MANAGEMENT OF INDUSTRIAL ORGANIC SOLID WASTE THROUGH VERMI-CULTURE BIOTECHNOLOGY WITH SPECIAL REFERENCE TO MICROORGANISMS

A THESIS SUBMITTED FOR THE AWARD OF DEGREE OF
DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY
IN THE FACULTY OF LIFE SCIENCE AND ENVIRONMENT

PRAKASH M MUNNOLI
DEPARTMENT OF MICROBIOLOGY
GOA UNIVERSITY
TALEIGAO PLATEAU, GOA – 403206, INDIA

DECEMBER 2007

THIS STUDY DEDICATED TO MY MOTHER

SMT. SUSHILA MUNNOLI

WHOM I LOST DURING THE COURSE OF MY STUDY

Certificate

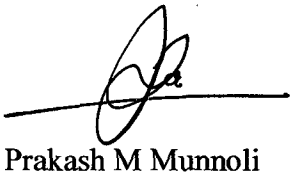
CERTIFIED that this report entitled "Management Industrial organic solid waste through vermi-culture biotechnology with special reference to microorganisms" Submitted by Shri. Prakash M Munnoli, in partial fulfillment of the award of Doctor of Philosophy in Microbiology, Goa University, is a record of students own original work carried out under my supervision and guidance, except as acknowledged in the text. This study report has not submitted in any other University or Institution for the award of such degree.

Supervisor/ Guide

Dr Saroj Bhosle
Reader,
Microbiology Department
Goa University
Taleigao Plateau- 403206

DECLARATION

I hereby state that this thesis entitled "Management Industrial organic solid waste through vermi-culture biotechnology with special reference to microorganisms" for the Ph.D degree is my original work and that it has not previously formed the basis for the award of any degree, diploma, associate ship and fellowship or any other similar title to the best of my knowledge and information.



Prakash M Munnoli

Candidate

Department of Microbiology

Goa University

Taleigao Plateau, Goa – 403206.

Evaluated

4/3/09

Shyam Sr.
04/03/2009
(External Examiner)

ACKNOWLEDGMENTS

Initial credit must go to Dr. Sunila Mavinkurve, Head Department of Microbiology Goa University who initiated the project, and to Dr. Saroj Bhosle Reader, Department of Microbiology Goa University, who agreed to be a supervisor. Shortly thereafter, Dr. Mavinkurve retired from the service and the full responsibility of supervisor is taken by Dr Saroj Bhosle , She has been a constant source of encouragement and consolation; not only has she kept the project on track and checked the reports, she has been a constant source of inspiration.

Support was received from numerous people along the way. Especially, the core of solid waste management by using earthworms, from Punjab, State Council for Science and Technology, Chandgarh, Dr. J.K Arora , Dr. Marwaha. Dr. Krishnamurthy [Rtd.] Principal, NITTTR, Chandigarh.

I am especially grateful to Dr.P.V. Desai Dean Life Science, Head Zoology Department Goa University, for his valuable suggestions, and guidance in identification work and successful completion of the work.

My Sincere thanks to Dr G.N Nayak Head, Marine Science Department Goa University, for his suggestions and constructive comments during the course of study.

I extend my sincere thanks to Prof. Dr D.J. Bhat Head Botany Department Goa University, for his guidance and words of encouragement during the course of the study, and providing me an opportunity to present my works during refresher courses in the university.

I sincerely thank Dr. S.Dubey, Reader & Head of Microbiology Department, Goa University for providing the necessary support and facilities to carry out the research work. I am also thankful to Dr Sandeep Garg, Dr I. Furtado, and Dr S. Nazereth for their valuable suggestions and encouragement.

I would like to express my sincere thanks to al the library staff of Goa University for being supportive in the course of formulation and compilation of the thesis.

I am sincerely thankful to Asst. Prof. Poleshi A Department of Soil science University of Agricultural Science, Dharwad, for his valuable guidance during course of study and specially during the testing of samples.

I am also thankful to Dr.R.S Giraddi Entomology Department agricultural university for his valuable suggestions and guidance during the course of study.

I/ We acknowledge the funding received from Council for Advancement of Peoples Action and Rural Technology {CAPART} for implementation of Vermi-composting Project in South Goa, during which the experimentation works, was carried out.

I extend my special thanks to Rev. Fr. P.M.Rodrigues Director, Agnel Technical Educational Complex Verna, Goa, for giving me permission to undertake the study.

I also thank our Principal Shri Subhash Lotlikar for his encouragement and support given during the course of study and Ex Principal Shri Jaime Alfonso for his words of encouragement during the course of the study.

I extend my sincere thanks to all supporting staff of Microbiology, Chemistry, and zoology Departments for their timely help in carrying out experimental works.

I acknowledge my sincere thanks to the Manager Shri Nikhil Dessai, Sanjeevani Sugar Factory Dayananad Nagar Goa for giving his whole hearted support to undertake the field trials at the factory site.

I am also thankful to all the supporting staff for helping me in field trials and drawing the samples for study regularly during the field trials.

I am especially thankful to all the research scholars of microbiology Department for their help and encouragement during the course of study.

I am also thankful to all my colleagues of the Agnel Polytechnic for there support during the course of my study. I am especially thankful to the staff members of Civil

Engineering Department and community Polytechnic for their support and help during the course of study.

I am in a special way would like to thank shri Shrikant Yalagi, Shri R.C Dharwad and shri Kubasad R.B for the support and encouragement given to me during the course of my study. My special thanks to all my friends who have wished me all the time during the course of study.

I sincerely thank my father in law and mother in law and all relatives for their wishes and blessings to complete the research work.

My Pranamas to Father Shri Mallappa Munnoli Brothers and Sisters have supported me and encouraged me through out the student carrier and shri. Basavaraj Jigalur & family for their support and encouragement.

While I have been preoccupied with this project work, my wife Smt. Sunanda P. Munnoli has taken much of the responsibility of caring for Children Sneha and Basavaraj .

PRAKASH MUNNOLI

LIST OF ABBREVATIONS

G	Specific gravity
w	Water content.
e	Voids ratio
n	Porosity.
$\sqrt{}$	Bulk density
$\sqrt{}_d$	Dry density
$\sqrt{}_{sat}$	Saturated density
S_r	Degree of saturation
a_c	Air content.
n_a	Percentage air voids
cfu	Colony forming units
^0C	Degree centigrade
d/w	Distilled water.
Fig.	Figure
g	Gram(s)
mm	Millimeter
µ	Micron
nm	Nanometer
OD	Optical density
rpm	Revolutions per minute
CMC	Carboxy methyl cellulose
MT	Million tones
Y^{-1}	Per Year
R.T	Rom temperature

List of tables

List of figures

List of Plates

CONTENTS

Introduction and Scope of study

1.0 INTRODUCTION AND SCOPE OF STUDY

1.1 Introduction: The major causes of environmental degradation are liquid, gaseous and solid [Wastes] pollutants generated by human activities. Until date, both developed and developing countries have been focusing more on management of liquid and gaseous pollutants. However, the large quantities of wastes being brought in by the increase in population, industrialization, urbanization with modern packaging materials and agricultural practices have recently become a cause of concern for the Government, environmentalists, researchers and general public.

The biodegradable matters present in the wastes contribute profoundly towards the environmental degradation as it has resulted in:

Communicable diseases spread by unmanaged solid wastes. Human and animal excreta mixed with industrial wastes can spread diseases like typhoid, cholera, dysentery etc. hookworm infections, skin infections, and infective hepatitis etc. Biological wastes containing pathogens be disinfected, to safe guard the community against the outbreak of diseases.

Co-disposal of hospital wastes and Municipal wastes has created sites with infectious diseases. The dumpsites create obnoxious and unhygienic conditions.

Damage to the aesthetics of land and water as the wastes are openly dumped on the ground and allowing the natural environment to deteriorate with foul smell etc. in turn affecting the quality of life of the proximate residents. Mixing municipal and industrial solid wastes causes toxicity and handling of wastes is further, made a difficult task. Ground water pollution due to flow of leachates reaching the ground

water sources like wells, nallas, rivers etc., and further due to overload of nutrients in the water bodies, leads to eutrophication. Organic pollution of soil ecosystem dissolves the soil minerals leading to ground water pollution.

Place of habitat for pathogens / rodents, pests, insects like flies and mosquitoes breed in organic wastes, cause nuisance and act as a disease spreading agents. Proximate habitats get affected; in turn act as pollution transfer agents. Space goes wasted by piling up wastes. Treated solid sludge's, fly ash of thermal power plants, slag from metal industries occupy more and more scares space around the industry.

Therefore, there is an urgent need for the design of proper disposal methodologies to treat municipal and industrial solid wastes. Even though there is governmental efforts to enhance the existing waste disposal facilities with a view to cope up with the alarming increase in quantum of solid wastes, however due to dearth of appropriate disposal/management technologies, it is clear that the air- soil -ecosystem is not able to respond in its natural way, leading to greater environmental degradation.

The Government of India has set up the National waste management council [NWMC] vide circular no. 170(1)-870/PL. HMSD dated 25.1.1990 to render special advice to government on all matters concern with disposal and utilization of wastes NWMC emphasizes on the need for efforts to be taken up for conversion of solid wastes into usable materials.

1.2 Problem definition: India is a agro based country, hence there is an all time effort by the government to improve the agricultural practices to increase the crop production with the advent of major, minor and lift irrigation systems. The farmers have

accepted the challenge of growing commercial crops like sugar cane, paddy, wheat, cotton etc., This has necessitated the setting up of more and more agro based industries like sugar factories, food/vegetable processing industries.

As on today there are about 566 sugar industries in India generating a huge quantity of solid wastes, of the order 43.13 MTY^{-1}baggasse, 5.5MT/Y^{-1} press mud [NWMC,1992] which are organic in nature. These waste though rich in organic matter are presently being disposed on land creating environmental problems.

In this context the study of press mud (PM) [Filter mud] of the Sanjeevani Sugar Factory Dayanand Nagar Goa is included for carrying out lab scale as well as field experimentation in assessing the amenability of press mud to vermi processing, and to ascertain what is the role of microorganisms in the complete process of vermiculture system. A visual on present disposal method presented in Annexure I.

1.3 Rationale

With the advent of industrialization, treatment of industrial wastes has become an essential part of life in all the developed and developing countries. The unsystematic and hazardous way of management of these wastes however, leads to serious environmental degradation. As mentioned earlier the NWMC addresses the below mentioned issues.

i] Identification of wastes

[ii] To suggest alternative technologies for reduction/reuse/recycling and R&D,

[iii] To determine categories of areas where specific measures be needed

[iv] To formulate action plans concerning taxes, legislation and incentives.

The sugar industry belongs to the most important processing industries in India. According to the Indian Directorate of Economics & Statistics, India produces on average 270 million tonne's of sugar cane per year. During the production process, considerable amounts of by-products such as bagasse (b), press mud (p), and trash (t) are produced. Part of these byproducts are utilized for the production of molasses and alcohol; however, there remains a considerable amount of waste products to be disposed of.

The recovery of nutrients by modifications of these huge quantities of solid wastes is becoming increasingly important in order to result in its judicial management and make these industries more viable to comply with strict rules of pollution control.

The use of chemical fertilizer has no doubt revitalizes the crop production, which is also associated with the fallowing ill effects.

I. Soil Compaction and destruction of soil.
II. Low organic matter in soil leads to soil erosion.
III. Poor water holding capacity.
IV. Increase salinity.
V. Poor quality product
VI. Excess/residual fertilizes are pollutants.
VII. Induce residual toxicity.
VIII. Increased hazard and outbreak of pests & diseases and weeds

Further, with the ill effects of modern agriculture, increase in cost of chemical fertilizers and their deleterious environmental impacts, the production of bio fertilizers out of wastes generated from food/agro industries, agricultural residues and biomass considered as a simple and economically viable solution.

Agro industrial and food /vegetable wastes are recalcitrant to biodegradation due to presence of complex natured polymeric constituents such as lignocellulose's (Nand, 1995). The mechanism of recalcitrant or resistance to biodegradation is yet a new field for researchers (Madhu Arora, 1998).

The mechanical and chemical energy supply are the major concern in the industrial era. The biotechnological era is the present day focus. This is the time for us to rethink about our biological resources and processes. The soil -plant -air -water ecosystems needs to be focus of study with their interrelationships. There has been a rise in the studies of relationships between invertebrate, micro-organisms and plant/soil development (Edwards et al., 1988; Crossley *et al.*,1991) .Earthworms are the important component of soil ecosystem and there has been works reported by Edwards and lofty(1977), Lee and Foster,(1995) and Stephenson(1930).

The research work aims at demonstrating the use of the by-products of sugar cane processing as ideal substrates for breeding/rearing of earthworms and microorganisms. Preliminary studies have shown that earthworms are capable of converting the waste material into organic manure, which is rich in calcium, nitrogen, phosphorous, potassium, humus, and humic- acids.

