

DECLARATION

I hereby declare that the thesis entitled “**Efficiency optimization of vermitechnology for generation of sanitized end product: An in-sight on PAH (polycyclic aromatic hydrocarbons) detoxification and microbial activity**” submitted to the **School of Sciences** Tezpur University in part fulfillment for the award of the degree of Doctor of Philosophy in **Environmental Science** is a record of research work carried out by me under the supervision of **Dr. Satya Sundar Bhattacharya**, Department of Environmental Science, Tezpur University, Assam - 784028. No part of this work has been presented for any other degree or diploma earlier.

Date:

(Jinnashri Devi)

Place:

Department of Environmental Science
School of Sciences
Tezpur University, Assam India



तेजपुर विश्वविद्यालय/ TEZPUR UNIVERSITY

(संसदकं अधिनयमकारंस्थाधिकं तं
यशिश्वविद्यालय)

(A Central University established by an Act of Parliament)

तेजपुर-784028 :: असम/ TEZPUR-784028 :: ASSAM

Dr. Satya Sundar Bhattacharya

Associate Professor

Department of environmental science

School Sciences

Tezpur University

Email: satya72@tezu.ernet.in

Tel: +91-3712-275610-5610

Certificate of the Supervisor

This is to certify that the thesis entitled “**Efficiency optimization of vermitechnology for generation of sanitized end product: An in-sight on PAH (polycyclic aromatic hydrocarbons) detoxification and microbial activity**” submitted to the **School of Sciences** Tezpur University in part fulfillment for the award of the degree of Doctor of Philosophy in **Environmental Science** is a record of research work carried out by **Ms. Jinnashri Devi** under my supervision and guidance.

All help received by her from various sources have been duly acknowledged.

No part of this thesis has been submitted elsewhere for award of any other degree.

Place: Tezpur

(**Dr. Satya Sundar Bhattacharya**)

Date:

ACKNOWLEDGEMENT

I take the opportunity to extend my heartfelt gratitude to my Supervisor, **Dr. Satya Sundar Bhattacharya** for his constant guidance, warm encouragement, healthy criticisms, and moral support.

I earnestly thank the Honorable Vice-chancellor Tezpur University for extending necessary infrastructural facilities to carry out my research work.

I also convey my sincere regards to the head of the department **Prof. R.R. Hoque** and other faculty members Prof. A.K.Das, Prof. A.Devi, Prof. K.Marimuthu, Dr. N.Gogoi, Dr. S.Handique, Dr. A.Prakash, Dr. N.M.Gogoi, Dr. S.Kalita, Dr. P.Deka in the Department of Environmental Science, Tezpur University for their constructive comments and advice.

I also take the opportunity to thank my doctoral committee members, Dr. Ashalata Devi and Dr. Suman Dasgupta for their suggestions and keen monitoring of my work progress.

I would like to thank **Dr. Utsab Deb, Defense research laboratory, Tezpur**, offering me constant assistance and help in conducting my experiments.

I also thank the technical staffs of the Sophisticated Instrumentation and Analytical Centre, and Department of Environmental Science, Chemical Science, and Physics for rendering their active involvement in sample analysis.

I am thankful to my lab mates Linee Goswami, Subhashish Das, Soma Barman, Pallabi Das, Nazneen Hussain, Ananya Mondol, Sarmishtha Paul, Ratan Choudhury , Ratul Pegu, Himadri Mandal and Dhriti Sundar Boro for maintaining a good working environment and helping me in various ways.

I would also like to thank Mr. Mofijul Ahmed for his assistance in conducting vermicomposting experiments.

I cannot finish without thanking my family. Here I would like to make a special mention of my mother, my husband and my in laws for their constant support and motivation in all aspects of my life. My sincere regards are also extended to all my

friends and well wishers who supported me in this journey till the end. A special thanks to my late father for his blessings.

My sincere gratitude is also to the Department of Science and Technology, Government of India for conferring the project fellowship that enabled me to complete my work

Last but not the least; I thank the Almighty for keeping me in good health and granting me the capability to proceed successfully.

