STRATEGIES FOR PRODUCTION AND PURIFICATION OF MYCOPHENOLIC ACID

THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE AWARD OF DEGREE

DOCTOR OF PHILOSOPHY

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Dedicated to My Beloved

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Abstract

Immunosuppressants are chemical or biological agents purposefully used to reduce or suppress the immune system. These drugs have an array of uses from preventing the body from the rejection of organ transplant to treating various autoimmune disorders and various non-autoimmune inflammatory diseases. In recent years, microbial metabolites have become a hotspot of scientific research. Secondary metabolites mainly isolated from a microbial source. Pharmaceutical industries commonly use several fungal fermentation processes for the large-scale production of drugs. Mycophenolic acid (6-(4-hydroxy-6-methoxy methyl 3oxophthalanyl)-4-methyl-4-hexenoic acid, $C_{17}H_{20}O_6$, MPA) is a fungal secondary metabolite. MPA has been produced by many *Penicillium species* as well as other fungi. Mycophenolic Acid and derivatives of mycophenolic acid, such as mycophenolate mofetil and sodium mycophenolate, are used in the treatment of patients with organ transplantation and autoimmune disease by inhibiting the enzyme inosine monophosphate dehydrogenase (IMPDH). MPA and its derivatives are commercially used as frontline immunosuppressive agents to prevent the rejection of transplant organs. Commercial immunosuppressants based on MPA include CellCept (Mycophenolate mofetil; Roche) and Myfortic (mycophenolate sodium; Novartis). B and T lymphocytes entirely depend on IMPDH for the synthesis of nucleotides, while other human cells use different pathways for this synthesis, and are less affected by the anti-proliferative effect of MPA. Due to this reason, MPA is highly selective and have fewer side effects as compared to other immunosuppressants drug. Mycophenolic acid also exhibits several therapeutic roles and is useful in the treatment of various autoimmune, cancers, fungal, and viral diseases. This study is an attempt to optimize the MPA production through fermentation process using *Penicillium brevicompactum*. One

variable at a time (OVAT) approach was used to optimize media components for production of mycophenolic acid. In the presence of glucose and peptone production of MPA was enhanced. The highest MPA concentration was observed at 200 rpm and 2 vvm, which were about 1.73 g/L and 1.76 g/L respectively. The viscosity of fermentation broth reached a maximum of 55.12 cp at 144 h. It was found that the consistency index increased from 0.019 to 0.067 $Pa.s^n$, while the flow behavior index decreased from 0.71 to 0.45 during MPA production. Volumetric oxygen transfer coefficient (K_La) value was 66.08 h^{-1} and 51.50 h^{-1} at 2 vvm and 1 vvm respectively. In fed-batch fermentation, the MPA concentration obtained was 1.91 g/L higher than the value obtained in batch culture, 1.55 g/L, while in continuous fermentation, 1.67 g/L was obtained. The mycophenolic acid productivity obtained in continuous fermentation process was 0.025 g/L/h, which was maximum MPA productivity. Purification of crude mycophenolic acid produced from batch fermentation process was done by using column chromatography. Response surface methodology (RSM) using central composite design (CCD) was employed as a statistical tool to investigate the MPA purification process in column chromatography. Under optimum conditions, the experimental yield was observed to be 84.12%, which matched well with the predictive yield of 84.42%. High-performance liquid chromatography (HPLC) and Fourier-transform infrared spectroscopy (FTIR) analysis was carried out to confirm the presence of mycophenolic acid.

Keywords: Immunosuppressant; Mycophenolic acid; Bioreactor; Response surface methodology; Central composite design; Flow behaviour index; Consistency index.

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SYMBOLS USED

α	Coefficient of growth associated product formation
β	Coefficient of non- growth associated product formation
η	Viscosity
γ	Shear rate
k	Consistency index
k_La	Volumetric oxygen transfer coefficient
m_s	Specific maintenance coefficient
μ_{max}	Maximum specific cell growth rate
n	Flow behavior Index
P	Product Concentration
q_p	Specific product formation rate
r.f.	Retention factor
R^2	Coefficient of Regression
S	Substrate Concentration
τ	Shear stress
X	Biomass concentration
X_{max}	Maximum biomass concentration
$Y_{p/s}$	Yield of product per unit mass of substrate
$Y_{p/x}$	Yield of product per unit mass of dry cell
$Y_{x/s}$	Yield of dry cell mass per unit mass of substrate

ABBREVIATIONS USED

ALR	Air Lift Reactor
ANOVA	Analysis of Variance
CAGR	Compound Annual Growth Rate
CCD	Central Composite Design
CIFC	Central Instrument Facility Centre
CNIs	Calcineurin inhibitors
DNS	DiNitroSalicylic acid
DO	Dissolved Oxygen
FTIR	Fourier Transform Infra Red Spectroscopy
H/D	Height to Diameter
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
GI	Gastrointestinal
IMPDH	Inosine Monophosphate Dehydrogenase
IL-2	Interleukins-2

MMF	Mycophenolate Mofetil
MPA	Mycophenolic Acid
mTOR	mammalian Target of Rapamycin
NMR	Nuclear Magnetic Resonance
OVAT	One Variable at Time
PKS	Polyketide Synthase
RFB	Rotating Fibrous Bed
SCSC	Squamous Cell Skin Cancer
STR	Stirred Tank Reactor
US-FDA	United States Food and Drug Administration
vvm	volume of air per volume of media per minute

PREFACE

"A scientist is happy, not in resting on his attainments but in the steady acquisition of fresh knowledge."

- Max Planck

A therapeutic compound known as mycophenolic acid drew my attention as it was reported to have several functions including immunosuppression. Patients suffer a significant impact as a result of expensive transplants followed by lifelong immunosuppression. Thus, I choose this study to find some options for increasing mycophenolic acid production using a *Penicillium brevicompactum* fungal strain. Different process characteristics that could affect the fermentation system were used to develop the strategies.

Firstly, I tried to optimize the medium composition for mycophenolic acid production. The optimized medium was then used for further studies in shake flask and bioreactor. The kinetic analysis of mycophenolic acid production was carried out in stirred tank bioreactor. Once the kinetic behaviour of fermentation process was analyzed, then different strategies were employed to study their effect on mycophenolic acid production.

Then the production in stirred tank bioreactor was studied by using different dissolved oxygen concentrations.

Another strategy which compared the effect of different fermentation process was done for mycophenolic acid production.

In order to validate the production of mycophenolic acid, purification was carried out. The purified sample was then characterized using different analytical techniques.

Thus, with immense support and guidance of my Ph.D. supervisor, Prof. Pradeep Srivastava, I have compiled my efforts in the form of this thesis. The thesis has been divided into five chapters:

1. **Introduction:** Details the importance of mycophenolic acid as a therapeutic agent
2. **Review of Literature:** Describes the studied done so far in the area of bioprocess development of mycophenolic acid and other antibiotics.
3. **Materials and Methods:** Provide the information about the chemical reagents and other aids utilized during the study. It also describes the methodologies which have been adopted for the study.
4. **Results and Discussion:** Gives an insight into the findings of this study and their implications.
5. **Conclusion:** Summarizes the work as well as provides the future scope of this work.

List of publications have been attached at the end.

I hope this research report would be interesting for the researchers working in the area of Biochemical and Bioprocess Engineering.