Earthworms are the natural bioreactors, which proliferate, growth of microorganisms and provide required conditions for the continuous sustenance of microorganisms in turn leading to biodegradation of these wastes through microbial activity. Vermi-culture biotechnology, which involves use of earthworms for biodegradation is tried for the treatment of the press mud .The laboratory and field trials for the treatment of press mud of Sanjeevini Sakhar Kharkhana are selected.

Within the frame, this project the application of earthworms (vermi- cultures) for the production of vermi-compost by using surface feeder species *Eudrilus eugenae, Eisenia fetida* and the deep burrower species of earthworm *Megacolex megasecolex* shall be optimized and standardized.

Further, to know the water holding capacity, aeration, porosity, air content, bulk density, dry density, degree of saturation and other soil aggregation characteristics laboratory experiments be conducted on the vermicompost prepared form the press mud with deep burrower and surface feeder species. The relation between these geo technical parameters and their relation to microorganisms in terms of required necessary conditions for the growth of microorganisms will be evaluated.

The vermi composts of filter mud and soil be subjected to microbiological and physicochemical analysis during and after vermi-processing. The microorganisms will be isolated from the vermi composts and subjected to various enzyme activities. The filter mud are treated with consortia of cellulose degrading microorganisms. The soil microorganism's relation ship with respect to aggregation is studied with a focus to give solutions to soil pollution problems. Moreover, the effects of vermi-compost produced from press mud sugar cane residues on the growth of garden plants (e.g. *Diffen bachia* and *Aglonima)* be studied at laboratory scale.

1.4 Objectives and Scope of work:

The fallowing are the objectives of the study

- To analyze and select, suitable soil cow dung proportion for vermi-processing.
- To select efficient earthworm species for soil cow dung vermi composting.
- To determine the time required for the conversion of press mud to vermi compost.
- Microbiological and physicochemical analysis of press mud and soil during and after vermi processing
- Isolation, purification, identification and characterization of microorganisms associated with earthworm and vermi compost of press mud
- To demonstrate the role of microbes (bacteria) in biodegradation of filter mud with and without earthworms and soil aggregation.
- Design a process package for the treatment of press mud.

Scope of the study

- Laboratory tests to characterize the organic solid wastes
- Laboratory study to find the suitable proportion of cow dung and soil proportion for surface feeder and deep burrower species
- Laboratory biodegradation study to find bioconversion time of press mud, with selected species of earthworms.
- Site investigations and collection of soil samples to check suitability of bed soil for vermi-processing.
- Study properties of bed soil for its suitability.
- Feeding trials with selected species of earthworms, select the efficient species for the treatment of filter mud.

- Laboratory study for analysis of microbiological and physicochemical changes during and after vermi-processing.

- Isolation, purification, and identification of earthworm-skin-adheared and vermi compost microorganisms.
- Prepare consortiums of cellulose degrading organisms. The same is used to study the extent of biodegradation
- To study the role of microorganisms on bioconversion of press mud, [Filter mud] and aggregation of soil, and odor problems.
- Design of vermi-compost bins for the industries
- To study the impact of vermi-compost on salinity characteristics and plant growth and soil aggregation.

The scope of study is of inter disciplinary nature involving microbiology, ecology, environment, biology of earthworms, biotechnology and civil engineering etc.

Keeping these objectives the work was initiated with a preliminary literature survey on generation of wastes, technologies for the treatment, and with special reference to vermi-culture biotechnology. The literature information available on the role played by microorganisms and its enzyme activities etc., are collected, complied and presented in the next chapter.

CHAPTER I

REVIEW OF LITERATURE

1. Review of literature:

1.1 Introduction:

Recognizing the world is finite and that the continued pollution of our environment, if uncontrolled, will be difficult to rectify in the future, the subject of solid waste management is both timely and important. The overall objective of solid waste management is to minimize the adverse environmental effects caused by the indiscriminate disposal of solid wastes [Pevey *et al.*, 1985].

The ideal objectives of integrated solid management can be defined as the selection and application of suitable techniques, technologies and management programmes to achieve specific objective of waste management (Tchabanglous et al., 1993). Waste disposal using earthworms is one of the sustainable technologies to upgrade the value of original waste materials so that, the product be sold as useful materials, to provide the upgraded materials *in situ* and to obtain final product, free of chemical or biological pollutants (Bouch, 1979)

In the last twenty years, our mother earth has been forced to come under furious threat due to environmental pollution caused by population pressure, urbanization and industrialization, greater mechanized agricultural activities. The attention has been focussed on the solid wastes developed by the urban areas and industrial activities by the environmentalists, scientists, and public in general. Treatment of solid wastes has become an essential part of life in almost all over the world, as the environmental problems due to solid waste disposal are complex in nature, and disturbs the ecosystem.

Even though there are governmental efforts to tackle the problems of solid wastes, the alarming increase in solid waste quantity, and the mixing of biodegradable and non biodegradable wastes at the generation point, and making the complication, to handle these wastes with the limited available resources.

To assess the management possibilities and feasibility, for this waste {Resource], the literature pertaining to the total industrial solid waste generation and its management /treatment, the literature with respect to various available technologies along with detailed information with respect to vermi-culture biotechnology have been reviewed.

A special attempt has been made to collect the literature on microorganisms associated with earthworm casts.

Lignocelluloses are the most abundant organic compounds in biosphere, approximately accounting to 50% of the in the world. The annual production is estimated to be of the order of 10 to 50 x 10^9 ton's arises out of agricultural, forestry and fruit and vegetable wastes. Large quantities of biomass in the form of wastes accumulate every year causing environmental degradation and loss of potentially valuable resources. A significant contribution could be made to overall problem of biomass recycling and conservation.

Biomass in the form of cellulose, hemi cellulose and lignin provides a means of collecting and storing solar energy and hence represents an important energy and material resource (Khuhed and Singh., 1993). It has also been reported that the waste lignin derived from the pulp and paper industry is of the order of 30 to 50 x 10^6 tons/year.

The organic matter decomposition of lignin, the structure of which is three-carbon side chain unlike starche, cellulose, protein, and lignin is not formed by specific enzyme, rather, by the poly condensation of the phenolic precursors of this compound in a chemical reaction that includes free radicals. The structure involves many multi dimensional linkages as well as C-O-C (ether) linkages. These together with high aromaticity make the structure very difficult to decompose/biodegrade hence recalcitrance (Madhu Arora, 1998). Similarly, humus formed by poly condensation of free radicals with amino acids contain nitrogen, whereas lignin usually does not contain nitrogen ,is recalcitrant due to presence of different types of bonds, its aromaticity, three dimensional configuration which makes it to decompose slowly.

The total annual availability of saw dust in India has been estimated to be 2.1×10^6 t (Roy, 1989). Composting of woody materials as reported by Saha *et al.,* (2000) requires a lot of time if traditional composting is followed because of its high lignin and cellulose content. Two step composting technique as a noble solution for utilization of woody materials, and studied the decomposition of soft wood simul wood dust by selected lignolytic fungi.

There has been a significant increase in Municipal Solid Waste (MSW) generation in India in the last few decades. This is largely because of rapid growth and economic development in the country. The per capita of MSW generated daily in India ranges about 100g in small town to 500g in large towns and the industrial sector generates about 100 MTY^{-1} of non-hazardous solid waste. The growth of MSW in urban centers has out placed the population growth in recent years (CPCB, 2000). Food and fruit processing industries, hotels, pilgrim house, hostels, housing colonies, community

kitchens, vegetable markets, etc. generate large amounts of organic waste containing cooked food remains, raw fruit and vegetables, fruit peels, etc. Management and disposal of these wastes have becomes a problem for them and the concerned authorities. The TEAM (TERI enhanced acidification and methanation) process shows a useful way to turn that waste in to wealth (Rajeshwari, 1998).

The quantity of MSW generation was estimated to be 40 million tones per year in the year (Alone *et al.,* 2002). India generates annually 25 MT of saw dust ,325 MT of agricultural residues, 210-270 MT of cattle manure, 3.3 MT of poultry manure and proper utilization of thee wastes improves land ,water, air environment along with quality of human life (Ramaswam,1998,2002;Surekha, 2007). Growing urbanization and industrialization have led to generation of large quantities of wastes, which can be broadly classified as MSW (municipal solid waste) and ISW (industrial solid waste). In India, the daily per capita solid waste generation ranges from about 100 g in small towns to 500 g in large towns. It is estimated that the industrial sector generates about 100 million tonnes of non-hazardous solid waste in a year.

The SWM by vermi-composting has been suggested by many workers/scientists. In the recent years, earthworms have been used to convert sugarcane trash into manure. (Ramalingam and Thilagar 2000; Abbasi and Ramaswamy, 2001)

The composition of MSW in a locality in Goa has revealed that 65% to 75% of the total solid waste is mainly arising from vegetable wastes (garbage) which are of driver category [Dutta & Mahendra, 1997] and a major contribution is conveying from market vegetable wastes which are in consumable or not sold due to a variety of reasons.

The present consumption rate is standing at less than 25 gm per hand against the minimum consumption 280g.

According to Daliwal *et al.*,(1996) at least 50% of total solid biomass are dumped over the soil or water bodies uncovered, and become breeding ground for harmful organisms like flies, mosquitoes, worms etc. Also according to Patra, (1997) the major source of soil pollution is the unscientific disposal of solid waste on land.

Adding to this Seasonal population structure of earthworm was significantly influenced by seasonal pattern in soil salinity and other chemical and physical factors in semi arid tropical grassland soils (Reddy and Pasha, 1993). Earlier, Kale and Krishnamurthy (1978) reported that soil moisture, salinity and organic matter concentration were the principal factors controlling the distribution and bionomic of earthworm population around Bangalore. Barley (1961) reported that the underlying un productive soil undergoes biochemical changes and brought to the surface as a fertile soil.

Inoculation of earthworms with organic manures and heavy mulching of organic wastes can be used successfully for improving saline soils. Bhawalkar (1992) reported a case study of vegetable farmer who grew successfully, tondali- vegetable crop on saline soils with saline irrigation water by adopting *in situ* vermiculture biotechnology. They also reported good results in sugarcane grown on saline soil with saline ground water irrigation and *in situ* vermi-culture.

On the industrial front there is no reliable data available on the solid waste generated in various industries, (Nand 1995) has reported that about 18550 food processing industries with a total production capacity of 171.49 lakh tones resulting in a waste generation of

55.9 lakh tones, with emphasis that these wastes are recalcitrant to bio degradation Madhu (1998). By nature, the fruits and vegetables are susceptible to infection by bacteria, fungi and viruses. Microbial invasion of plant tissue can occur during various stages of fruit and vegetable development and hence, to the extent the tissues are invaded, the likelihood of spoilage increases, contributing to solid waste before reaching the industry Pelzar (1993). There is an added solid waste due to mechanical handling producing breaks in tissues, which facilitates invasion by microorganisms.

Also sugar industries have been reported to produce 43.13 MT per year of baggasse, 5.5 MT per year of press mud (NWMC 1992) and some agro industries are producing 28.3 MT per year of waste in the form of rice husk, willow dust, molasses, saw dust etc. (Singh, 1997). The conversion of distillery sludge into organic manure has been reported by Senappa *et al.,* 1995. Kalpana,(1978) reported the possibility of mixing sewage sludge and other industrial waste materials like paper pup sludge or lignin rich wastes and composting the mixture by earthworms.

Taken on annual basis the sugar industries are producing filter mud $9mty^{-1}$, Food and food processing solid wastes $4.5mty^{-1}$, Dairy wastes 60 MLD^{-1}, and agricultural residues $350mty^{-1}$. These estimated recyclable waste presently available for organic farming (Selvaraj *et al.,* 2005; Ramanathan, 2006) can be efficiently be used for treatment through microbial technologies

Bhattacharya (2007) reported the use of agro industrial by products oil cakes, paddy husk and barn, bagasse, filter mud, saw dust, fruit and vegetable wastes, cotton wastes from plantations crops such as tea, coffee, cocoa, spices, and grapes and malt wastes from

brewing industries. It has been estimated that the total available nutrient value of organic resources in India is 12.796 mt and trapped amount is 8.953 mt and present utilization is 3.75 mt approximately and agro industrial wastes for organic farming is also reported by Surekha (2007) and Kitturmath (2007).

The potential for using paper mill sludge enhanced with spent yeast, from brewery industry as a feedstock for soil dwelling earthworms was investigated using *L terristris,* with 66:1 proportion of wet paper mill sludge: dry east extract (Butt 1993).

The bioconversion of pulp mill sludge's and primary sewage sludge, mixed with nitrogenous materials, dairy sludge's by *E andrei* was reported by Elvira *et al.,* (1995, 1996, 1997, and 1998).

Treatment of paper mill solid waste by using *Eisenia fetida,* bioaccumulation of any metal was not observed in the body of the earthworms hence no harm in using earthworm from treatment units for fish bait. The metal content in vermi-compost was well within the permissible limits (Deolkar 2005).

There are reports on distillery waste using suitable substrate by earthworms; *L mauritii* used to treat sugar industry distillery effluent in a two tier filter unit consisting of gravel sand and soil by using decreasing organic carbon load of effluent Sundervadivel *et al.,* (1995). Delvi and Seenappa (2000) also studied the distillery effluent by sing earthworms and concluded that 4.3 Kg of earthworm biomass is required to convert 300 liters of distillery effluent which will produce 90 kg of vermi-compost.

