pending subsequent analyses. Sampling was performed at 60 h of fermentation from each bottle for biotransformation process.

The supernatant and the cell pellet were separated by centrifugation at 2,822 × g (5,000 rpm) for 15 min. The supernatant obtained was analyzed for pH level by using pH meter, TSS by hand refractometer, sugars (glucose, fructose and sucrose), acetic acid and ethanol was analyzed by HPLC with refractive index detector and 20 min of run time. The column used was Aminex ®HPX-87H Ion Exclusive 9 μm particle size with 5 mM H_2SO_4 in distilled water. Flow rate was set at 0.75 ml/min. Temperature of column oven was set at 37°C. The refractive index was measured with Refractive Index Detector with 20 min run time. Volume of injected sample was 20 μl. The pellet obtained was washed once with distilled water, followed by centrifugation at 2,822 × g for 15 min. The washed cells pellet was analyzed for dried biomass weight, optical density at 600 nm.

The errors were calculated from 5 replicates which collected from each time point. The statistic comparison was made by the same hypothesis testing as section 3.7.3.

3.9 The comparison of phenylacetylcarbinol production by whole cells from three microbial strains

The experiment of two phases emulsion system for PAC biotransformation was conducted by adopting whole cells of *S. cerevisiae* TISTR 5606, *C. utilis* UNSW 709400 and 709700 cultivated in three conditions, namely, the batch cultivation with dried longan extract at 48th h of fermentation period, fed batch cultivation with DLE and DDLFH at 60th h of fermentation period. The whole cells concentration were adjusted to dried biomass equivalent level of 12.24 g/l. The experiment was done with 5 replicates (overall experimental design: 3 (microbial strains) x 3 (conditions) x 5 (replicates) = 45 members). Because each member contained two phases, the overall analysis would be 45 x 2 = 90 analyses.

The condition for biotransformation reaction was as following. The organic phase contained 1.75 M benzaldehyde in octanol with the total volume of 5 ml. The aqueous phase of equal volume consisted of 300 mM sodium pyruvate, 1 mM thiamine pyrophosphate (TPP) and 1 mM $MgSO_4.7H_2O$ in 1.2 M phosphate buffer. After addition of whole cells, each biotransformation bottle was placed in a rotary tumbler mixer (Fabix,)at 10 rpm, 4^{o}C for 72 h as shown in Figure 3.2.

The microbial growth in the collected sample was slowed down by immersion in 4°C ice/water mixture for 5 min and kept frozen at -20°C pending subsequent analyses.

Figure 3.2 The biotransformation bottle was placed in a rotary tumbler mixer at 10 rpm, 4^{o}C for 72 h

The phases obtained were analyzed for pH level after 72 h of reaction by pH meter, volume of each layer after centrifugation to calculate the volume ratio, PAC, benzaldehyde and benzoic acid were determined by using HPLC with diode array detector (DAD) and ultra violet (UV) detection wavelength at 263 nm for benzyl alcohol and 283 nm for other species with 20 min of run time. The column used was Altima™ C8 5 μm particle size with 32%(v/v) of acetonitrile and 0.5% (v/v) of acetic acid in distilled water. Flow rate was set at 1.0 ml/min. Temperature of column oven was set at room temperature. Volume of injected sample was 5μl as described by Rosche *et al*. (2001). Acetoin was analyzed by using HPLC with HPX-87H column. Pyruvate concentration was determined spectrophotometically by enzymatic NADH + H^{+} coupled assay method with LDH based on the modified method from Czok and Lamprecht (1974) (Appendix H). Acetaldehyde concentration was determined in the same manner as pyruvate concentration with the replacement of LDH with ADH based on modified method from Bernt and Bergmeyer (1974) (Appendix I).