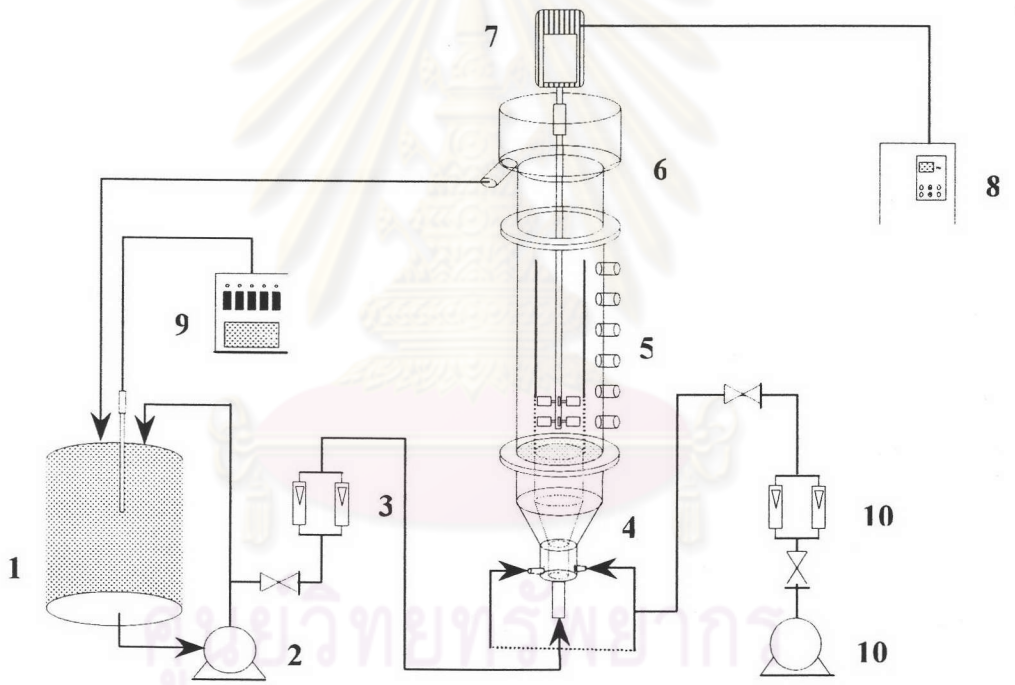
CHAPTER 5

EXPERIMENTS

5.1 Experimental Set-up

A Three-phase fluidized bed with agitator used in this work was schematically shown in Figure 5-1.



1. storage tank 2. liquid pump 3. flow meters 4. distributing section 5. fluidized bed section 6. disengaging section 7. impeller and motor 8. inverter 9. thermocouple and temperature indicator 10 . air compressor

Figure 5-1. Schematic diagram of three-phase fluidized bed employed in the experiment

The detail of the fluidized bed system and the fluid flow direction inside are shown in Table 5-1 and Figure 5-2, respectively.

Table 5-1. The detail of fluidized bed system.

Capacity of fluidized bed unit	9 L
Capacity of circulating tank	20 L
Diameter of glass beads	991-1,397 μm
Load of bead	0.5 kg
Speed of impeller	0-3000 rpm
Gas flow rate	0-40 cm/min
Liquid flow rate	0-40 cm/min

This fluidized bed unit consists of three sections. The first section is distributing section in which a sieve plate with holes of 500 μm is used as gas and liquid distributor. The distributor is placed on top of the distribution coaxial cone, which was packed with beads for a purpose of uniform distribution of gas and liquid phase. Liquid is fed through the base of the inner cone, while gas is injected through the outer cone. Fluidized bed section is the second part consisting of outer column and coaxial draft tube. The column is 24 inch in height and 5 inch in inner diameter with five sampling tubes at fixed axial positions. The coaxial draft tube is 16 inch in height and 3 inch in inner diameter. The wall of draft tube is bored with 1 mm diameter holes. The annular of fluidized bed unit is filled with glass beads with mean diameter ranged between 991-1,397 μm. Moreover, the stainless steel agitator made of a vertically mounted central shaft with twice impeller of 1 inch height and 2.5 inch length is also equipped inside the column. The base of agitator is fixed at the central of the fluidized bed column at a high of 2 cm from the bottom. Gas liquid disengaging section is the last point, where the liquid is collected and recycled to a circulating tank.