Bhawalkar and Bhawalkar (1992) used different residues viz., sugarcane trash, press mud, sugar wastewater, boiler ash, bagasse and spent wash from sugarcane processing

activities. These were vermi stabilized into vermi-castings and applied to the field for triggering the soil biology. However, they did not mention the superiority of any residue in the biodegradation and biomass production process.

Jambhekar, (1992) investigated on recycling of sugar industry by-products like press mud and bagasse and mycelia and assessed the ability of earthworms *Eisenia fetida* , *Eudrilus eugeniae* and *Prionyx arboricola* to biodegrade the wastes . The results revealed that earthworms could be efficiently used for producing humus with the help of some industrial solid wastes and agricultural wastes.

Aquino *et al.*, (1994) studies the earthworm reproduction in manure and sugarcane bagasse. Two experiments were conducted to evaluate the reproductively of adult earthworms. The first experiment was conducted with manure and chopped bagasse at different ratios and second experiment was conducted in litter pot using manure and chopped bagasse at different ratios. The highest numbers of earthworms were found at 1: 1 and 3: 1 ratios, for chopped bagasse. No mortality was found for the ground bagasse treatment.

Hedge (1995) investigated the suitability of different crop residues along with sugar industry wastes like press mud and bagasse for earthworms *E. eugeniae* under laboratory condition. Cow dung and paddy straw were proved most suitable food materials for worm's multiplication, biomass and vermicompost production. Press mud and sunflower stalk were the next best wastes fallowed by vegetable waste, wheat straw, jowar stalk, maize stalk and bagasse. However, potato plant waste was found to be least suitable for earthworms.

Sugar industry by-products such as press mud and surplus bagasse were composted through vermi-composting technique into valuable organic rich vermi-compost. This was tested as carrier for bio-fertilizers. The maintenance of bacterial population was better in vermi-compost than lignite a widely used carrier for bacteria Muthukumarasamy *et al.*, (1997).

Giraddi (2002) suggested a combined integrated approach to sugar industry wastes including attached distillery effluents using, composting, vermi-composting, bio gas sprinkling of spent wash on windrows, proper leachate management system etc. Mala *et al.*, (1998) described a procedure for obtaining a valuable bio-compost, based on sugar industry by-products. In a clean pit of size 31 * 11 * 3 ft was placed a mixture of 6 t. press mud, 1. 5 t. coir pith and 1. 5 t. fly ash, 5 doses of 250 lit. Of vinasse were incorporated at intervals of 10 days, plus 3 Kg bio inoculums, local species of earthworm (*Lampito mauritii*, 500 / t) were introduced. Physical and chemical characteristics of the finished product were assessed.

Total microbial population and enzyme activity in the press mud and press mud vermicomposts *L. mauritii* and *E. eugeniae* (fresh, 15 and 30 days old) had been determined. The fresh press mud vermicasts of both worms showed relatively higher microbial population and activity than un ingested press mud (Parthasarathi and Ranganathan, 2000).

Giraddi and Tippannavar, (2000) reported the bioconversion of sugar industry by-products using earthworms. It was evident that press mud was completely degraded in 85 days while bagasse could not be degraded completely even after 90 days. Sugarcane trash, which was fully accepted by the earthworms, recorded maximum number of

earthworms and collected of vermi-compost. They suggested that for biodegradation of press mud and bagasse using earthworms, sugarcane trash is to be mixed in order to reduce the period of degradation and to harvest higher quantity of vermi-compost.

Use of synthetic fertilizers and other agro-chemicals has many limitations such as loss of top soil due to the indiscriminate use of fertilizers and pesticides has an ecological and economic implications, viz. Biomagnifications of chemicals in food chains and food web, environmental pollution, pest and disease outbreak and increased cost of fertilizers. Therefore, the need of the hour is to popularize organic manures, the production of which would be cost effective and environmental friendly.

Therefore the recovery of nutrients by modification of this huge quantum of organic solid wastes is becoming increasingly important in order to result in proper waste management system, and make the municipalities/industries more viable and comply with strict government regulations

The production of organic manure out of the waste generated from municipalities, industries, production, agriculture and animal residues and biomass available is being considered as simple and economically viable solution to compete with the increasing cost of chemical fertilizers and their deleterious impacts.

1.2 Process Recovery Options:

Processing and recovery are interrelated terms. Some of the processing techniques are used to improve the solid waste management system, while other are used to recover materials from solid waste or to convert solid waste to products of value. Depending upon the type of solid waste, source, quantity generated, availability of recoverable

materials in solid state, final processing and recovery technique should be tested, but before deciding the final system their merits and demerits should be properly evaluated (Trivedi and Raj, 1992)

The Andhra Pradesh State Pollution Control Board adopted the three broad approaches which emerged with cleaner production technologies and waste minimization techniques for industrial productions.

- Applying waste minimization technologies involving raw material substitution, process enhances, improved house keeping, and equipment redesign and product reformation.
- "End of the pipe" treatment options involving recovery, of raw materials, water, energy and marketable by-products.
- Waste utilization technologies involving reclamation and utilization of wastes as secondary raw materials in other industrial units Surya Prasad and Raghavendra Rao, (2007).

1.3 Categories of treatment processes for organic solid waste management:

The fallowing are the different categories of treatment processes for organic solid waste.

(i) Physical process (ii) Biochemical process (iii) Thermal Process

Organic solid waste depending upon their carbon content can be used to make fuel pellets through pelletization process. Based on the calorific value they can have a good substitute for wood and coal (Jain and Garg, 1997; Tchbanoglous *et al.*, 1993)

Indian sugar and General Engineering Corporation Yamuna Nagar has already developed agro waste plant/ briqueeting machinery (Anonymous, 1994). Solid waste

of food-processing industries at pilot or full-scale methane generation has not been extensively studied except for few reports.

Constant *et al.*, 1989 have reported that potato peeling in potato chips factory in Finland are utilized for Biogas generation in 10^6M^3 plug flow digesters and the gas produced is directly used in a boiler as a substitute for energy.

Food processing and other organic solid waste has not been extensively tried for biogas generation. Lane, (1979) has attempted to utilize fruit and vegetable processing waste, especially citrus, at laboratory and bench scales. It has been reported that the loading rate of four Percent of citrus processing solid waste could be used for methane generation and higher level of it would result in a failure of anaerobic digestion (Lane, 1984)

Fruit and vegetable waste are seasonal and are available only for a short period 2 to 3 months. During this period, varieties of wastes are generated in the processing industry. It is, therefore, necessary to operate the digester with the waste available in this season. Viswanath *et al.*, (1992) studied the effect of feeding different fruit and vegetable waste in a 601-biogas digester.

Jackshe, (1995) and Nand, (1995) reported that organic wastes of food processing industries can be treated by anaerobic waste digester process to derive energy from renewable resources.

The Biomethanisation of industrial effluents, of agro based industries, municipal solid wastes, sewage, and agro-residues accounted to 80% of global waste which are being treated to generate methane rich (60%) gas. Use of water hyacinth has been used to generate methane by many countries Nair (1995).

Organic wastes from food processing/municipalities can also be treated through a technology called celrich-technology which adopts microbial aerobic fermentation (Anonymous 1994).

Solid wastes rich in organic wastes could also be converted into organic fertilizer/manure through composting which is a biological process for conversion of solid organic wastes to humus like products (Jain *et al.,* 1997; Trivedi and Raj, 1997)

Zuccani and Betrodi (1986) have studied composting of various raw materials like waste water sludge's, industrial sludge's, industrial wastes and residues, food processing wastes and agricultural residues.

Composting of solid wastes effectively reduces the waste volume by about 40-60 percent depending on the content of compost-able materials contained in the wastes. (Gotass, 1962)

Bhoyar and Bhide (1983) have suggested C: N ratio takes an important controlling factor in composting. Lower C/N ratio have been reported to expedite the composting process.

It has been reported that large quantity of cattle dung is burnt in the field to prepare it for the next crop cultivation. (Sinha, 2000)

The total annual availability of sawdust in India has been estimated to be $2.1x10^6$ tonnes (Roy, 1989) 196.8x106t cereal straw. Total organic matter available is 1.3569×10^9 tonnes.

If only 10.20% of the organic is recycled, it will contribute significantly in solving food crisis enhancing soil productivity and in controlling soil pollution Nagaraj (1981).

The water hyacinth, weed has tremendous reproduction potential under ideal conditions it is a prolific multiplier. A single plant could produce the incredible number of 24800 daughter plants I just 90 days at a growth rate of 0.5 tonnes per day per hectare. Eradication of this weed will provide a new source of energy. (Nair1995)

Use of industrial effluents dairy and distillery was tried along with municipal solid wastes which has shown an increase in micro and macro nutrient with earthworm inoculums *Eudrilus eugeniae* Hemalatha, (2006).

Soil Pollution by metals is known to have detrimental effects on soil macro invertebrates. Most of the eco-toxicity tests or field studies have shown negative effects on metals on feeding activity, biomass .and reproduction of earthworms. [Nahamani, 2005; Nahamani, 2002; Karmaz, 2000; Khalil *et al.,* 1996]

The effect of pesticides, when used in used in soil,ultimately interfere with soil microbes and microbiological processes like soil respiration, nitrification, phosphate solubilisation etc., and inhibit the normal activities(Kalam *et al.,* 2004).

Earthworms have the capability of scavenging several heavy metals/pesticides and accumulate them. Therefore it is advised to avoid sites of heavy metal concentration affected with excess chemical fertilizers/pesticides (Singh and Sinha, 2001)

1.4 Technologies for Organic Solid waste treatment / Management:

The biotechnological exploitation of microbes is becoming more and more important both for industrial purposes and in the treatment and utilization of solid organic waste materials. Agricultural and industrial organic residues contain substantial amounts of cellulose and have the potential of serving as growth substrates for either ruminants or microorganisms Chang (2005).

1.4.1 Development of biodigestible environment friendly seed pots:

Bio-digestable environment friendly seed pots and blocks are made out of coconut pith, an agro waste duly processed, and waste paper for extensive use in agro horticulture field. The co-co pith/waste paper pulp mix is vaccume formed into different product needs. Pulpers, molding machines, moulds, drier and vacuums /pricumatics are needed besides other facilities. Mechanical Assembly Systems (India) Pvt. Ltd., Charthala, Alleppey Dist. Kerala State, India (Anonymous, 1994), has developed this.

1.4.2 Celrich Technology:

Converting organic waste into soil enrichers, from fiotech Constorium India, New Delhi adopts microbial aerobic fermentation. Waste from municipalities/food processing industries is heaped in rows and inoculated with a slurry containing microbes. The temperature of heap gradually rises to 60 to 80c within a week. The heap is turned mechanically alter 11 days. After three cycles of 11 days each (i.e. one week). The volume of heap is reduced to one third. The heap is then silted and graded into bio-organic soil enrichers.

Advantages to this technology are: Converts wasteland fills material into useful bio fertilizers; cost alternative and economical process which is not labor intensive (a 100 TPD plant costs approx 1 crore effectively masks obnoxious odors in five hours.

Areas in application of this technology are heterogeneous municipality garbage; food/vegetable/fruit Industrial waste (Anonymous, 1994).

1.4.3 Agro – waste Briquette Plant/Machinery:

Agro waste materials have proved to be good non conventional renewable sources of energy. Indian Sugar and General Engineering Corporation, Yamane Nagar, India has developed a agro-waste briquette plant/machinery for processing agro-waste material. It is a binder less process. The briquetteing achieved by the application of pressure and process heap. As a non-conventional renewable source of energy, it converts agro waste material into desired fuel briquettes for easy transportation and storage. It can be applied in small and medium capacity gate type industrial boilers and rural household. (Anonymous, 1994).

1.4.4 Microbial composting technology:

Composting is the process of converting organic residues of plant and animal origin into manure, rich in humus and plant nutrients. It is largely a microbiological process based upon the activities of a host of bacteria, actinomycetes, and fungi. All kind of organic residues amenable to the enzymatic activities of the microorganism can be converted into compost by providing optimum conditions for biodegradation. Unless strictly controlled, composting employs the activities of both aerobic and anaerobic microorganisms.

Effective harnessing of the great biochemical activities of microorganisms for bioconversion processes has become more imperative today as modern societies are generating huge quantities of wastes. Unless these waste are prudently managed and recycled, not only will the dwindling resources become further scare, but environmental quality will also deteriorate to an intolerable level. In this background, conversion of organic wastes into value-added products through microbial technologies appears to be an extremely useful approach.

Recycling of organic residues through composting is an age-old practice. Yet, tremendous renewed interest has been shown in this process of late. In developed countries, practicable technologies have now been worked out for the composting of troublesome wastes such as sewage sludge. Rapid composting processes based upon specific engineering designs have been proposed. Even patented composting methods and apparatuses have been designed. In developing countries, investigators have been engaged in improving conventional technologies of rural composting by certain mineral and microbial inputs. Widespread interest is been shown in evaluating the role of earthworms in composting. Furthermore, some commercially available microbial starters have to be found to be useful in the composting of industrial wastes. In view of these noticeable developments in composting Methodologies, it is pertinent to make an appraisal of existing status of the technology of composting with particular emphasis on the microbiological aspects of the process.

