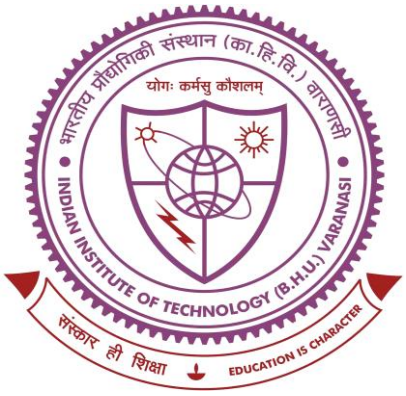
STRATEGIES FOR THE PRODUCTION AND PURIFICATION OF HYALURONIC ACID



THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE AWARD OF DEGREE

DOCTOR OF PHILOSOPHY

By

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It is further certified that the student has fulfilled all the requirements of Comprehensive, Candidacy, and SOTA for the award of Ph.D. Degree.

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DECLARATION BY THE CANDIDATE

I, ***"Priya Shukla,"*** certify that the work embodied in this thesis is my bonafide work and was carried out by me under the supervision of ***"Prof. Pradeep Srivastava" and "Prof. Abha Mishra"*** from ***"July 2018" to "August 2023"***, at the ***"School of Biochemical Engineering,"*** Indian Institute of Technology (BHU), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credits to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not wilfully copied any other's work, paragraphs, text, data, results, *etc.*, reported in journals, books, magazines, reports, dissertations, theses, *etc.*, or available on websites and have not included them in this thesis and have not cited as my work.

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It is certified that the above statement made by the student is correct to the best of my knowledge.

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Acknowledgments

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.

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Priya Shukla

This Thesis is Dedicated to My Beloved Parents

NEELAM SHUKLA

&

AMAR NATH SHUKLA

(Without whom none of my success would be possible)

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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

List of Abbreviations

HA	Hyaluronic acid
GAG	Glycosaminoglycan
MW	Molecular weight
MWCO	Molecular weight cut-off
ALR	Air Lift Reactor
ANOVA	Analysis of Variance
CAGR	Compound Annual Growth Rate
CCD	Central Composite Design
CIFC	Central Instrument Facility Centre
DNS	Dinitro-Salicylic acid
DO	Dissolved Oxygen
FT-IR	Fourier Transform-InfraRed Spectroscopy
NMR	Nuclear Magnetic Resonance
CTAB	Cetyl trimethyl ammonium bromide
XRD	X-ray Diffraction
MTCC	Microbial Type Culture Collection and Gene Bank
SEM	Scanning Electron Microscopy
TMP	Transmembrane Pressure
SEC	Size Exclusion Chromatography
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
MALDI	Matrix-Assisted Laser Desorption/Ionization
TGA	Thermogravimetric analysis
ESI-MS	Electrospray Ionization Mass Spectroscopy

PREFACE

"An expert is a person who has made all the mistakes that can be made in a very narrow field."

– Neils Bohr

A natural biopolymer known as hyaluronic acid drew my attention as it reportedly has several clinical, aesthetic, and biomedical functions. Owing to its numerous properties, it has earned potential roles in several surgeries related to eyes, knees, skin, and drug delivery. Thus, I chose this study to find options for increasing hyaluronic acid production using the *Streptococcus zooepidemicus* bacterial 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 used for further studies in the shake flask and bioreactor. The kinetic analysis of mycophenolic acid production was carried out in a stirred tank bioreactor. Once the fermentation process's kinetic behavior was analyzed, different strategies were employed to study their effect on hyaluronic acid production.

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

A scale-up study was also performed to encounter and solve the problems during the enhanced hyaluronic acid production.

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

Thus, with the immense support and guidance of my Ph.D. supervisors, Prof. Pradeep Srivastava and Dr. Abha Mishra, 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 study done in the bioprocess development of mycophenolic acid and other antibiotics.

3. **Materials and Methods:** Provide 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.

A list of publications has been attached at the end.

I hope this research report will interest Biochemical and Bioprocess Engineering researchers.