# CHAPTER V

# CONCLUSION AND SUGGESTION

A 5 MHz QCM sensor was developed for detection of *Vibrio harveyi*, a bacteria causing luminous in shrimp. Immobilization of monoclonal antibody (MAb) against *V. harveyi* onto gold electrode of quartz crystals was successfully accomplished through a three-step procedure. The first step involved a formation of self-assemble monolayer of carboxyl-terminated alkanethiol. The QCM and water contact angle data suggested that the optimal condition for SAM process was to use 10 mM of MPA and 24h immersion time. The second step was an activation of the monolayer, using EDCI and NHS to convert the terminal carboxyl group on the surface to an active NHS ester by 15:45 mM of NHS/EDCI and 30 min activation time. The third step was a coupling of MAb against *V. harveyi*. The identified optimal condition for this step was to use the MAb concentration of 0.1 mg/mL and immobilization time of 15h. Characteristic functionalities of SAM of MPA both before and after MAb immobilization and *V. harveyi* binding were also verified by reflection-absorption infrared spectroscopy.

It has been demonstrated that the binding of *V. harveyi* of the MAb-immobilized substrate was significantly improved after the treatment with 1% BSA to block the unreacted or non-specific sites. Effect of density of surface-immobilized carboxyl-terminated alkanethiol on attachment of the antibodies was investigated. Mixed MPA-CE SAM can bind more MAb than the homogeneous SAM of MPA suggesting that the antibody can bind more efficiently when the packing of MPA was not too dense. But for mixed MPA-ME, only a ratio of 80%MPA:20%ME can improve the MAb immobilization and the subsequent bacteria binding.

Results from the detection of *V. harveyi* suggested that the QCM-based immunosensor gave a working range of $10^3$ to $10^7$ CFU/mL and was specific to *V.*

*harveyi* with a slight cross reactivity to *V. vulnificus* but not specific to *V. paraheamolyticus*.

In addition, the immunosensor can be partially regenerated by 0.1 M glycine/HCl buffer solution (pH 2.3). In order to achieve a better regeneration efficiency of the sensor, others parameters such as type and concentration of acid affecting the regeneration has to be thoroughly investigated.