1.5 Methods of composting:

Any system or design that ensures efficient decomposition of organic matter can constitute a composting method. However, some methods, worked out long ago, have been in vogue over the past several years with a little or no modification. These methods are based upon simple manipulations of the raw materials and conditions of composting, and are being followed in most practical situations. Conventionally two methods of composting are known in India. A brief description of these methods is given below.

1.5.1 Indore Method:

This method is worked out by Sir Albert Howard, a British agronomist in India, at Indore in Madhya Pradesh during 1924-30. It is a systematization of the traditional composting practices being followed in China and India for centuries. The Indore method is an aerobic process and hence precludes adequate supply of oxygen during the decomposition process. Waste organic materials such as straw, garbage, leaves, and plant clippings are laid in a heap or pit in alternate layers with animal manure and soil. Accordingly, a 15cm thick and 1.5-12.0m wide layer of organic materials to be composed is placed on a hard upland place. This is followed by a 4-5cm layer of animal manure (dung), which is in turn covered by a sprinkling of surface soil mixed with a small quantity of lime or wood ashes, about 30mm in all. The layers are repeated until the heap reaches a height of 1.5-2.0 m. The final layer should be of the compostable material covered by a thick layer of soil, about 60mm. To provide optimum moisture (60-70%), water is sprinkled over each layer. The same procedure is followed if composting is carried out in pits of about 1 m deep. 1.5-2.0m wide, and of suitable length. The heap is turned thoroughly at intervals of 3, 6, and 12 weeks the finished compost is ready in about three months if the materials are properly shredded and layered. Generally it takes about 4 months for the compost to be ready (Refer figure 1.1). The Indore method has the disadvantage that it demands considerable labor in construction of the heap, turning of material, and maintenance of adequate moisture. Loss of nitrogen as ammonia gas also takes place, which can be considerably reduced. Economy in use of water is also possible if composting is carried out in pits instead of heaps. (Abbasi and Ramaswamy, 2001)

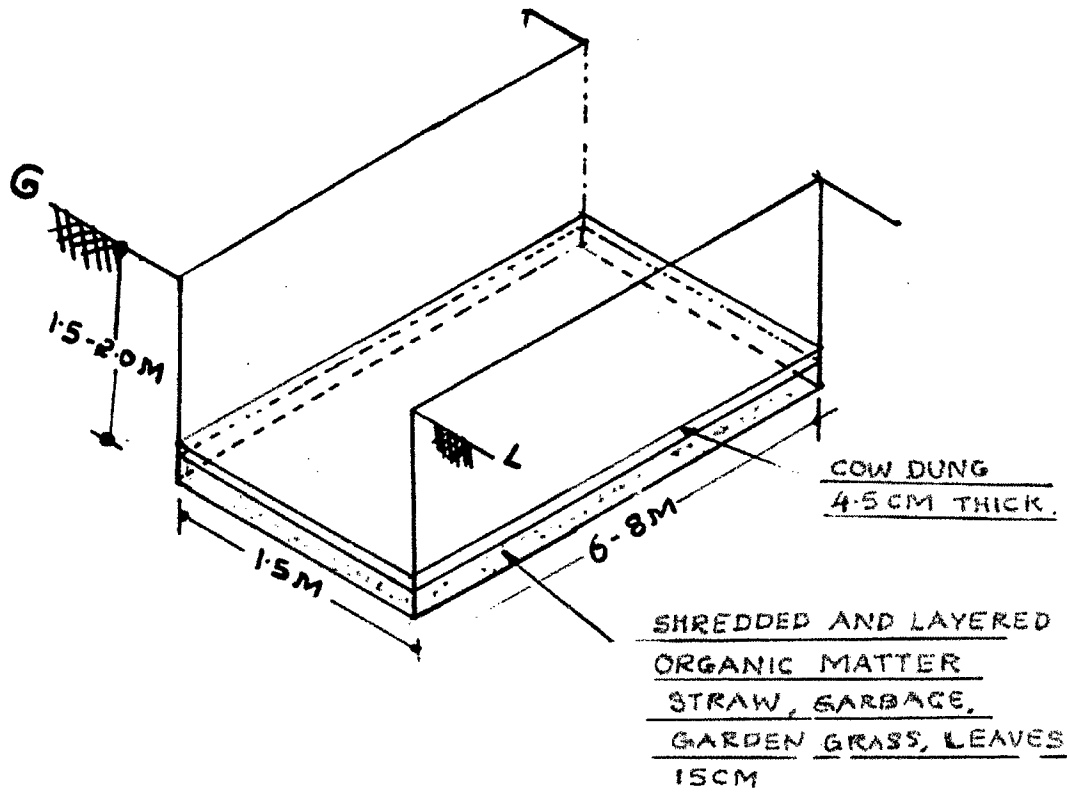


Fig. 1.1 Indore method composting

1.5.2 Bangalore Method [Hot fermentation Method]:

This method was firstly worked out, firstly to overcome some of the disadvantages of the Indoor method, and secondly to process night soil and city refuse. In fact before the method was developed, the disposal of night soil collected from dry latrines in Indian towns was a real problem. Although it was known as an aerobic method, in reality, it is aerobic to start with followed by aerobic decomposition later. This method is also known as hot fermentation method, as heat loss during decomposition is considerably reduced. Though initially worked out for towns, it can be used for compost – making from conveniently available organic materials. Under rural conditions, animal dung can be used to substitute night soil, for night sail is generally not collected in rural areas.

To carry on composting by the Bangalore method, trenches of about 1m depth, 1.5-2.5m width, and of any length are made at an appropriate place, generally on the outskirts of the city. If material is limited, one can go in for pits of 1m depth, 1.5 m width, and 3-4 m length. The compost-able refuse \is dumped into the trench or pit and spread out with rakes or forked shovels to make a layer of about 1.5cm thick. Night soil or dung is then placed over the refuse in a layer of about 5 cm the process is repeated until the trench or pit is filled up to about 30cm above the ground level and a final layer of compost able material is placed on the top. At each layering, water is sprinkled over the material to make it optimally moist. The above ground material is made into a dome shape and covered with about 2.5 cm mud-plaster (Fig.1.2).

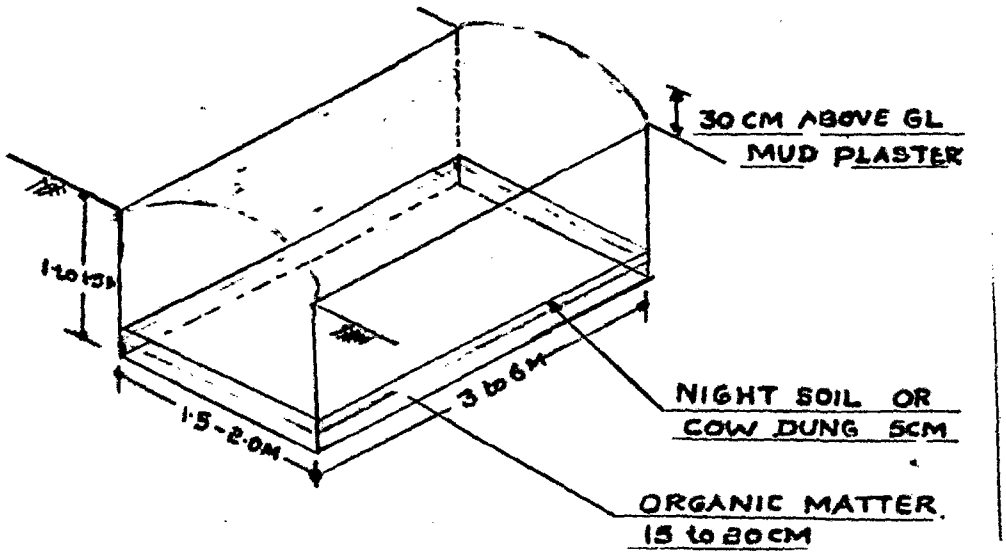


Fig. 1.2 Bangalore method composting

If all operations are properly carried out, the compost is ready in about five to six months, a period of about one-and-a half times longer than that for aerobic composting by the Indore method (Abbasi and Ramaswamy, 2001).

1.6 Anaerobic-Waste Digestion

Fully enclosed systems are used for digestion, odor emission are minimal. Digestion as an element of biological waste management has grown along with the concept of energy made from renewable resources. Digestion works self sufficiently and unlike composting is independent of the structure of the material. This is especially advantageous in seasons (winter, spring) where mainly wet organic material are collected liquid manure or other industrial (eg. Food processing industry) biodegradable inputs can also be treated (Jaksche, 1995).

1.7 Vermiculture Bio-technology

Vermi means earthworms and culture means rearing or forming of earthworms essentially, every specialized farming (fish farming, poultry farming etc.) involves the following steps, procuring seed stocks/ culture of paper variety and providing optimum conditions of food, moisture, air and temperature. Accordingly vermiculture has started gaining attention as a specialized technology called vermiculture biotechnology.

Vermicomposting involves biooxidation and stabilization of organic material through the interactions between earthworms and microorganisms. Although microorganisms are mainly responsible for the biochemical degradation of organic matter, earthworms play

an important role in the process by fragmenting and conditioning the substrate, increasing surface area for growth of microorganisms, and altering its biological activity (Domínguez, 2004; Domínguez and Edwards, 2004).

Earthworms are well known as soil inhabiting animals, having cylindrical body and well masked external and internal metaeneric segmentation. They do not have any apandages or suckers but have a few hook like chaetae for gaining hold on the substratum. Hence they are called Oligochaeta oligo, few chetae – hair a group of phylum annelida to which they belong. Earthworms are hermophorodifies and sexually matured. Worms have a distinctive epidermal ring shapes called clitellum, which has gland cells that secrete materials to form the cocoon. Earthworms are major components of the soil fauna in a Wide variety of soils and climates and are involved directly or indirectly in organic matter decomposition and stabilization, nutrient turnover, and modification of soil physical properties (Edwards and Bohlen, 1996; Lavelle and Spain, 2001).

The production of bio-fertilizers through vermi-culture has a bright future in India and the world. However, it is very essential to select suitable species of earthworms capable of consuming rich organic matter, stress resistant, efficient decomposers, sustaining adverse environmental conditions, and having high fecundity rates Mohammad, (1993); Edwards and Lofty, (1977).

More than 4200 species of oligichaetes are known in the world, of these, 280 are microdrili and about 3200 belong to Megadrili (earthworms). In the Indian subcontinent earthworms also form the bulk of the oligichaeta fauna. They are represented by 509 species and 67 genera, indicating a high degree of diversity in this region as compared to

other areas. Though majority of the forms have specific habitat preference, a few ubiquitous species also occur. It is important to critically assess existing diversity in Indian earthworms and their ecological requirements for evolving suitable vermicomposting techniques. As per Bouch, (1977) earthworms are classified as Epigies: Litter or dung dwellers characterized as high rate of cocoon production and short life cycle. Endoges: Dwellers of top organo-mineral soil, tolerant to disturbances moderate to high rate of cocoon production, intermediate life cycle, and small to large body size with weakly pigmented. Aneciques: deep burrower ,construct burrows, lay cast at surface, move in night in search of organic material, intolerant to disturbance, low rate of cocoon production and long life cycle, large body size; pigmented at anterior and posterior ends. Commonly adopted worms in vermiculture are *Bimastos parvus, Dendrobeana rubida, Eisenia fetida, Eisenia hortensis* belonging to the family Lumbricidae.

Eudrilus eugeniae family Eudrilidae, *Amnthus diffringes, Lampiito mauritii, Metaphire anomala, Metaphire brimania, perinyx excavatus, Perionyx sansibaricus, Megascolex megasclex, notoscolex* etc., belonging to family *Megasclecidae.* Many mentioned above and other species available in the sub continent are yet to be explored for their potential use in vermi-culture Julka (1993).

The other species reported are *Drawida willis, Dichogaster boloui, Lumbricus rebellus* by (Dash and Senapati, 1985; Singh, 1997). The deep borrower *Pheritima elongate* has been used for industrial wastes as reported by Singh (1997).

Gautam Bhattacharjee (2002) reported seven species of earthworms, viz.*Perionyx excavatus* Perrier,*Lampito mauritii* Kinberg,*Polypheretima elongata* (Perrier),

Pontoscolex corethrurus (Muller), *Eutyphoeus gammiei* (Beddard),*Dichogaster modiglianii* (Rosa) and *Drawida nepalensis* Michaelsen are presented. The peregrine earthworms such as *Perionyx excavatus, Pontoscolex corethrurus, Dichogaster modiglianii,* and *Polypheretima elongata* are considered to e continuous breeders with high fecundity. Native *Lampito mauritii* and *Drawida nepalensis* are semi-continuous and *Eutyphoeus gammiei* discrete breeders. High rate of cocoon production, short development time with high hatching success, as well as continuous breeding strategies in the epigeic species *Perionyx excavatus* and *Dichogaster modiglianii* and the top soil endogeic species, *Pontoscolex corethrurus, Drawida nepalensis* and*Lampito mauritii,* indicate their possible usefulness in vermiculture. The giant anecic worm, Eutyphoeus *gammiei,* which has a very long cocoon development time, discrete breeding strategy and very low rate of cocoon production, is not a suitable species for vermiculture.

