

CHAPTER IV

MATERIALS AND METHODS

The purpose of the following experiments was to study the selective resolution of racemic menthol by lipases in aqueous/organic solvent system. The detail of experimental investigation is as follows:

4.1 Materials

Racemic menthol (\pm)menthol, > 99% purity) as the substrate was purchased from Aldrich Chemical Company, Inc. Ethyl acetate, butyl acetate, and hexyl acetate as acyl donors and monobasic sodium phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) and dibasic sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) were purchased from Aldrich Chemical Company, Inc. Five kinds of organic solvents were used : hexane, heptane, 1-octane, cyclohexane, and benzene. All of organic solvents were purchased from Ajax Chemicals. All chemicals used in this work were of analytical grade.

Three kinds of lipases from *Candida cylindracea*, Porcine pancreas, and Hog pancreas were used. All the enzymes were purchased from Aldrich Chemical Company, Inc.

4.2 Experimental Equipments

4.2.1 Stirred tank reactor with the dimensions shown in figure 4.1.

4.2.2 Temperature controlled water bath model HC from Julabo co., ltd.

4.2.3 Motor for the reactor paddler was model DM 60 C from Japan Servo co., ltd. and an adapter model SA 1000 from Protech Electronic co., ltd. for stirring speed control.

4.2.4 Shaker model D3006 from Gesellschaft fur Labortechnik (GFL) co., ltd.

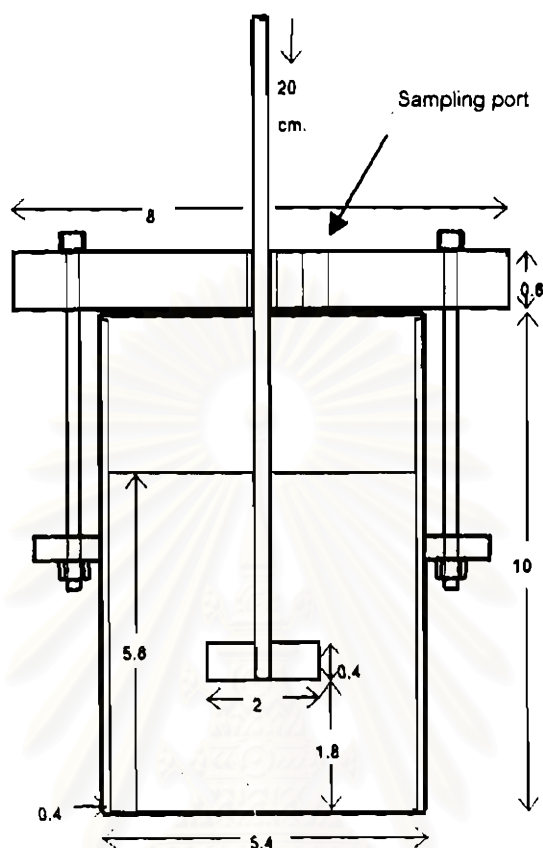


Figure 4.1 Drawing of the reactor. The dimensions shown are in cm.

4.3 Experimental Procedures

4.3.1 Transesterification in organic system

(±)Menthol and an acyl donor were added to a 125 ml organic solvent contained in a 230 ml stirred tank reactor to the desired concentrations. This reactor was submerged in a temperature controlled bath. The reaction was initiated by an addition of an appropriate amount of lipase, and the enzyme-free organic solvent was taken as samples at appropriate time intervals before storing in a -15°C freezer. These samples were then analysed by gas chromatography. Stirring speed of a paddler were varied

from 95 to 215 rpm. The results shown are an average of three separate identical experiments, and the coefficient of variation found was $\pm 10.32\%$.

4.3.2 Transesterification reaction in aqueous-organic two phase.

The organic phase with both substrates was prepared as in sec. 4.3.1. The aqueous phase comprised the lipase from *Candida cylindracea* dissolved in pH 7 buffer solution. The two phases were added together in 250 ml volumetric flask and then incubated in a rotary shaker at approximately 200 rpm and room temperature. Samples were taken from an organic phase every 5 hour intervals and analysed by gas chromatography. Results shown were an average of three injections.

4.4 Determination of Product Concentrations

Menthyl acetate was determined by gas chromatography using a Shimadzu Model 14A equipped with a flame ionization detector. Separation took place in a 2 m x 2 mm glass column packed with 0.3% CARBOWAX 20M/80/100 Carbopack C (from Supelco, Inc.) and nitrogen was used as carrier gas and its flow rate was set at 40 ml/min . Both the injector and detector temperatures were fixed at 240 °C, while the starting oven temperature was 90 °C with an increasing rate of 10 °C/min to the final temperature of 220 °C. The data obtained from a C-R6A recorder integrator showed a retention of 21 minutes for menthyl acetate. The coefficient of variation obtained from three injection of the same sample was $\pm 3\%$.