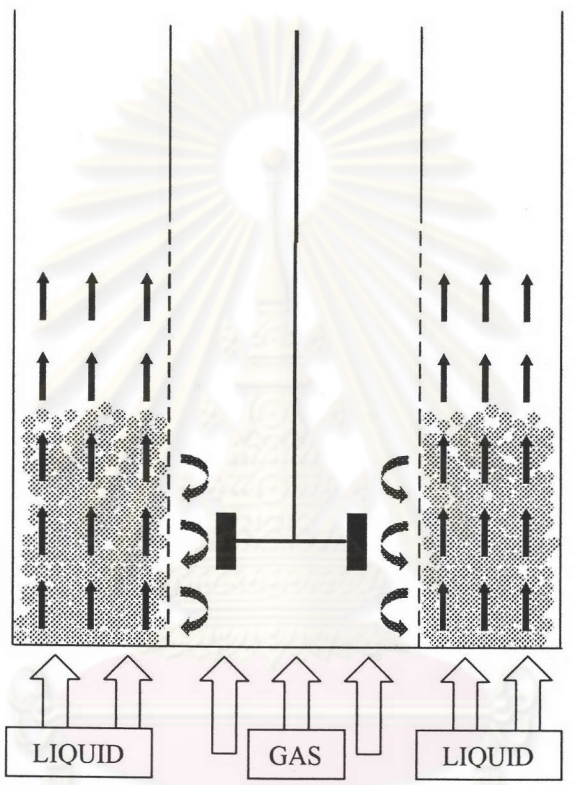


Figure 5-2 Diagram showing the inside of three-phase fluidized bed with agitation

5.2 Experimental Procedure

Three microalgal strains, *Chlorella ellisopedia* TISTR 8260, *Chroococcus sp.* TISTR 8623 and *Chlorococcum sp.* TISTR 8509 were obtained from Microbiological Resources Centre (MIRCEN), Thailand Institute of Scientific and Technological Research (TISTR). *Chlorella ellisopedia* was cultured in modified N-8 medium. *Chroococcus sp.* and *Chlorococcum sp.* were cultured in modified BG-11 medium. The cultivation of microalgae was performed in a 10 liters carboy, incubated at $24 \pm 1^{\circ}C$, and continuously illuminated by cool-white fluorescent lamps at the light intensity of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. The culture was sparged with air. Three types of microalgal strain were removed from the culture when the cell concentration was high enough to dilute to (9-15) $X10^6$ cells/mL of 8 or 22 liter final volume.

In this work, volume of suspension employed in continuous operating is about 22 liters while in batch operation it is only 8 liters. Moreover, the suspension was carefully prepared with 10°C water.

In continuous system, cell suspension was filled in to the circulating tank and column in prior to starting each experiment. At operating time, liquid flowed from circulating tank through a centrifugal pump and flow meter and then to the bottom of the cone of column. After that the liquid flowed back to the circulating tank of which temperature was controlled by cooling jacket. Air flowed from a compressor through a rotameter, and was fed to fluidized bed section. During to operation, temperature was manually controlled within a range of 10-24 $^{\circ}$ C to avoid the thermal disintegration process. For batch system, suspension was filled in column and only air was fed into the column already filled with glass beads of a certain weight.

Each experiment was conducted for a period of 180 minutes and samples were taken at least 3 times every 15 minute at fixed position. Glutarldealhyde of 2 % final concentration was used to preserve the sample for analysis of cell suspension.

5.3 Measurement of Cell Analysis

5.3.1 Cell Counting

An immoderate number of microalgal cells in sampling is required to ensure the counting reliability. To perform cell counting in this study, Segwick-Rafter counting chamber and microscope (Olympus, CX31) were applied. For convenient and effective cell counting, sampling from each time were diluted to 20-40 cells per unit counting area of Segwick.

Procedure

1. Sigwicks-Rafter counting chamber with 1 mL of sample was divided into 1000 squares. Pipette 1 mL of well mixed algal suspension into the chamber. Slide the special coverslip across the top of the chamber, making sure there were no bubbles trapped beneath the coverslip. Place the chamber on the microscope stage and let microalgal cell sink to the bottom of chamber.

2. Randomly count the number of cell that appears in 50 squares. After that determine the mean number of cell per unit area and convert to the number of cells per mL using the dimensions of the chamber.

Percent of cell disruption can be determined by using the following equation.

Percent of cell disruption

$$= \left(\frac{\text{number of living cells at 0 min.- number of living cells at intended time} \quad X100}{\text{number of living cells at 0 min.}} \right) \qquad (5\text{-}1)$$

5.3.2 Cell Size Determination

Cell micrographs were captured by SLR camera (Panasonic, WV-CP240/G) that support microscopy and cell size was determined using images processing program (Image Pro V. 3.0). Each sample containing at least 100 cells was determined twice. For the determination of the cell size, width and length of each cell were measured and then the pre-calculated value of cell volume base on the ellipsoidal formula is employed. Cells size at operating and starting time were obtained from the transformation of averages cell volume

5.3.3 Chlorophyll A Determination

Chlorophyll A was also used for confirming the cell disruption in this work. 1 mL microalgal suspension was sampled and 4 mL alsolute acetones were then added. After vigorous shaking the samples was kept at room temperature for 10 min. The extinctions of clear supernatants obtained after centrifugation (10min, 3000 X g) were then measured at 664 and 647 nm. Finally the amount of Chlorophyll A was calculated from the light absorption intensity by using the following equation.

$$\text{Chlorophyll A} = 11.93A_{664} - 1.93A_{647} \qquad (5\text{-}2)$$

5.3.3 Microscope Observation

Cell ruptured were evaluated qualitatively by microscopic method (Olympus, CX3). Morphology of microalgal cells was also observed at the magnification of 1000 for *Chlorella ellisopedia* and 400 for *Chroococcus sp.* and *Chlorococcum sp.*