Earthworms vary greatly in size though not in shape. In India, some peregrine species like *microscolix phosphorus* (Drugs), *Dichogaster Saliens* (Beddard) and *Bimastos parvus (Eisen)* are even less than 20mm long, while some endemic geophagus forms, such as *Drawida nilamburensis (Bourne)* and *Drawide grondis (*Bourne) may reach upto one meter in length. *Mgascolides acestralis* (mclor) from Australia is reported to attain a length of over 4m and the worm *Microchaetus microchaetus* (Rapp) found to attain a length of seven meters (Mohammad, 1993).

The information available on consumption rates of few of the earthworms in India and world as studied by a number of workers (Table 1.1) (Van-Rhee, 1967; Satchell, 1983; Marinissen and Bouch, 1992; Miyo and Grinval, (1997).

It is of importance to note that the utilization of all the types of species is not reliable until and unless the suitability with specific substrates is studied. The species good for one region may not necessarily be good in any other regions especially the deep burrowers which require more time to acclimatize to new environment. As such deep burrowers undergo diapose when subjected to shocks of vibrations, therefore it is advisable to transport the vermi-compost containing earthworms and then transfer with vermin- compost itself in the new environment. In case of surface feeders, the biomass of earthworms or even cocoons can be easily transported, placed in new environment.

Table 1.1: Consumption rate of earthworms

Earthworm species	Consumption rate mgg-1	Food substrate	Reference
Allolobophora caliginosa	80	Dung	Bareley,(1961)
Allolobophora caliginosa	200-300	Soil	Bareley,(1961)
Allolobophora longa	20	Soil	Satchel,(1967)
Eisenia fetida	10-5000	Activated sludge	Mitchell,(1978)
Eisenia fetida	200-20000	Activated sludge	Hartenstein *et al.,(*1979)
Eudrilus eugeniae	2000-5000	Activateed sludge with deasd leaves	Jaashankar,(1994)
Eudrilus eugenae	3000-7000	Activated sludge mixed along with sludge	Balaji,(1994)
Lampito mauritii	700-2800	Soil	Dash *et al.,(*1980)
Lmbricus rubellus	27	Litter	Fronz and Leitenberger,(1948)
Lumbricus terristris	27-80	Elm leaves	Neetham1957)
Lumbricus terristris	10-30	Soil	Satchell,(1967)
Millsonia anomala(adult)	4000-7000	Soil	Level *et al.,(*1980)
Millsonia anomala (young)	6000-18000	Soil	Level et al.(1980)
Octolasium sp	29	Soil	Cross *et al.,*(1971)

Source: Thomas Sonia, (2002)

The management of organic solid wastes through composting is time consuming. The vermiculture biotechnology utilizes earthworms as the versatile natural bio-reactors to convert organic waste into value added products the Vermi-casts (EPK, 1980) at a faster rate and can be applied to industries producing organic wastes (Bhawalkar, 1995; Singh 1997 Surekha and Mahadev Kumar,2007: Bhattacharya,(2007). The vermi-culture biotechnology in its true form is the synergistic effect of microorganisms and earthworm which falls under the distinction of biotechnology given by Spinks, 1980, [Roig, 1993] as application of organisms, systems or biological processes in the manufacturing, industrial and services.

Vermiculture, which began in the United States in 1930's, developed in Italy only from the second half of 1970's. The treatment of organic wastes was realized in late 1970's in USA. In India the importance in organic waste management has been

realized only in the last 15-20 years.(Piccone *et al.*, 1986, Raganathan, 1996). Biotechnology which has entered the domestic and industrial marketing in the countries of Canada, the USA, Italy, Japan, (Senapati, 1985).

In the year 1995, the community rural vermi-composting have been initiated by Punjab State Council for Science and Technology and in Goa by Agnel Charities, a NGO working in the rural development has undertaken vermicomposting in rural areas of South Goa in the year 2003.

Methods of setting vermicompost units.

i) Circular Vermi-composting Unit.

ii) Rectangular Vermi-composting Unit.

iii) Strip Method.

The first two methods require a complete roof so that no direct sunlight enters the vermi beds, covered with jute bags to enhance reaction and maintain moisture.

The advantages of organic farming are many to increases the soil fertility level, water holding capacity, and aeration.This technology eliminates pathogens, does not produce odor and also heavy machinery not required. The general health of soil increases. No chemical sprays are required and the vermi-compost itself is insect resistant.

The various designs adopted based on the topography, type of soil and amount of rainfall for vermiculture are given below.

1.7.1 Pit method:

Features

- Below ground level
- Depends on type of soil

Advantages

- Low cost
- Pits of different dimensions can be chosen as per the individual requirements

Disadvantages

- Open at ground level makes entry of predators easy like rats, benit cakes, ants centipedes
- Difficult to take any measures to tackle predator
- Direct entry of ground moisture
- Worms can move out if they get favorable moisture
- Not suitable for periodic harvesting

Sustainability

- No Sustainability as the pit may close by itself by ground water/ soil.
- In turn disturbs worms/micro flora

Suitability/ Remarks

- Suitable only where moderate rains, with many pits under common roof
- Not advisable for Goa, Sandy silt soils and rain intensity of 3000 mm per year.

1.7.2 10 cm thick rectangular tank with holes left:

Features

- Above ground level.
- Brick measure has to be carried with skilled labor.

Advantages

- It will be low cost in other regions where cost of transport is not there
- Under a common roof many can be built

Disadvantages

- Holes serves an entry predators
- Also the worms move out of the system
- Constraint is also not effective
- It is not a stable structure
- Wet materials exert horizontal thrust on walls
- Short life

Sustainability

- 10 cm thick wall building or open spaces does not give any stability

- Only can be adopted for internal vermi-composting with full roofing material cover. Against heavy coastal rains efflorescence is the main problem, wear out, collapse
- Bricks will absorb moisture from compost and become weak

Suitability/ Remarks

- Not suitable for Goa, in open field.
- Very good for research work with a common glass and Asbestos sheet roofing with sides protected from predators
- Not practicable for villages

1.7.3 10 cm thick complete wall without plaster:

Features

- above ground level
- skilled labor is required

Advantages

- low cost if bricks are locally available
- under common roof many can be built

Disadvantages

- 10 cm thick is not a stable structure against horizontal liquid pressure of wet materials.

Sustainability

- 10 cm thick wall building on open spaces does not give any stability
- If protection from rain with roof cover
- Even 20 cm thick is not suitable if absorbs moisture.

Suitable/Remarks

- Not suitable for Goa for outside composting

1.7.4 20 cm thick laterite masonry:

Features

- Above ground level.
- Masonry has to be carried out with skilled labor.

Advantages

- As compared to wire cut bricks this is low cost in Goa.
- Suitable for individuals.
- Safe against predators.
- Long life.
- Permanent in nature
- Can be continuous economic resource for farmers
- Good for the research on agricultural residues.
- Very good for periodical harvesting.
- Withstands liquid pressure
- Temporary roof with locally available materials is suitable.

Disadvantages

- Cost is on higher side but cannot be compared against various advantages it is offering and break even can be achieved within six months.

Sustainability

- Structure is completely sustainable
- Very good for both periodical as well as one time harvesting

- Even watering of beds is missed by farmers. Vermi-bed can sustain by itself more days as compared any other types.
- Sustainability through retraining is possible.

Suitability/ Remarks

- Suitable for any region with heavy rains
- Suitable for Silty-Sands and loamy soils.

1.7.5 Heap method:

Features

- It should be completely indoors for protection purpose.

Advantages

- Depending on the space inside many heap rows are possible
- Very good for large scale production under green glass house.

Disadvantages

- Rooms should be reserved for this sole purpose only.
- Dispersal of earthworm.
- As it is inside Red ant will be a problem.

Sustainability

- It is sustainable for rich farmers, but not for marginal/land less farmers

Suitable/Remarks

- Suitable for field farm houses/sheds.

1.7.6 Indoor vermi-composting:

The whole system completely protected with a thatch or bamboo roof or carried out inside the existing cowsheds.

A full proof in vessel composting made up of high-density polythene material of capacity 50 liters. A small aerator arranged through a central pipette to give the airflow for the biodegradation of the wastes. The exhaust gas carbon dioxide comes out of perforations provided at both the ends of the drum Haug *et al.,* (1993).

1.7.7 Industrial vermi-composting:

Depending on the quantity, type, rainfall, soil type any one of the suitable method with modification to suite the local requirements has to be adopted.

Experiments with worms have successfully been conducted for recycling the utilizable organic wastes arising out of household garbage, city refuse, and sewage industries (Mitchell and Horner, 1980)

Sharma, (2002) has reported that MSW and MSW with cow dung were treated with *Eudrilus Euginiae* showed a degradation time of 40 days, with substrate preference of MSW plus cow dung and MSW.

A similar experiment was carried out with *plerotus* harvested spent straw and spent straw with cow dung alone with, *Eudrilus eugniae.* The waste material with cow dung showed preference as compared without cow-dung and showed a degradation time of 40 days with both *Eudrilus eugniae* species.

Earthworms vary greatly in size though not in shape. In India some peregrine species like *Microscolix phosphorus* (Drugs), *Dichogaster saliens* (Beddard) and *Bimastos parvus (Eisen)* are even less than 20mm long, while some endemic geophagus forms, such as *Drawida nilamburensis (Bourne)* and *Drawide grondis (*Bourne) may reach upto one meter in length. *Mgascolides acestralis* (mclor) from Australia is reported to attain a

length of over 4m. The world's largest known worm *Microchaetus microchaetus* (Rapp) which is found in South Africa has a length of seven meters (Mohammad, 1993).

It has been a well-established and reported fact that earthworms are used as living plough and regarded as safe cheap and tireless farmhands (Darwin, 1881).

Earthworms upturn the soil and provide much needed air to microorganisms and roots. Through their constant borrowing, mixing and digesting, earthworms significantly improve the composition of soil.

Guild (1948) found that light and medium loams had higher populations of earthworms than did clays or gravelly sand, but that these extreme soils had different species compositions. In the U.S.A., Hopp, (1973) speculated that sandy soils contained fewer earthworms than clay soils. El-Duweini and Ghabbour, (1965) surveyed a wide area in Egypt and concluded that populations decreased with increasing sand content, this they related to soil moisture rather than soil texture. Vernon *et al.,* (1981) observed higher populations in wet loam and wet clay and lowest in dry loam.

It is observed that only after three days of earthworm's action, test soil beds had over 50% larger aggregates, clumps of silt, clay and sand, particles, which form in earthworms gut, these aggregates, are an essential component of productive soil which remains aerated and resists erosion.

Their body exudates improve the water holding capacity of soil and promote the establishment of micro-organisms. (Kale, 1994 Trivedy, 2002)

Due to earthworm activity extensive burrows are termed, which resulted in loose and porous soil, these macro pores improves the water absorption, drainage, aeration for roots and propagation. (Nobel *et al.,* 1970Earthworm burrows up t a depth of 1 m was reported for the species *A Calignosa,*in unpolluted zones of soil Nahamani (2005). ; Bahl, 1950; Baig, 2003; KOzolovskaya, 1961).Bareley (1961) showed that earthworms trans locates the sub soil to top layers. This unproductive subsoil undergoes chronicle changes during its passage alimentary canal of earthworm and it is immediately available for crop growth Kretzschmar (1991).

1.8 Species, borrowing, nutrients of vermicasts:

There are about 509 species of earthworm fauna placed in 67 genera and 10 families were reported in Indian subcontinent (Julka, 1993).

The species *Perionyx excavatus* has been used for the treatment of tannery waste. It is available in India and Tamil Nadu commonly and adaptable to organic wastes like sugar cane trash coir waste, paper pulp, faecal matters, cow, sheep, horse activated sludge, and Biogas sludge of poultry droppings. (Govindan, 1998; Shahul Hameed, 2002; Ramalingam, 2000)

Ranganath Reddy (2002) has reported the consortium of earthworms namely *Eudrilus eugeniae, Perionyx excavatus and Perionyx sonsiboricus* solid urban waste after preliminary decomposition for 20-30 days involving macerating or mixing

The earthworm species are *Perionyx excavatus, Lamito maurtie, Octocraetana servuta, Eisenia fetida, Eudrilus eugeniae* as reported by Senthil Kumar, (2000); Bhatacharya, (2007) has reported the species of earthworms *Eisenia fetida, Eudrilus eugeniae,*

Perionyx excavatus, Denadrobaena veneta for industrial solid waste treatment.

