

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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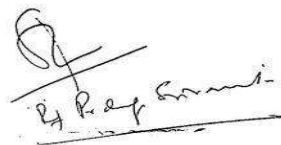
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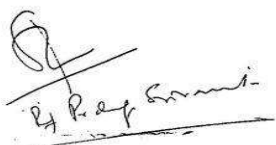
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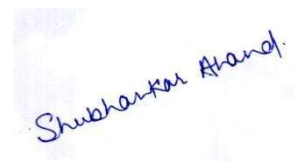
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Dedicated to My Beloved

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Acknowledgements

Through this page, I offer my salutation, to the creator of this universe 'Almighty God'. I would also like to bow my head to the founder of this pious seat of learning, Bharat Ratna - Mahamana Pt. Madan Mohan Malviya Ji.

I take this as an opportunity to express my deep sense of gratitude and indebtedness to my thesis supervisor, Prof. Pradeep Srivastava, School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi for his valuable suggestions, co-operation and nurturing motivation which has led to the successful completion of this study. I am thankful to him for his constant academic guidance and parental support throughout the research period.

I am grateful to express my sincere thanks to the Coordinator of the School, Prof. Vikash Kumar Dubey, and Ex Coordinators, Prof. Subir Kundu, Prof. S.K. Srivastava, Prof. R.M. Banik and Prof. (Mrs.) Mira Debnath (Das) for providing the required facilities and administrative support throughout my research work.

It is a great privilege for me to express my gratitude to all the RPEC members Prof. V. L. Yadav, Department of Chemical Engineering and Technology, IIT (BHU), Dr. Abha Mishra and Dr. Sanjay Kumar, School of Biochemical Engineering, IIT (BHU) Varanasi for their technical suggestions and valuable guidance.

I take this as an opportunity to express my hearty thanks to all the other faculty members of School of Biochemical Engineering, IIT (BHU), Prof. R.M. Banik, Dr. Sanjay Kumar and Dr. Vishal Mishra for their support and constant motivation.

I express my thanks to Technology Business Incubator (TBI) at IIT (BHU) Varanasi for providing the fermentor facility. I extend my gratitude to the Coordinators (Prof. P. K. Mishra and Prof. Pradeep Srivastava), Manager and staff of TBI who supported me in every possible way to carry out the work. I would also like to express my sincere thanks to Central Instrumentation Facility Centre (CIFC), IIT (BHU) Varanasi for providing access to sophisticated instruments.

I also acknowledge the Ministry of Human Resource and Development (MHRD), Govt. of India, New Delhi, and Director, IIT (BHU) for the financial support in the form of teaching assistantship and other grants.

I also owe a lot to all the non-teaching staff of the School of Biochemical Engineering, IIT (BHU) including Mr. Suchit Verma, Mr. Ramashankar Singh, Mr. Dinesh, Mrs. Usha, Mr. Arun, Mr. Deepak Sinha and Mr. G Jagan Mohan as this work would have never been completed without their support.

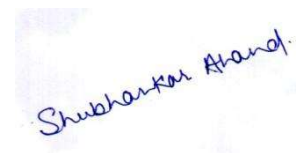
Acknowledgements

My heartfelt thanks go to my labmates, Dr. (Mrs.) Rupika Sinha, Dr. Sarada Prasanna Mallick, Ms. Namarata Yadav, Mr. Satyavrat Tripathi, Mr. Divakar Singh, Ms. Preeti Kaur, Ms. Priya Shukla, Mrs. Shikha Kumari, Mr. Rahul Ranjan, Mr. Aditya Anand, and Mr. Virendra Singh. I learned the team work and other organizational skills from them. I express my thanks to every student (IDD, M.Tech and Ph.D.) of School of Biochemical Engineering for being directly or indirectly part of my learning as a research scholar and teaching assistant.

I would like to thank my seniors and friends, Dr. Ashish, Dr. Sukhendra Singh, Dr. Moti Lal, Dr. Ipsita Chakravarty, Mr. Rahul Kumar and Ms. Anupam Chauhan for their care and encouragement. The journey was eased because of their presence and company as they always stood as pillars whenever I faced personal or academic issues.

It is impossible to express in words, my gratefulness towards my parents Mr. Sohan Lal and late Mrs. Sarla who were the foundation of my strength and endeavor with never ending motivation, blessings and faith. I would like to express my gratitude to my sister's Mrs. Ekta, Mrs. Shweta, and Mrs. Archana that provide me all the wisdom and strength to carry on this journey which at times became very tough and tedious. Also my loving brother's, Dr. Shubhesh Kumar and Mr. Vishisht Kumar always deserves my gratitude for helping me with his affectionate nature.

Lastly, I express my gratitude to everyone who directly or indirectly helped me in pursuing my studies, doing research and being motivated. I deeply apologize for any omissions or mistake in expressing my thankfulness.



Shubhankar Anand

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.sⁿ, 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.