**Ampicillin production proposed protocol:**

To produce semi-synthetic β-lactam(Ampicillin), there are two proposed methods: One put-one step synthesis(1P1S) and one put two step synthesis while the second has showed a most overall yield then the first:

**1P1S:**

Pen G: 15 ml of 20 mM

D-PGME: 60 mM in 100 mM Phosphate buffer;PH7

iPGA(99.2 UPenG)

We add all the materials in round bottom flask on magnetic stir plate at To(22-25oC) about 160min

**1P2S:**

Pen G: 7.5ml of 40mM

Phosphate buffer: 100mM; PH7

iPGA:124UPenG/gram of carrier

We add all the materials in round bottom flask on magnetic stir plate at To(22-25oC). Then after about 60 min we add D-PGME(7.5 ml/120 mM).

Then after 160 min the PH was adjusted with NaOH from approximately 6.4-7.0

**!**

**The two-enzyme system with iPGA and AEH outperformed the systems that used only iPGA, thus demonstrating the clear advantage of using AEH**(1)**.**

**This result can be shown at the figure below :Figure 1**

**AEH:** Soluble amino ester hydrolase from *Xanthomonas campestris pv. Campestris*

**iPGA:** Eupergit-immobilized penicillin G acylase from *Escherichia coli*

**D-PGME:** (D)-phenylglycine methyl ester hydrochloride



Figure : Reaction profile of the enzymatic conversion of penicillin to ampicillin using 99.2 UPenG of iPGA and 2.2 UAmp AEH. Both the A) 1P1S and the B) 1P2S profiles are shown. D-PG (+), 6-APA (●), PAA (◆), AMP (∎), D-PGME (▴), PENG (\*).