The species identified for economic multiplication of earthworms and vermi-composting are i) *Esenia fetida* – Europeon worm, ii) *Eudrilus eugeniae* - African worm, iii) *Perionyx excavatus* - indigenous are prolific breeder, high multiplication rate, voracious feeders, easy to handle, having one to three years longevity, survive under aberrant weather conditions possibility of household waste treatment Kale and Sunita, (1992).

Ghatenkar (1999) has suggested the following species of earthworms *Lumbricus rebellos, Eudrilus eugeniae, Eisenia fetida, Pheritima posthume.*

Trivedy (2002) has reported 3000 species in the world. About 385 species have been recorded in India out of the total 4500 species. 280 are called Microdrilli (small in size). Reported species are, *Eudrilus Eugeniae, Eisenia fetida, Pheritima posthume, Lumbricus rebellus, Drawid Willsi, lampito Mauritii, Octochaetara Serrato* Species in Indian climate are lampito *Mauritii, Dichogaster boloai, Dravida Willsi, and Perionyx excavatus* (Dash and Senapati, 1985, Singh,1997). where as Subba Rao(1979) reports 3000 species of earthworms in the world and about 500 in India alone. He also reported the species *L rubellus* and *Eisenia fetida* are thermo tolerant.

Roy (1990) has reported a total of seven thousand identified species requiring a PH range of 5.0-8.4 for their best survival.He also reported borrowing species (up to a depth of 6 meters,20 feet) of *Lumbricus terrstris, Aporrectodea caliginosa* which have been successfully tried for the treatment of board mill sludge

Narayan (1999) in his studies concluded that the exotic species *Eudrilus euginae* has shown better results as compared to local worms and control. As given in Table1.2.

Table 1.2 Biodegradation of kitchen waste using *Eudrilus eugeniae.*

	Exotic	Local	Control
pH	7.4	6.8	6.4
Minerals	0.3	0.14	0.11
Organic Carbon	10	0.25	0.10
N	5	3	1
P	32	32	14
k	130	170	100

In case of tannery waste which consists of salt, hair, flesh, myrob dust, buffing dust and trimmed waste, the studies conducted by Shahul Hameed (2002) in Tamilnadu reported the nutrient values of vermicompost using *Peronyx excavatus* (Table 1.3)

Table 1.3 Chemical characteristics of vermi-compost of tannery waste

Parameter	Vermi-compost (Tannery waste)
pH	7.4
Ec (mho cm-1)	0.79
Organic carbon (%)	17.6
Nitrogen (%)	1.42
Phosphorus (%)	1.28
Potassium (%)	0.33
Iron(pap)	18.2
Manganese(pap)	16.5
Zinc(pap)	10.7
Copper(pap)	4.9
C/N ratio	12:1

Jeevan Rao and Rama Lakshmi (2002) have reported nutrient values for a good vermicompost based on their study on urban wastes Table 1.4.

Table 1.4 A good quality vermicompost will have the fallowing composition.

Parameter	Vermi-compost
pH	7-8.5
Organic carbon (%)	20-30
Nitrogen (%)	1.5-2.0
Phosphorus (%)	1-2
Potassium (%)	1-2
Calcium (%)	1-3
Manganese(pap)	1-2
Sculpture (%)	<1
Moisture (%)	15-20
C/N ratio	15-20:1
Micronutrients(pap)	200

It is estimated that earthworms feed 4.5 times of their own body weight daily. In other words, earthworm decomposes approximately 4.5 kg of organic waste in 24 hours. (Venkateswarah, 1995)

Due to earthworms activity extensive borrows are formed, which result in loose and porous soil. These macro pores improve the water absorption, drainage, aeration for roots and root propagation (Nobel *et al.*,1970).

Experiments have shown that degraded soils with earthworms alone, or in combination with plants, can develop decreased bulk density and enlarged porosity and structural stability (McColl *et al.*, 1982; Aina, 1984; Shaw & Pawluk, 1986; Stewart *et al.* 1988; Springett *et al.*, 1992; Zhang & Schrader, 1993).

Abbasi and Ramaswamy (2001) have reported the brrowing activity of *L.terristris* and *Alolobophora nocturna* up to a depth of 150-240 cms showed that the earthworms translate the subsoil to the top layers. The unproductive subsoil undergoes chemical changes during its passage through alimentary canal of earthworm and it is immediately available for crop growth.

According to Edwards & Tomson (1973) earthworms can act as bio concentrates for heavy metals and toxic materials. These toxic materials are stored in the tissues of earthworms, which help in detoxification of polluted soil.

The resinous substances excreted by earthworms together with humus produced, helps in increasing water-retaining capacity of soils. Besides bulk density of soil was reduced to the extent of 30% and which shows vast internal spaces for accommodation of air and moisture and enormous surface upon which hydrolytic and oxidative catalyses can be affected by soil micro-organisms, enzymes and humid substances (Kolher, 1995).

A decrease in bulk density from 1.60 to 1.34 , Ec from 0.32 to 0.22 when soil is treated with 100% NPK + 20 THa-1+ 10 Kg Ha-1 is being reported by Neeraj Kumar *et al.*, (2007).

Ranganath Reddy, 2002, has reported the increase in N, P, K decline in organic carbon, C/N ratio over time. The results are similar to the findings of (Edwards, C.A.1995 and Kale, 1993)

The materials ingested by earthworms undergo biochemical changes in the digestive track and in the ejected cast, the plant nutrients and growth substances are rendered in plant assemeable form. The fertility is attributed to enzymatic activity and microbial activity that associated with earthworms. (Kale *et al.*, 1987)

Loquet *et al.*, (1977) reported that the earthworm burrows lined with earthworm costs are excellent media for harboring and fixing bacteria.

According to Kale *et al.*, (1992) significantly higher number of microbes was observed in experimental plots treated with vermi-compost. Nitrogen fixing bacteria are also higher in vermi-compost applied plot after harvesting of the crop. Higher microbial load was also observed in paddy applied with vermi-compost.

The castes also contain enzymes such as protease, amylases, lipases, celluloses, chitinase as 10 to 20 times high microbial activity as compared to the soil and organic matter the worm ingest and cast contents more micronutrients, like N, P, K, S, Fe, Man, Cu, Zn, Molybdenum, more boron (Trivedy,2002).

Prabhakar *et al.,* (2006) in his studies on enrichment of soil has reported the increase in N,P,K by using *Octochaetona serrata* when treated in experimental pots with organic wastes like potato, tomato, mixed vegetables and cow dung.

Bhawalkar, (1989) in his study included that the waste could be gradually bio-processed by earthworms, into balanced plant nutrients. He further stated that the earthworms biosystems enrich the growth of beneficial bacteria and actinomycetes in its surroundings.

According to Roy (2006) in vermi-composting the effect of micro organisms along with earthworms are the prime work force to process organic wastes. These are 10 times faster in their work as compared to the process of composting. The grain and malt quality and grain yield of barley was also increased greatly with application of vermi compost.

Bhattacharya *et al.,* (2000) has reported the comparison of various techniques of compost. The vermi-compost prepared by using *Eisenia fetida as* on predigested traditions compost and samples drawn after 84 days shows

Decrease in	PH	8	to	7.5
Org C.		23.44	to	16.43
Increase in	N	0.64	to	1.0
Decrease in	CL2	37.74	to	10.43

Ismail (1993 and 1997) in his study suggested that the waste converted to organic manure will have a long term impact for sustainable agriculture by improving the soil

fertility. He stated that the earthworm's casts contained a good percentage of available nutrients.

According to Bano *et al.,* (1987a) the nutrient status in the vermi-compost found to be rich in all minerals. And the same is supported by Choudhary *et al., (*1993) for sericulture farm waste vermi-compost. Where as the comparisons made by Pawar (1996) between farm yard manure and vrmicompost showed higher nutrients in vermi-compost.

Asha gupta,(1997) reported higher nutrient in vermi-composts prepared out of vegetable waste, cow dung, leaf litter , cloth, grass and leaves of neem and mango showed higher micro and macro nutrients. At the same time the studies carried out by Jayabal and Kuppuswamy (1997) and Muthukumarswamy *et al.,* (1997) showed higher nutrients in vermi-composts prepared from coir pith press mud, water hyacinth, cow dung and press mud, bagasse respectively.

A report from Kale and Bano,(1988) cultured earthworms by using gram bran and wheat bran with cow dung in 3:1 proportion showed increase biomass and hastened the growth of the worms.

Balaji (1994) reported that the vermi-compost obtained from garbage, neem waste was in good nutrients content and the vermi-compost supported luxuriant growth of *Amaranthus* plants

Ramesh baba (1995) reported that apart from the available nutrients, the plants growth promoter substances are found in the worm casts. Tamil Selvi (1998), from her study concluded that the high percentage of NPK in utilizable form and the required amount of calcium and magnesium were found in vermi-compost obtained from garbage, coir pith

and pongumea leaf litter. High nutritive value vermi-compost was prepared from different solid wastes by Ravichandran *et al.*, (2001).

Shweta and Mamta (2001) studied a qualitative variation of vermi-compost prepared from different substrates and by different earthworms. Cow dung, Kitchen waste, leaf litter were used individually and / or in combination utilizing two different types of earthworms *Eisenia fetida* and *L mauritii.* The analysis showed the vermicompost prepared by *Eisenia fetida* from cow dung had more organic C, K and Fe.On the other hand the vermi-compost prepared by *L mauritii* from cow dung had more K.P and Zn and that fro leaf litter had more Cu.

Senthil Kumar (2002) has reported that plenty of agriculture waste, consisting of crop straw, husk, and sugarcane trash; groundnut shell, animal remains etc. amounting to nearly 320 million tones. Annually, of which sugarcane waste alone amounts for 27 million tones, dung and poultry droppings constitute 4 million tones. Of 60% of this tremendous waste is composting using earthworms, 210 million tones

Sundararajan *et al.*, (1996) have reported that a two-phase anaerobic digester of 20L capacity of each phase was operated at room temperature using kitchen – refuse from a student's hostel as feed stock material at 24=70C. The maximum biogas produced was 0.324m3/kg VS added / day in summer at the loading rate of 3.2 kg VS/m3 of digester per day, and minimum volume of biogas of 0.156 m3/kg VS added per day at the loading rate of 2.0 Kg VS/m3 of digester per day was obtained in winter.

Adejvyigbe, (2006) has reported the biodegradation / decomposition and release of N, P, K, Ca and Mg for the litter released followed the fallowing order with microorthoprods

and earthworms> with earthworms > with microorthopods. No faunal addition.

According to Reaganold (Washington State University), organic matter plays a major role in maintaining soil quality. It improves soil quality and productivity by enhancing their granulation, porosity, water holding capacity nutrient supply rate and activity of soil biota. Also improves soil texture, structure and proliferate useful microorganisms. Sengar and Salini Gupta, (2006). It is a well-known fact that endogenic anecic earthworms influence soil macro porosity and aggregation Lee and Foster, (1991).

Improved productivity of soils in many environments are reported by (Edwards and Lofty, 1977; Satchell, 1983; Lee, 1985; Kretzschmar, 1992).

Population and behavior study of *Aporrectodea calignosa* and *Lumbricus terristris* has been reported as a negative with board mill sludge both in laboratory and field trials Kevin *et al.,* (2005).

Allen (1957) reported the enumeration of total C/W, bacteria, actinomycetes and fungi and found to be higher in vermi-composts.

The loamy soil (sand) with different substrates/habitats like vegetable garden, flower garden, grassland in Benghazi, Libya, have shown abundance of the fallowing type of earthworm species namely *Allolobophora caliginosa* , *Allolobophora rosea* , *microscolex dubis* (Achuthan Nair, 2005). He also reported the soil loamy sand has almost a neutral pH value in case of grass lands where as it is 7.7 -7.5 in case of vegetable garden and flower garden.

1.8.1 Feeding additives for better management of wastes:

Kale *et al.,* (1986) reported the suitability of neem cake as an additive in earthworms feed and its significance in the influence in the establishment of micro flora. Earthworm, *Eudrilus eugenae* was found to be tolerant to the neem cake in the culture medium up to a concentration of 6.4 %. 4 to 1.6 % neem cakes in the medium had positive effect on earthworm biomass production.

The earthworm *E.eugenae* was mass cutured on six different feed formulae prepared by mixing cow dung, sheep and horse with other organic waste such as rice polish, wheat barn, and green gram bran vegetable waste and eggshell powder in various combinations. The worm casts obtained from six different feeds were analyzed for pH, Ec, organic C, N.Pand K. all casts recorded slightly acidic to neutral pH. The percentage nitrogen remained the same more or less in all the casts. However, Ec, organic C, P_2O_5 and K_2O contents varied greatly Bano et al., (1987b).

A laboratory scale study was conducted to assess the suitability of powdered rubber leaf litter as vermi-culture substrate for *P. excavatus, E.eugeniae, E.fetida.* The earthworm mortality, biomass production and reproduction were measured during investigation.

Singh Kumar (2001) cultured the earthworm *Eisenia fetida* by mixing cow dung with wheat bran, rice bran and gram bran in pits of different depth and the casts were analyzed for nutrient status.

