CHAPTER 4

CONCLUSION

This is the first report on metabolic engineering of lactic acid bacteria for 1,3-propanediol (1,3-PD) enhancement. A heterologous alcohol dehydrogenase, yqhD, has been expressed in L. reuteri. A reasonable increase in 1,3-PD specific productivity and molar yield has been achieved in the recombinant strain. A considerable shift in the metabolite profile was observed in the engineered strain, leading to enhanced synthesis of ethanol and lactate. The insight that selective utilisation of NADPH by YqhD has increased the absolute flux through phosphoketolase pathway, leading to enhanced ethanol and lactate levels, has been revealed by flux analysis studies. The enhanced ethanol (and lactate) levels are correlated with decreased native 1,3-PDOR activity. Ethanol inhibition studies have further emphasized that the enhanced ethanol levels have curtailed native 1,3-PDOR activity by decreasing NADH/NAD\(^+\) ratio. The active role of a redox-sensing global regulator that tunes the expression of target genes to maintain the cellular NADH/NAD\(^+\) ratio is inferred from bioinformatic analysis of L. reuteri. An unstructured kinetic model was adopted to describe adequately, the growth, substrate consumption and product formation in both control and recombinant L. reuteri strains. However, this simple model needs to be extensively improved to reflect the mechanism of enhancement of 1,3-PD specific productivity in the recombinant strain. This work has highlighted the role of redox and energy balance in cellular adaptation and the importance of tinkering at the global regulatory level to achieve significant enhancement in
1,3-PD levels. For scaling up the process, it has been shown that cell density can be enhanced by fed-batch process in the native strain. However, productivity needs to be improved by optimization. Further, the tolerance of the organism to high concentrations of 1,3-PD has been demonstrated. In future, process development strategies need to be adopted in the metabolically engineered strain for translating this work into a technology.