(Jinnashri Devi)

LIST OF TABLE

Table No.	Caption	Page No.
2.1	Difference between Composting and Vermicomposting	18
2.2	An account of vital physico-chemical attributes for assessing the composting and vermicomposting status	21
2.3	List of some end product quality assessment of previous works	23
2.4	Waste-based feedstock composition and verified earthworm species for successful vermicomposting	30
2.5	Vermicomposting of different biogenic and lignocellulosic feedstocks using different earthworm species	32
2.6	Different treatments used for PAH removal from different sources	35
4.1	Physico-chemical characteristics of the spent mushroom substrate (SMS) and cow dung (CD) used for the study. Values represent mean \pm standard deviation	69
4.2	Transmittance values (T%) and the main absorbance bands in FTIR spectra of the composted and vermicomposted samples along with their assignments	72
4.3	Count of total bacteria (TBC), total fungi (TFC), P solubilizing bacteria (PSB), and N fixing bacteria (NFB) in the composted and vermicomposted SMS at 60 days of incubation. Values represent mean \pm standard deviation	77
4.4	Comparison between the composted and vermicomposted SMS extracts on relative root (RRG), shoot growth (RShG), relative seed germination (RSG), and germination index (GI) of green gram (<i>Vigna radiata</i>)	81
5.1	Pearson Correlation coefficients (r) and level of significance depicting the relationships among microbial attributes in vermibeds	110
6.20	Basic chemical details, abbreviated names, and QA-QC information of the studied PAHs	123
6.1	Temporal variation in the concentration (mg kg ⁻¹) of 3-ring Acenaphthylene (AceN) under various treatments. Values are presented as mean \pm standard deviation	126
6.2	Temporal variation in the concentration (mg kg ⁻¹) of 3-ring Fluorene (Fl) under various treatments. Values are presented as mean \pm standard deviation	127

Temporal variation in the concentration (mg kg ⁻¹) of 3-ring Phenanthrene (Phn) under various treatments. Values are presented as mean ± standard deviation	127
Temporal variation in the concentration (mg kg ⁻¹) of 3-ring Anthracene (Anth) under various treatments. Values are presented as mean ± standard deviation	128
Temporal variation in the concentration (mg kg ⁻¹) of 4-ring Pyrene (Pyr) under various treatments. Values are presented as mean ± standard deviation	128
Temporal variation in the concentration (mg kg ⁻¹) of 4-ring Chrysene (Chrys) under various treatments. Values are presented as mean ± standard deviation	129
Temporal variation in the concentration (mg kg ⁻¹) of 4-ring Benz(alpha)anthracene (BaA) under various treatments. Values are presented as mean ± standard deviation	129
Temporal variation in the concentration (mg kg ⁻¹) of 5-ring Benzo(alpha)pyrene (BaP) under various treatments. Values are presented as mean ± standard deviation	130
Temporal variation in the concentration (mg kg ⁻¹) of 5-ring Benzo(k)fluoranthene (BkF) under various treatments. Values are presented as mean ± standard deviation	131
Temporal variation in the concentration (mg kg ⁻¹) of 5-ring Benzo(beta)fluoranthene (BbF) under various treatments. Values are presented as mean ± standard deviation	131
Temporal variation in the concentration (mg kg ⁻¹) of 6-ring Benzo(g,h,i)perylene (Bpl) under various treatments. Values are presented as mean ± standard deviation	132
Temporal variation in the concentration (mg kg ⁻¹) of 6-ring Indeno (1,2,3-cd) pyrene (Ip) under various treatments. Values are presented as mean ± standard deviation	132
Temporal variation in the concentration (mg kg ⁻¹) of 6-ring Dibenzo(a,h)anthracene (DbA) under various treatments. Values are presented as mean ± standard deviation	133
Removal efficiency (%) of the 13 PAHs as observed under the different	134

feedstocks of both composting and vermicomposting pathways	
Accumulation pattern of the 13 PAHs by <i>E. fetida</i> and <i>E. eugeniae</i> in the spiked feedstocks	141
Budget and detailed apportionment of the 6-ring and 5-ring PAHs under composting and vermicomposting systems. Values are represented as mean \pm standard deviation	145
Budget and detailed apportionment of the 4-ring PAHs under composting and vermicomposting systems. Values are represented as mean \pm standard deviation	149
Budget and detailed apportionment of the 3-ring PAHs under composting and vermicomposting systems. Values are represented as mean \pm standard deviation	151
Pearson's correlation matrix among the PAHs and biochemical attributes of the vermicomposted and composted feedstock samples	156
7.1 Comparison between the composted and vermicomposted feedstock extracts on relative root (RRG), shoot growth (RShG), relative seed germination (RSG), and germination index (GI) of green gram (<i>Vigna radiata</i>)	188

