CHAPTER 4

CONCLUSIONS

Flow injection cell-based biosensors for monitoring of chlorophenols have been developed. 2,4-Dichlorophenol was used as a model compound in this study. The immobilized mixed culture microbes from two sources, Thai and Swedish, were used as biological sensing elements in these biosensor systems. Swedish mixed culture bacteria were immobilized by entrapment between membranes, whereas Thai mixed culture microbes were entrapped in alginate gel beads. The measurement was based on the respiratory activity of microbes. When mixed culture microbes assimilated chlorophenols as a carbon source and energy in aerobic condition, the respiratory activity and the consumption of oxygen increased. Therefore, the concentration of dissolved oxygen in the solution would decrease and can be determined by a Clark type oxygen electrode transducer.

Mixed culture microbes was first enriched and acclimated into 2,4-dichlorophenol at optimum concentration. After it reached the exponential growth phase, the microbial cells were harvested and used in biosensor system. The optimum concentrations of 2,4-dichlorophenol to cultivate Swedish mixed culture bacteria and Thai mixed culture microbes were 50.0 and 60.0 mg l^{-1}, respectively.

The second part was to find the optimum conditions of each biosensor, operated in a flow injection system. The optimum conditions for Swedish mixed culture bacteria, placed on the oxygen electrode, were:

Conditions	**Optimum values**
Flow rate	0.10 ml min^{-1}
Sample volume	100 µl
Buffer pH	7.50
Buffer concentration	100 mM phosphate buffer

Thai mixed culture microbes was entrapped, packed into a column and placed prior to the oxygen electrode in the flow system. Its optimum conditions were:

Conditions	**Optimum values**
Flow rate	0.10 ml min^{-1}
Sample volume	500 µl
Buffer pH	6.50
Buffer concentration	100 mM (Tris-HCl buffer containing 10 mM $CaCl_2$)

Table 22 summarizes the performances of these two biosensor system.

Table 22 Performances of biosensors

Sensor performances	Swedish mixed culture	Thai mixed culture
Linearity	0.01-0.30 mM (1.6-48.9 mg l^{-1})	17.0-61.0 mg l^{-1}
Limit of detection	0.02 mM (3.3 mg l^{-1})	0.01 mg l^{-1}
Analysis time	7-21 min	30-60 min
Selectivity to chlorophenols	Selective	Not selective
Stability	7 days	3 days

When Swedish mixed culture bacteria was used in a cell-based biosensor system, the linear range, lower limit of detection and analysis time were 0.01-0.30 mM, 0.02 mM and 7-21 min, respectively. The sensor can be used more than a week and retained approximately 61 % of initial response with total analysis of more than 90 times. Furthermore, the sensor is quite selective to phenol and chlorophenols.

Thai mixed culture microbes showed linear range, limit of detection and analysis time for 17.0-61.0 mg l^{-1}, 0.01 mg l^{-1} and 30-60 min, respectively. It did not show good selectivity to phenol and chlorophenols since it also responded to

benzene and benzoic acid. At the beginning of the measurement (1^{st} – 3^{rd} day), the sensor showed good stability and the sensitivity reduced after that. The sensor showed relatively good precision, when eight injections at same concentration of sample were injected into the sensor system. However, when compare between 4 reactor columns, three out of four gave similar responses while the responses from the other column were lower. This might be the effect of the different amount of cells entrapped in the alginate gel, although the same amount of alginate gel beads (5.20 g) were packed. When this type of sensor was applied to analyze chlorophenols in environmental sample, the sensor responses were obtained. However, GC/MS did not show the chromatogram of chlorophenols. Therefore, the observed sensor response might be the effect of other aromatic compounds in real sample.

From the study, the analysis time of Swedish mixed culture bacteria was shorter than Thai mixed culture microbes. Since Swedish mixed culture bacteria were immobilized directly on the top of transducer, the product from the reaction can be quickly detect by the transducer. In case of Thai mixed culture microbes, the immobilized cells were packed in a reactor column, where the sample took a longer time to pass through. In addition, the changes occurred in the reactor column need time to move to a transducer and this is the cause of a long analysis time.

The selectivity of microorganisms from two sources was different. Swedish mixed culture bacteria provided higher selectivity to phenol and chlorophenols, whereas Thai mixed culture microbes can give sensor response to phenol, chlorophenols and other aromatic compounds.

The lack of selectivity of Thai mixed culture microbes can be improved by prolongation of acclimation time of mixed culture microbes in chlorophenols. The metabolite products obtained from mixed culture microbes can inhibit the activity of other strains. The application of pure culture can also improve the stability of the sensor to prevent the inhibition by toxic metabolite of other strains. Unlike Swedish mixed culture bacteria (3.3 mg l^{-1}), Thai mixed culture microbes (0.01 mg l^{-1}) could reach the permitted concentration of phenolic compounds (1 mg l^{-1}) in industrial effluent. The increasing of amount of immobilized cells can solve this problem. In fact, immobilized cells packed in a reactor column should give lower limit of detection than the immobilization on the top of the electrode, because the

sample has longer time to retain in the reactor column and contact to the immobilized cells. Therefore, the use of immobilized cells packed in a reactor column would be recommended for the analysis of chlorophenols at very low concentration. However, the active mixed culture microbes with long acclimation time are needed. Immobilization by membrane entrapment is suitable for process control, where short analysis time is required and does not need to go to very low concentration.

Compare to other works where pure culture bacteria were applied, their limit of detections were in the same range as Thai mixed culture microbes. The lower limit of detection was 0.2 and 0.7 mg l^{-1} using *Pseudomonas* and *Rhodococcus* strains, respectively. However, the use of immobilized *Pseudomonas* on graphite electrode allowed only 10 consecutive measurements (Skládal *et al.*, 2002). On the other hand, *Rhodococcus* gave very longer stability (21 days) (Riedel *et al.*, 1993), when compared to Swedish mixed culture bacteria (7days) and Thai mixed culture microbes (3days).

At current state of this study, this type of cell-based biosensor can be used as a screen method for phenolic and other aromatic compounds. It provided a cost effective method, due to mixed culture microbes can collect from the environmental wastewater. It is also easy to immobilize and operate.