The number of earthworms in Park Grass, a perement pasture fertilizer experiment was three times greater in plots receiving $35tha^{-1}$ of dung than in unmanured plots. Where as in Barn field, an arable cerement growing mangolds, there were about fifteen times more

earthworms in plots receiving dung annually than in unmanured plots Edwards and Lofty (1977).

Satchell (1955) and Edwards and Lofty (1977) reported an increase in number of earthworms in Barn field Rothemsted (Grass land) and Park grass Rothemsted (Arable land) as given in table 1.5

Table 1.5: Increase in number of earthworms.

Species	Grass land Park grass Rothemsted		Arable Land Barn field Rothemsted	
	Unmanured	Dung	Unmanured	Dung
L. terristris	13.1	22.5	023	10.8
L. castaneus	16.0	59.6	-----	-----
A. caliginosa	2.9	8.0	0.8	15.4
A. chlorotica	1.6	-----	3.2	44.6
A. rosea	10	21.3	-----	0.23
A. longa	----	------	0.46	1.8
A. nocturna	1.3	18.9	----	----
O. cyaneum	6.9	24.5	----	-----
Total	51.8	154.8	4.69	72.83

1.9 Earthworm Gut Morphology and contents:

The earthworm gut is basically a straight tube extending from the mouth to the anus, its different region are a muscular pharynx, oesophagus, intestine and associated digestive glands. The oesophagus may be further differentiated into two bulbous chambers a muscular gizzard and a thin wallet crop. There may be more than one gizzard depending upon the species. Various modifications in the digestive system in different worms depending upon the food taken by them.

Gizzard is generally absent or rudimentary in earthworms, which thrive on liquid or semi liquid diet. Litter feeding species lack a typhlosole whereas it is well developed in soil feeding worms. (Senapati, 1993)

The gut contents usually comprise mucus, organic and mineral matter and organismal components (micro flora and micro founa). A variety of digestive enzymes have been also reported from the alimentary canal of the earthworms. Their enzymes are usually co-related to the preferred diet of the organism. (Wallwork, 1984)

Analysis of gut contents in earthworm has revealed the occurrence of different kinds of symbiont like microfungi, bacteria, protozoans etc. maximum numbers of micro fungal species are found in the foregut, gradually decreasing in number in the mid and hind gut with a minimum number of freshly laid casts. (Dash *et al.,* 1985)

It is well established that the earthworm gut provides suitable conditions for the development of bacterial colonies since earthworm cast contained significantly higher counts of bacteria than in the surrounding soil. Microorganisms may constitute an important part of the diet of earthworms, which can feed on them selectively (Edwards 2004; Moody *et al.,* 1995).

The analysis of gut content of earthworms has revealed the occurrence of different kinds of symbiotic micro fungi, bacteria, protozoa's etc. Fold increase of 13.76, 572.96 times in actinomycetes and 700.8, 927.78 times bacteria in the mid gut and Hindgut as compared with fore gut respectivel has been reported Parle (1959).

The presence of digestive enzymes like amylase, cellulase, protease, lipase, chitinase and lichenase in the intestine signifies digestive ability of earthworms, these enzymes operate in a medium with remarkable stable pH ranging between 6.3 and 7.3 (Wallwork, 1984)

Zhang (1993) reported the presence of enzyme activities and the strongest enzyme activities were located in the fore gut and mid gut. The activity of lichenin, cellulase, starch, glucomannan and glactomannan are found to be predominant. He also concluded that cellulase and mannase activities are mainly due to microorganisms.

Dash and Senapati (1986) have also reported the gut enzymes of the earthworms as given in Table 1.6

Table 1.6: Gut enzymes of earthworms.

Earthworm species	Gut enzymes				
	Amylase	Cellulase	Chitinase	Protease	Urease
Dichiogaster boaiui	+	+	-	+	+
Drawida calebi	+	+	-	+	+
Drawida willsi	+	+	-	+	+
Eutyphoeus sp	-	-	-	+	+
Lampito mauritii	+	+	-	+	+
Dendrobaena octoedra	-	+	-	-	-
Eisenia fetida	-	-	-	+	-

1.10 Application of vermicompost and microbial population:

Soil microorganisms play an important role in improving soil fertility and crop productivity due to their capability to fix atmospheric nitrogen, soluble insoluble phosphate and decompose farm wastes resulting in release of plant nutrient (Tewatia *et al.,* 2007). Owing to the importance of soil microorganisms and the advantage of adding vermi-compost brings in the benefits of soil fertility in terms of nutrients and microbial population, in the following paragraphs the reports available on the same are presented.

Teotia (1950) reported a bacterial fold increase of 3.4 to 5.4 times as compared to surrounding soil. Where as Atlavinyte and Lugauskas (1971) reported a five-fold increase in microorganisms in earthworm introduced pot tests. Ghilarov (1963) has reported in his study on number of microorganisms in earthworm casts and soil and the fold increase is 1.64,1.35,1.97 times in three different fields namely oak forest, Rye field and Grass field respectively. The moisture content of vermi-compost becomes an essential environmental condition for survival of the beneficial micro organisms, Irrespective whether earthworms continue to live or not. The decrease in moisture content will bring down the (colony forming units) cfu. Organic carbon,(Scheu, 1987: Parthasarathi and Rang Nathan, 2001; Parthasarathi, 2006).

The study on microbes in the gut of earthworm reveals the increase in number of bacteria, actinomycetes as compared to those in soil, follow exponential law (Parle, 1957). The above literature survey showed that the increase in microorganisms in the gut and vermi-casts of earthworms contain higher number of microorganisms is taken as the one of the measure to evaluate the vermi-composts.

It also enables us to say that earthworms are important in inoculating the soil and their casts are the foci for dissemination of soil microorganisms, which will elevate the overall fertility of the soil.

Atlavinyte and Lugauskes (1971) have reported that earthworm increases the number of microorganisms in soil as much as five times. Earthworms are therefore important in inoculating the soil with microorganisms and their casts are foci for dissemination of soil microbes.

Monson *et al.*, (2007) has reported the increase in nutrient of vermi-compost of kitchen waste by using *Eudrilus eugeniae* the increase in N from 1.31% to2.12%; P from 0.121% to.7%; K from 0.45to 0.48% and C/N ratio decreased from 32.45 to13.66%.

A significant higher number of microbes were observed in experimental plots treated with vermi-compost. Nitrogen fixing bacteria was also higher in vermi-compost applied plot after harvest of the crop. Higher microbial load was also observed in paddy, applied with vermi-compost (Kale *et al.,* 1992).

The effect of vermi-compost on microbial population in soil environment is reported to be best with vermi-compost prepared out of the combination of leaf litter, straw, grass and water hyacinth (VC1) as compared to vermi-compost of leaf litter (VC2), Home garbage (VC3) and partially decomposed cow dung VC4 when applied at the rate of 5%(w/w). The fold increase is 2.16,1.83,1.71,1.69 in bacteria,1.49,1.30,1.52,1.40 in actinomycetes, 2.89,2.76,2.38,2.47 in fungi for VC1,VC2,VC3,VC4 respectively(Sahu, *et.al.,* 2000).

Loquet *et al.,* (1977) reported that the earthworm borrows lined with earthworm casts are excellent medium for harboring nitrogen fixing bacteria

Bhattacharrya (2000) has reported an increase of microbial count of Vermi Compost as compared to traditional compost.

	Traditional compost	Vermi Compost
Bacteria	$143 \times 10^{+7}\ g^{-1}$	$167.29 \times 10^{+7}\ g^{-1}$
Fungi	$39.61 \times 10^{5}\ g^{-1}$	$96.25 \times 10^{5}\ g^{-1}$
Actinomycetes	$365.27 \times 10^{6}\ g^{-1}$	$419.62 \times 10^{6}\ g^{-1}$
PP solution	$195.61 \times 10^{5}\ g^{-1}$	$168.20 \times 10^{5}\ g^{-1}$
N_2 fixing bacteria	$92.58 \times 10^{5}\ g^{-1}$	$96.62 \times 10^{5}\ g^{-1}$
Thio Sulphate oxidizer	$315.38 \times 10^{5}\ g^{-1}$	$569.29 \times 10^{5}\ g^{-1}$

Polysaccharide content of earthworms cast was much higher than the soil but did not vary with changes with stability of total and mineral nitrogen. Major part of inorganic nitrogen occurred as ammonia, which was rapidly converted to nitrate (Parle, 1963)

Santhil Kumar (2002) reported the following vermi-compost contents.

Humus	30 to 50%
N	0.72 %
K	0.74%
Carbon	40-57%
Hydrogen	4-8%
Oxygen	33-54%
pH	4 to 9
C/N	20

Water holding capacity of soil was increased due to increase in colloidal materials like earthworm mucus, a good absorbing agent in vermicompost. Earthworms increased water-holding capacity of New Zealand soils by about 17% (Stockdrill and lossens, 1966) Sahu *et al.*,(2000) has reported that the improvement in soil environment was best with vermi-compost prepared out of the combination of leaf, straw, grass and water hyacinth (VC1) as compared to vermi-compost of leaf litter (VC2), home garbage (VC3) and partially decomposed cow dung (VC4) is applied at the rate of 5% (W/W) in soil were investigated. The vermin composts recorede ratio of carbon to nitrogen VC1 was highest followed by VC2 VC3 and VC4 corroborated the earlier reports of Fragoso *et al.,* (1993) Castings of *Eisenia fetida* from sheep manure alone and mixed with cotton wastes were analyzed for their properties and chemical composition every 2 week for 3 months and compared with the same manure without earthworms. The results showed that earthworms accelerated the mineralization rate and gave castings with higher nutritional value and degree of humification, suggesting that this kind of industrial waste can be used in the vermi-composting (Albanell, *et al.,* 1988).Similarly accelerated mineralization and humification of solid paper pulp mill sludge with earthworms in comparison to without earthworms was reported by Elvira *et al., (*1996).

Athanasopoulos (1993) used of earthworm biotechnology for the management of an-aerobically stabilized effluents of dried vine fruit industry were successfully treated in earthworm filters using *L rubellus*. The COD removal was 95% with loading 0.1 and 0.15 Kg COD/m-2d.

Bird and Hale (1982) undertook work on sludge contaminated with heavy metals from industrial sources and found the increase in heavy metal concentration in vermi-compost applied soil above the prescribed limits. They suggested that vermicomposting of waste contaminated with much less glass, metal etc. The best results of cotton waste with cattle manure is being reported by Zajonc and Sidor, (1990).Where as Edwards and Bater (1992) tried vermi composting using urban and industrial sources. Madhukeshwar *et al.*, 1996) says any kind of organic waste generated in an agro based industry or biotechnology unit would be a resourceful vermi-compost when it is treated with earthworm activity.

In Columbia, more than one m.t of coffee pulp produced every year is treated by composting method by turned piles led to low quality compost. When started treating with *Eisenia fetida* in vermin composting process resulted in increase of P.Ca, and Mg and decreased K (Orozco et al., 1996).

Giraddi and Tippannavar (2000) studied the techniques of biodegradation of organic solid wastes using earthworms. Waste from fruit pulp, biscuit industry and sugar industry are bio-degradable in field designs using *Eudrilus eugeniae*, *Eisenia fetida*, and *P excavatus for* the waste management. The wastes were bio-converted to compost in 40-90 days. The quality of the compost obtained had increase micro and macronutrients.

Waste from olive oil industry alone or mixed with cattle manure is a suitable substrate for vermicompost Moreno *et al.*, (2000).

There are some reports on pulp and dairy industries, Kalpana, (1978) reported the possibility of mixing sewage sludge and other materials like pulp paper sludge or lignin

rich waste and inoculating earthworms for biodegradation. Butt, (1993) explored the possibility of treating paper mill sludge with spent yeast from brewery industry using the earthworm species *L terrestris.* Where as the same industrial waste was treated with *E Andrei* Elvira *et al.,* (1998) They also investigated the vermi-composting of sludge's from paper mill and dairy industry mixed with cattle manure using *E Andrei* in six month pilot scale experiment. The number of earthworms and bio mass increased significantly. The vermi-composts were rich in N, P, and K and had good structure, low level of heavy metals, lower conductivity, high humic acid contents and good stability and maturity.

They also reported the growth study of *E andrie* by using paper mill and dairy mill sludge's in pure waste by mixing with different proportion of cattle manure Elvira *at al.,* (1999). Studies of possible usage of paper and dairy mill sludge's during vermi-composting confirmed that such a material might be a valuable component of breeding medium for *Eisenia fetida* earthworms. , But the contents of mineral nitrogen and total potassium were low.

1.11 Organic Agriculture:

Organic agriculture started in the year 1920. The growth over last seventy years is classified into three eras 1924 – 1970 period of struggle, 1970 – 1980 period of organic symbols and 1980 onwards acceptance of organic agriculture. (Kamalabai, 2000)

Press mud though a good organic amendment contains an appreciable amount of toxicants, heavy metals as their constituents use of this waste in agriculture though have

some nutrient enhancement / or beneficial effects on soil physico chemical characteristics but the danger of toxicant built up is always there , and needs constant monitoring (Pagaria,2007). The use of bio fertilizer prepared from filter mud will have beneficial effects on soil without any build up of toxicants.