LIST OF FIGURES

Figures No.	Caption	Page No.
	Schematic representation of the overall research plan	8
	Schematic representation of the thesis organization	9
	Graphical representation of the experiment	19
	Bacterial diversity identified within the intestinal wall of earthworms	32
	The sources of organic pollutants in the environment	34
	Schematic representation of the work done under phase I	48
	Schematic representation of the work done under phase II	49
	Structural deformation in the spent mushroom substrate (SMS) under the vermicomposting and composting treatments as verified from the crystallinity index.	70
	Temporal variation in pH, total organic C, total Kjeldahl N, and available P of spent mushroom straw based feedstocks under the composting and vermicomposting system	74
	Changes in microbial biomass C, compost respiration, microbial quotient, and microbial metabolic quotient under various bio-composting treatments	76
	Phospholipid fatty acid (PLFA) identified microbial groups in the composted and vermicomposted SMS-based feedstocks at 60 days of incubation	79
	Percentage composition of different types of fatty acids in the vermicomposted and composted feedstocks detected through Phospholipid fatty acid (PLFA) analysis.	80

	Temporal variations in earthworm count (1a) and body weight (1b) in the lignocellulosic waste-based feedstock under different treatments (initial stocking densities of earthworms) during vermicomposting.	97
	Changes in Crystallinity index (2a), pH (2b), Total organic carbon (2c), Humification factor (2d), Total Kjeldahl nitrogen (2e), Available Phosphorous (2f), and Potassium (2g) under different treatments	101
	Variation in microbial community structure Microbial biomass carbon (3a), Compost respiration (3b), Microbial quotient (3c),Biomass (total PLFA) (3d), Gram-positive (3e), Gram-negative (3f), Eukaryotes (3g), Anaerobes (3h), and Actinomycetes (3i)in lignocellulosic waste-based feedstocks under different treatments (initial stocking densities of earthworms) during vermicomposting.	105
6.4	HPLC Chromatogram of 13 PAH mixture(Polycyclic aromatic hydrocarbons)	122
	Changes in the concentration of PAHs during composting and vermicomposting and their corresponding removal efficiency at 30 days after incubation	137
	Health, proliferation, and PAH-bioaccumulation potential of <i>Eisenia fetida</i> and <i>Eudrilus eugeniae</i> : (a) earthworm count; (b) body weight; (c)bioaccumulation pattern of 3 & 4 ring PAHs; and (d)bioaccumulation pattern of 5 & 6 ring PAHs	139
	Temporal variations in the physicochemical and microbial attributes of the PAH spiked feedstocks under composting and vermicomposting. (a) pH; (b)total organic C; (c)total N; (d)available P; (e)available K; (f)microbial biomass C; (g)compost respiration; and (h)bacterial count	157

7.3.1	Only earthen no perforated walled vermireactor (EVR)	171
7.3.2	The solid model of prototype MSVR	168
7.3.3	The solid model of the shredder mechanism MSVR	168
7.3.4	The solid model of the inner structure of the prototype MSVR	169
7.3.6	Dimensions of the prototype MSVR	169
7.3.7	Clay and paper paste made perforated-walled truncated cone shaped vermireactor (CPVR)	170
7.3.8	Mechanized with shredder and watering device incorporated vermireactors (MSVR)	172
7.3.9	vermireactor experiment	173
7.3.10	Temporal variation in (a) earthworm counts and (b) earthworm cocoon counts in the feedstocks under the composting and vermicomposting system	176
7.3.11	Temporal variation in pH, total organic C, and total Kjeldahl N in the feedstocks under the composting and vermicomposting system	178
7.3.12	Temporal variation in (a)Avl P,(b) Avl K, and (c)MBC (Microbial biomass C) in the feedstocks under the composting and vermicomposting system	176
7.3.13	Relative abundance distribution of detected OTU by Taxon classification Genus	182
7.3.14	Abundance of genes in the vermireactors derived from sequenced data	183
7.3.15	Relative abundance distribution of detected OTU by Taxon classification Phylum	184
7.3.16	Alpha (Shannon and Simpson) diversity of bacterial	185

communities in the vermireactors

Rarefaction curves estimating the species richness in the vermireactors 185

Temporal variation in (a) Cd(mg/Kg), (b) Zn(mg/Kg), and (c) Mn(mg/Kg) in the feedstocks under the composting and vermicomposting system 187