

PURIFICATION, CHARACTERISATION AND STUDIES  
ON THE MECHANISM OF ACTION OF NAPHTHALENE  
OXYGENASE FROM *Corynebacterium renale*

BY  
S. MEERA

SUBMITTED  
IN FULFILMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF DOCTOR OF PHILOSOPHY  
TO THE  
INDIAN INSTITUTE OF TECHNOLOGY, DELHI

DEPARTMENT OF CHEMISTRY

FEBRUARY, 1980

CERTIFICATE

This is to certify that the thesis entitled "Purification, Characterisation and Studies on the Mechanism of Action of Naphthalene Oxygenase from Corynebacterium renale" being submitted by S. Meera to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy, is a record of bonafide research work carried out by her. S. Meera has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge, has reached the requisite standard.

The results contained in this thesis have not been submitted in part or in full, to any other university or institute for the award of any degree or diploma

(P.S. Rao)  
Thesis Supervisor,  
Assistant Professor,  
Chemistry Department,  
I.I.T. Delhi,  
New Delhi-110029

(R.D. Dua)  
Thesis Supervisor,  
Professor, Chemistry Department,  
I.I.T. Delhi,  
New Delhi-110029

## ACKNOWLEDGEMENTS

I express my profound gratitude to Prof. R.D. Dua and Dr. P.S. Rao for leading me into a comprehensive study of this problem of absorbing interest. The inspiring guidance, invaluable suggestions and critical supervision rendered by them during the course of this investigation as well as in the preparation of the thesis were a constant source of encouragement.

I should like to express my thanks to the authorities of the Solid State Physics Laboratory, National Physical Laboratory and University of Delhi for providing the facilities of ESR spectrometer, atomic absorption spectrometer and spectrophotofluorimeter, respectively.

I wish to express my sincere gratitude to Prof. R.P. Gandhi, Head, Chemistry Department for providing excellent research facilities and to my colleagues and friends who generously extended their help and cooperation, whenever sought for.

Mr. N.K. Nautiyal, who maintained the instruments in the Biochemistry Laboratory in good shape, needs a special mention here as his services helped me to finish the project satisfactorily.

A word of appreciation is due to Mr. S.L. Aneja for his neat typing of the manuscript and to Mr. N.L. Arora for skilfully making the diagrams.

I am obliged to the Director, Indian Institute of Technology Delhi, as well as Council of Scientific and Industrial Research for financial help for the project.

February, 1980

(S. Meera)

## CONTENTS

	Page
INTRODUCTION	1
Historical Development of Mono and Dioxygenases and their Purification	1
Significance of Oxygenases	7
Aliphatic Hydrocarbon Metabolism	10
Aromatic Hydrocarbon Metabolism	13
Aims and Scope of Present Investigation	23
MATERIALS AND METHODS	25
List of Chemicals	25
Growth of the Bacterium	28
Preparation of Crude Cell Free Extract	29
Protein Determination	30
Assay of Naphthalene Oxygenase Activity	31
Preparation of DEAE-cellulose Column	32
Preparation of Sephadex Column	33
Disc Gel Electrophoresis	33
Molecular Weight Determination	36
Fluorescence Measurements	41
Preparation of <u>cis</u> -1,2-Dihydroxy-1,2-dihydro- naphthalene	41
Preparation of <u>trans</u> -1,2-Dihydro-1,2-dihydroxy- naphthalene	45
Thin Layer Chromatography of Enzymatic Product	45
Isolation of Product(s) Formed Enzymatically from Naphthalene	46
Identification of Acid Treated Products of Enzyme Reaction	47

	Page
Oxygen Uptake Studies by Warburg's Method	48
Estimation of Iron	49
Electron Spin Resonance Studies	52
Identification of FAD as the Constituent of Enzyme by TLC	53
<b>RESULTS</b>	<b>55</b>
Purification and Characterisation of Naphthalene Oxygenase	55
Activity of the Crude Enzyme	55
Localisation of the Enzyme	55
Ammonium Sulfate Fractionation	57
Sephadex G-75 Column Chromatography	57
DEAE-cellulose Column Chromatography	58
Effect of Solvents	60
Effect of pH	60
Effect of Temperature	61
Validity of Assay Procedure	61
Determination of Kinetic Parameters	62
Molecular Weight Determination	63
Absorption Spectrum	64
Fluorescence Spectra	64
<b>MECHANISM OF ACTION OF NAPHTHALENE OXYGENASE</b>	<b>66</b>
Introduction	66
Tryptophon 2,3-Dioxygenase	66
Lipoxygenase	73

	Page
$\alpha$ -Ketoglutarate Dioxygenase	82
2-Nitropropane Dioxygenase	89
Benzene Dioxygenase	92
Results	95
Identification of the Products Formed by the Enzymatic Oxygenation of Naphthalene	95
Essentiality of Molecular Oxygen	97
Stoichiometry of Naphthalene Oxygenase Reaction	97
Effect of Metal Chelating Agents	98
Quantitative Determination of Iron	101
Influence of Metal Ions	103
ESR Studies	103
Determination of Valence State of Iron of Naphthalene Oxygenase During the Reaction	105
Effect of Atebrin	106
Effect of Thiol Group Inhibitors	111
Effect of Electron Donors	111
Effect of Catalase	114
Effect of H <sub>2</sub> O <sub>2</sub>	114
Identification of Product Formed when NADH was Substituted by H <sub>2</sub> O <sub>2</sub>	116
Effect of Superoxide Dismutase	119
Chemical Evidence for the Generation of O <sub>2</sub> <sup>-</sup>	121
Effect of ·OH Radical Scavengers	123
Studies with Non-enzymatic Model Systems Containing Naphthalene and H <sub>2</sub> O <sub>2</sub>	125
DISCUSSION	131
SUMMARY	143
BIBLIOGRAPHY	146