5. Summary and Conclusion

Mycophenolic acid production was done by using *Penicillium brevicompactum* MTCC 549 procured from IMTECH, Chandigarh, India. Microbial fermentation was performed using complex media with growing conditions of 200 rpm, 5.5 pH and 28°C temperature in shake flask.

Fermentation medium was optimized for better production of mycophenolic acid using one variable at time (OVAT) method. In the presence of glucose, greater levels of mycophenolic acid and yields were found. Glucose enhances the growth of *Penicillium brevicompactum*. In the presence of peptone, production of mycophenolic acid enhanced than other nitrogen sources. The increased production of mycophenolic acid was not due to different sources or concentrations, but rather to strong interactions among medium components. Addition of 0.5 g/L methionine and 9 g/L glycine showed the enhancement in production of mycophenolic acid. It indicates that methionine and glycine are the precursors for mycophenolic acid biosynthesis.

Mycophenolic acid production was then carried out in 3.7 L stirred tank bioreactor with two impellers. Kinetic analysis was done for production of mycophenolic acid. Because the culture was active, the growth phase began nearly immediately after inoculation and lasted 120 h, during which time the maximal rate of mycophenolic acid synthesis was achieved.

The process parameters such as agitation rate and aeration rate were also optimized for better production of mycophenolic acid. The highest mycophenolic acid concentration was found at 200 rpm, which was about 1.73 g/L. The results stated that there is an optimum

between morphology and agitation for the fungal fermentation process, where agitation might also be internally regulated with the availability of dissolved oxygen to the cells. The high agitation speed increases the power consumption and creates heterogeneous mixing of nutrients. When the agitation rate is too high it increases shear forces that can damage fragile microorganisms and affect product formation. On the other side, when the agitation speed is too slow, the viscosity of the fermented broth will increase, leading to a reduction in mass transfer efficiency. Agitation in fermentation process interacts with the culturing environments, which affect the product formation. The effect of aeration rate on production of mycophenolic acid was examined during fermentation process and found that maximum mycophenolic acid 1.76 g/L was obtained at 2 vvm.

The two most essential factors for all rheological changes in the broth during the production process are morphological variations and biomass content. The clumped growth of *Penicillium brevicompactum* causes a substantial rise in broth viscosity, which limits free cell cultivation investigations for mycophenolic acid synthesis. The viscosity of mycelia increased with increasing cell mass content in the early hours of fermentation, peaked at around 55.12 cp at 144 h, and then dropped. The decrease in viscosity may be attributed to the differentiation of swollen hyphae fragments into arthrospores.

The culture obtained via 3.7 L STR was then subjected to rheological investigation, which revealed that the relationship between shear stress and shear rate is best characterized by the Power Law model. The rheological investigation utilized *Penicillium brevicompactum* broth exhibit similar pseudo-plastic non-Newtonian behaviour and conform to the power law model. The consistency index is shown to be substantially linked with biomass concentration.

Mycophenolic acid production was investigated at different dissolved oxygen concentrations of 30 %, 40 %, and 50 %. The maximum production of mycophenolic acid 1.61 g/L was observed at 40 % dissolved oxygen concentration. The findings imply that vigorous aeration is important for the formation of mycophenolic acid.

According to studies on volumetric mass transfer coefficients, k_La increases as gas throughput increases. In addition, as broth viscosity increased, the volumetric mass transfer coefficient reduced dramatically. An interface blockage by cells, which have lower oxygen permeability than the liquid media, explains the drop in volumetric mass transfer coefficient. In STR, k_La values have been measured experimentally as a function of fermentation age and aeration rate. The k_La was calculated at two aeration rates, and it was observed that as the aeration rate increases, the k_La and OTR increase as well. The use of a 2 vvm aeration rate resulted in a 22 % increase in k_La. K_La value was 66.08 h^{-1} and 51.50 h^{-1} at 2 vvm and 1 vvm respectively. It also reveals that increasing the mixing velocity within a bioreactor can promote mass transfer, which aids in the formation of mycophenolic acid. From the results, it was observed that k_La during late exponential phase can be decreases because of oxygen demand were high at the time of mycophenolic acid production. The decrease in k_La value probably results of the interaction between rigid pellets and oxygen bubbles.

The continuous cultivation yielded the highest mycophenolic acid productivity of 0.025 g/L/h, while the fed batch culture yielded the highest mycophenolic acid concentration of 1.91 g/L. The rate of mycophenolic acid production is highest during the stationary phase, which lasts 120 - 240 h. Mycophenolic acid production can also be boosted in the fed batch fermentation method, allowing the culture to remain in the stationary phase for longer. The organism's growth rate was maintained over a long period of time in continuous culture. In

contrast to batch cultures, continuous cultures had the highest rate of mycophenolic acid buildup. The best dilution rate for the continuous mycophenolic acid production process was found to be 0.015 h^{-1}. Batch cultivation was shown to be the least efficient method of operation, with the lowest mycophenolic acid productivity.

After that, the broth obtained from the batch fermentation was purified. The crude broth was extracted with ethyl acetate. Mycophenolic acid purification from fermented broth was done by using column chromatography technique. It was observed that alumina column gives the highest purity of mycophenolic acid. Polar impurities were removed first, and then non-polar mycophenolic acid was eluted.

Then purification process of mycophenolic acid using column chromatography was optimized. Different factors such as pH, flow rate of mobile phase and volume of eluent were used for the optimization process.

The results of the ANOVA analysis gave the optimized value for MPA purification using Alumina column. Under pH 6.05, flow rate of 1.96 mL/min and volume of eluent 149.49 mL, the maximum predicted percentage of elution of MPA was 84.42%. To confirm the predicted response, experiments were conducted in triplicates. Experimentally, the maximum percentage of elution was found 84.12%, which was close to it. The result of this study could be used to design and enhance the mycophenolic acid production.

The mycophenolic acid-positive fractions were pooled and concentrated. Finally, HPLC and FTIR were used to determine the sample's purity.

The current study is an attempt to conduct a comprehensive investigation into the synthesis of mycophenolic acid using several growth modes of *Penicillium brevicompactum* and various operating strategies that may be used to scale up the process.

This investigation will expand in the future to include immobilization and fed-batch research in the Airlift reactor. It's also possible to investigate the impact of other microorganisms produced in co-culture with *Penicillium brevicompactum*. Furthermore, metabolic flow analysis could provide information into modifying the biosynthetic pathway to increase mycophenolic acid production.

The intricate details of gene interaction and gene over expression during synthesis have been revealed by the molecular mechanism of biosynthesis. Further work on this project will focus on understanding the molecular processes involved in the production of mycophenolic acid in *Penicillium brevicompactum* MTCC 549. The expression of the genes associated with the production of mycophenolic acid can be changed by using genetic engineering techniques.