# CHAPTER 5

# CONCLUSIONS AND RECOMMENDATIONS

## 5.2 Conclusions

Extractive fermentation was a novel technology that overcomes product inhibition during fermentation. The reports about extractive alkaline protease fermentation were very limited. To select a suitable aqueous two-phase system is in need for extractive fermentation.
In present study, *B.subtilis* TISTR 25 was suitable for alkaline protease extractive fermentation using ATPs obtained by PEG X (4000, 6000, and 10000)/ potassium phosphate but not for PEG 1000/potassium phosphate system. PEG 4000 was found out as the most suitable molecular weight of PEG. After 71 hours of fermentation, some compositions were found out as an optimal composition for best parameters.

**Table 5.1** Compositions for best parameters.

| System compositions | | Partition coefficient of alkaline protease | Total alkaline protease (unit/ml) | Purification factor | Specific activity of alkaline protease in the top phase (unit/mg) |
|---|---|---|---|---|---|
| PEG 4000 % w/w | Potassium phosphate % w/w | | | | |
| 9.75 | 8.78 | **26.75** | -- | -- | -- |
| 15.68 | 7.18 | -- | **2.78** | -- | -- |
| 10.2 | 9.36 | -- | -- | **4.38** | -- |
| 13.6 | 9.25 | -- | -- | -- | **39.395** |

For alkaline protease production, the last composition was recommended as an optimal composition that given best quality for the alkaline protease in top phase, which was our target.

## 5.2 Recommendations

For further development, we suggest that:

- To find the limiting molecular weight of PEG is one of the most importances of extractive fermentation needed.
- The effect of oxygen transferstion should be deeper investigated in case of scale up fermentor.
- Need more experimental information for deeper understanding about the change of cell shape.
- Finally, improvement process is necessary to enhance total alkaline protease production as feedback, semi-continuous, continuous process etc.