Chapter 2.

Aims and Objectives
The developments of host-vector systems in *E. coli* for overexpression of target recombinant proteins coupled with better bioprocess design allows us to reach productivities a higher than what was possible earlier some of these techniques have been applied to the production of recombinant therapeutics including IFN-γ with reasonable success. We decided to study IFN-γ production using these high expression systems. The focus of the work was to use the recent developments in bioprocess strategies and get higher levels of productivity. This would not only have implications in developing a process technology for IFN-γ it would provide the guidelines for a successful bioprocess strategy with possible application to other recombinant proteins. Kinetic modeling would also provide insights into cellular physiology especially when the cells were under the high metabolic burden associated with recombinant protein production.

Parallelly it was decided to study alternative hosts namely the methylotrophic yeasts *Pichia pastoris* and *Pichia methanolica*. The reasons were two fold; firstly the native IFNγ is glycosylated while *E. coli* produces it in the non-glycosylated form. Secondly previous reports suggest that IFNγ is formed as inclusion body in the cytoplasm of *E. coli* thus requiring correct refolding before activity can be restored. Also as with all cytoplasmic proteins the initial methionine is not removed. A soluble glycosylated and possibly extracellular product would thus be a distinct advantage. The disadvantage would be obviously the lower productivities achieved in this system. Thus it would be interesting to compare these completely different expression strategies and determine which is better in terms of a comprehensive production process.

The objectives of this works thus were

a) Cloning of hIFN-γ cDNA

b) Expression studies in *E. coli* using a variety of host vector combinations.

c) Cloning and expression studies in *Pichia* in different host-vector combinations.

d) Comparative bioreactor studies on hIFN-γ expression in both the systems.

e) Optimization of the bioprocess using the best host-vector system.