

## CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

### 5.1 Conclusions

Media optimization of enrichment protocol was studied extensively to enhance the recovering and detection of *Listeria* spp. and truly understand the kinetics behind the enrichment step. The experiment was strategically designed into two parts; non selective enrichment broth and selective enrichment broth. In each part, the growth profiles and growth kinetics were measured by using the developed micro-inoculation technique (MIT) and micro-cultivation of *L. innocua*, *E.coli* and *S.aureus* in 96 deep well plates and 96-well micro plates. The proposed novel protocol was established to economize the use of media and supplies. Also this protocol enabled the significant reduction of the analytical time to multiply and detect *Listeria* spp.

The non selective enrichment experiment included 6 typical media to culture *L. innocua*. Among selected media, Tryptic Soy Broth (TSB) performed the best to resuscitate and amplify *L. innocua* number in the non-selective enrichment step. It gave the highest maximum growth rate value of *L. innocua* ( $0.21 \text{ h}^{-1}$ ) with a very good fit ( $R^2 = 0.989$ ) and very good final cell count ( $9.25 \text{ log CFU/mL}$ ).

The selective enrichment experiment provided a solid foundation in understanding the role of inhibitory effect of antimicrobial agents and the underlined kinetics to separate *Listeria* spp. from other contaminants. All selective agents generally formulated in typical gold standard handbooks were included in the experiments. Acriflavine and polymyxin B are very good inhibitors to use as selective agent for *Listeria* spp. Although lithium chloride didn't affect the growth and selectivity of *Listeria* spp., it included in optimization of inhibitor to study the combination and concentration on the growth and selectivity of *L. innocua*.

The optimization of the selective agent combination indicated that the use of each inhibitor affected the growth and selectivity of each microorganism differently. The concentration of polymyxin B influenced the efficiency of acriflavine. Besides, lithium chloride concentration affected the growth of *S. aureus* when used in conjunction with polymyxin B. Finally, the optimum combination that obtained from the experimental design is similar to the combination of Palcam broth.

## 5.2 Recommendations

The results from this study showed an opportunity to reduce *Listeria* spp. detection time and incidences of false negativity from the detection *Listeria* spp. in processing line by applying optimal non-selective enrichment and selective enrichment step and using the micro inoculation technique (MIT). However, the optimal non selective enrichment and selective enrichment media of this proposed detection procedure suggested from this study still achieved the experiment by using pure culture of food borne bacteria. The results from this study were only guideline for detection *L.monocytogenes* in food samples because the strain of *Listeria* which used in this research was *L.innocua*. All microorganisms that use to study were performed individually. Moreover, some selective agents weren't investigated in this study. So, for further study, microorganism should be combined to make the condition similar to real condition. In addition, ceftazidime and cycloheximide should be examined in the next study to make more precise and accurate result. And also the proposed technique should be compared with the gold standard to validate the efficiency of novel protocol.