The following are the renowned personalities in the field of organic farming

1) Rdolf Steiner from Austria [1861 – 1925] first person to start organic education informs of biodynamic agriculture.
2) Hans Multer [1891 – 1988] from German. Swiss importance is given to micro-organisms and their key role in improving soil fertility
3) Lady Eve balfour from Britan [899-1990] based on the principle that health of soil and health of man are inseparable
4) J. I. Rodale first to publish organic farm ideas & initiate to start organic farming in USA.
5) Masanobu Fukuoka his well known book is "One straw revolution"

1.12 Economic importance:

Organic matter identified, ingested by the worms is pulverized in their alimentary canal, and excreted as colloidal humus, which is rich in plant nutrients.

In addition, a large number of worm's die during unfavorable periods when the chemical demand in the soil is maximum due to growing plants. The microbial decomposition of dead worms releases considerable amount of locked up nitrogen and thereby making it available to the plants.

The global problem of increased quantity of organic wastes due to human activities in rural and urban areas and industries is a serious constraint in the maintenance of clean and healthy environment. Earthworms are most useful converters of these wastes. Experiments have been successfully conducted for recycling the organic wastes Michell & Horner, 1980, Singh 1997)

The bio fertilizer prepared out of organic wastes provides the fallowing advantages

- The general fertilility level of the farm/garden is greatly improved.
- Increased water holding capacity
- It makes ease of cultivation
- Eliminates valuable waiting time
- It reduces soil erosion and reduces flood hazards
- Increase the soil layer thickness every year
- It multiplies the microbial population
- Land can safely be plowed more deeply
- Hard pans will not form
- Heavy machinery not required
- The soil has greater aeration
- Soil made darker by humus absorbs heat more quickly and effectively.
- Dry Weather advantage
- It will actually improve rain conditions.
- It transpires less water through the leaves
- Continuous application of vermicompost reduces the further requirement of manures year by year.

- It has residual no effects
- Aesthetically farms look better
- It is weed resistant
- Less risk of crop failure
- It is much more immune to plant resistant
- The insect menace is reduced to minimum
- Very few poison sprays are required
- No chemical treatment are needed to seeds
- Farm animals fed on organically produced feeds are healthier
- Food tastes better when it is raised organically.
- Earthworms have high nutritional value to be used as medicine

1.13 National Status:

Work on organic solid waste management in India was initiated on late 1980's. Research and development on this technology is undertaken by many institutions and agricultural universities, and different aspects of this technology are being studied. The vermiculture biotechnology is still under development process. Sufficient published literature on this technology in context with India is not available; however some published literature is presented here.

Sharma (2002) and Madan *et al., (*1988) and reported that crop residues, cattle dung, urine, poultry waste, sawdust, household refuse, and night soil can be used for vermicomposting

Kavian and Ghatnekar, (1991) reported that Red American earthworm's *L. rebellus* could be used for recycling of dairy sludge. They found that, the sludge cake can support

earthworms without processing to compost and when supplemented with cellulose material, such as cow dung. *L. rebellus* produced cocoons 10 days after being transferred to the sludge cake feed, and hatching appeared in 30 days. They also found that the organic matter/ash ratio of the sludge cake feed decreased during the experimental period from 4.5 to 2 at 120 days a similar level that observed in national compost.

Some species of epigeic earthworm have been reported to work very well in nitrogen rich organic matter such as household litter, agricultural wastes, plant residues, sugarcane thrash, weeds, and hedge cuttings, dung of cattle, sheep, horse and pig.

Silkworm castings, saw dust husk and choir waste, paper pulp and slurry of cow-dung after its use in biogas plants at University of Agricultural Sciences. Bangalore (Kale, 1994)

Bhiday (1994) suggested that biogas plant, in association with the earthworm compost/culture, could be used for treatment of urban, ordinary, industrial, agricultural and food production waste. He recommended that pre treatment of wastes is essential before feeding to earthworms.

Bhawalkar (1995) has developed process for poultry residues, soya residues, city garbage, and onion residues as well as swage treatment process through vermiculture. He strongly recommends use of deep burrowing earthworm *phereteina elangata* instead of using *E. fedida, L. rubellus* etc for vermiculture.

Kavian *et al.,* 1996 studied bio management of paper mill sledge in different concentrations using culture of Red American earthworms (*L. rubellus)* and reported that

25 % concentration of sludge was ideal giving the highest microbial count and reproduction rate of earthworms.

Dubey *et al.,* (1996) has also suggested Vermiculture as the possible alternative to reduce municipal garbage and industrial wastes.

1.14 Status in Indian Institutions:

Indian Institute of Technology, Bombay has taken up a DBT sponsored project on vermiculture bioconversion of solid residues. Chemical engineering department of this institute has set up a demonstration plant processing 1 to 1.5 ton food waste of hostel. The plant is operated since May, 1990

Bhawalkar Earthworm Research Institute (BERI) Pune in collaboration with Biotech Consortium India Ltd. set up by DBT is actively engaged in promotion of vermiculture biotechnology. The institute has standardized technology packages for vermiculture treatment of diverse organic wastes.

Department of Zoology, University of Agricultural Science, Bangalore, using selected tropical species of earthworms has undertaken considerable research work on vermiculture biotechnology.

National Environmental Engineering Research Institute, (NEERI) Nagpur. Solid waste division has conducted laboratory studies on vermiculture treatment of food wastes from food processing industries. This division however, is suggesting the use of surface feeding worms rather than deep burrowing types.

Investigations on vermiculture are being carried out by Tamilnadu Agricultural University, Karnataka University and University of Agricultural Science Dharwad, and

Department of environmental Science, Bangalore University, Indian Cardomon Research Institute Myladumpara, Research Institutes attached to coffee and Rubber Board, Various scientists working in colleges in the city of Chennai, Vivekananda Mission Centers and private entrepreneurs.

Punjab state council for science and technology has done pioneering work for promotion of vermiculture biotechnology in the state and following programme has been undertaken in this regard.

i) Providing assistance to Brooke Bond Lipton India Ltd. Zahura Distt. Hoshiarpur to se up a vermi processing plant for treatment of tomato skin seed waste.

ii) The setting up of vermi processing units in more organic waste generating industries of Punjab in progress.

iii) The council has assisted a military unit of army cantonment at patiala to set up a community vermi processing plant to treat municipal solid waste.

iv) The setting up of vermi-processing plant for treatment of 10 times per day municipal solid waste has been included in "Satlej River Action Plan"

v) Initiations taken to undertake work on vermi-culture for agro based industries in Punjab.

Department of Agriculture, Punjab is promoting vermiculture biotechnology for live stock waste management.

A vermi processing plant near Kharar has been setting up by the department and if also plans to set up pilot vermiprocessing plants in every district for demonstration to farmers.

At I.I.T. Bombay, a project sponsored by the Govt. of India's Department of Biotechnology is undertaken where in 4 tons of garbage, mostly canteen waste from the hostel an I.I.T. campus are fed to earthworms everyday.

In Bombay's Jal darshan Housing Society at Nepean Secroad, A group of environmentally conscious youngsters have similar plans to treat the building waste. Such efforts at the community level, they feed, will go a long way in convincing the public and the civil authorities about the feasibility of this technology. (Staff reporter 1992)

In Goa University the department of microbiology and Zoology undertake the research and community oriented projects.

Project on implementation of vermicomposting by Agnel Charities through department of civil engineering Agnel Polytechnic Verna Goa was a success story, funded by CAPART (2001-2003).

1.15 International Status:

Cuba's Nation wide experiment has established the utility of vermicasting in realizing higher yields. New Cuba is having 172 full fledged vermi composting centers capable of launching the increase generating scheme (Nayak & Rath 96)

Many developed countries like USA, England, & Japan have made extensive studies and developed techniques by large scale manufacture of vermicompost from agricultural, domestic and industrial organic wastes on commercial bases (Ranganathan & Christopher, 1996).

Riggle (1998) Almost vigorous programs of research in earthworm ecology, vermiculture, and vermi-composting in the US today can be found at Ohio State University Coloumbus, Ohio.

In Austrelia, Green waste Technology Unit established in University of New south Wales Sydney under takes research on earthworm technology and Promoting Medium, large scale vermi-composting. In addition, in New-zeland vermi-culture was implemented from home worm bins to large scale composting units, which are becoming popular in treatment of food trimmings as reported by Applehof *et al.*, (1996).

Taiwan and Philippines also have adopted the treatment of organic wastes [Staff reporter TOI, 1992]

Abbott,J.and Atkins,I(1997) reported the waste streams for treatment through vermiculture , with specific example of meat processing plant and description of vermiculture facility design options for the waste streams.

Where as biochemical production from agriculture and food processing industries by different microbial strains; like xylitol a sweetener obtained from sugar industry effluents by using *Pichiaguillermondiib* and biomass protein starch from waste stream of starch industry used as a feed by using Micro-fungi (*rhizoopus*). Similarly Polyhydroxylalkanates a bio-plastic obtained from waste stream of food processing industry by using *Ralstonia eutrophac* is being reported by Raghunandan,(2007).

The review of literature has revealed that the earthworms are playing an important role in the organic matter decompositions and soil metabolism. The contribution is mainly through physical participation by feeding, fragmentation, aeration, turnover, and

dispersion, chemical participation by digestive organic substrate and contributing to participation by grazing over micro-flora. There is a need for pin pointing experiments for biodegradation/ bioconversion studies of different organic wastes with locally available species of earthworms. The literature survey does not give any work of using microbes alone for the treatment of solid wastes. Hence, there is an urgent need to develop technologies dependent only on microbes in order to undertake solid waste treatments at a faster rate and in an economically feasible way to bring profits and sustainability to industries.

Further the available information with respect to operational vermi processing plants in the industrial sector is being placed in the Appendix A2 .also a careful selection of the major waste producing industries will be of great help in targeting the waste management plans and specific strategies with microbial technologies more realistically,. In this regard, a WHO report provides a list industries in the food manufacture along with their UN classification codes is given in appendix A3. The opportunities for waste minimization as given by Khanna, (1996) given in appendix A4. Which shows the food processing industry with moderate opportunity for waste minimization? Meaning there is a lot of scope to work with these industries makes them comply with the requirement of the Government by providing new technological solutions and technical guidance at the same time bringing sustainability and profits to the industries.

The literature survey revealed that there is a substantial work in the field of vermi-culture biotechnology from agricultural residue point of view. Where as there needs a lot of work is yet to be carried out in the field of industries producing huge quantities of organic wastes. There are few or no pin pointing experimentation conducted with respect to industrial wastes. Therefore, it was felt that the work on vermi-culture and associated microbes has got tremendous scope in the field of waste management. Therefore, the work undertaken to standardize the vermi-culture with different proportion of soil cow dung and their experimental results are presented in the next chapter.

CHAPTER II

Characteristics of raw materials used for vermi-composting and optimization of soil cow dung proportion

Chapter II: Characteristics of raw materials used for vermi composting and optimization of soil cow dung proportion:

2.0. Introduction:

The vermi-composting, a system which consists of inputs [organic wastes], outputs [vermi-compost], and utilizing activities of earthworm and microorganisms along with soil processes. The microbial activity is governed by the presence of earthworms and vice-versa, which show a symbiotic relationship. The earthworms play an important role in forming bacteria by providing the necessary environmental conditions like pH, temperature and air circulation. The only other condition to be satisfied is moisture requirement. If moisture level of 70 -80 % maintained in the vermi beds depending on the external temperature, the earthworms will survive and multiply in number by feeding on the food substrate provided. Hence, the increase in number of earthworms and juvenile predominance are of significance in measuring substrate preference and biodegradation. The dynamics of earthworm- soil relationship and the physicochemical conditions in the soil environment will have a significant effect on the survival and growth of earthworms. To initiate the vermi-processing it is always advisable to start with cow dung as a substrate for earthworms and then slowly switch over to the selected substrates. Cow dung is an easily biodegradable substrate used for vermi-composting along with several agricultural residues and other substrates from industrial processes. To know the extent to which cow dung is being mixed with the soil is of importance because it gives an indicator of growth of earthworms. The earthworms live in soil with little or no organic

matter. They tend to move to a safe region of soil with organic matter. The collection of earthworms resorted by sprinkling of cow dung water continuously for three to four days and then dig the ground to pick the worms also indicates the liking of cow dung as a substrate. The vermi-composting of press mud was initiated using *Eisenia fetida, Eudrilus eugeniae*[surface feeders], *Megascolex megascolex*[deep burrower].Physico chemical and microbiological analysis required were determined for the raw materials, and proportion of soil cow dung to be mixed highest number of earthworms in a given time by setting experiment with different proportions of soil and cow dung. The soil obtained from the proposed vermi-processing site of the industry was sieved through 850-micron sieve.

2.1: Materials and Methods:

2.1.1: Raw Materials for vermi-composting.

A) Press mud solid waste from sugar factory:

Samples of fresh solid wastes, viz. Press mud [Filter mud] were obtained from Sanjeevani Sakhar Kharkhana Dayanand Nagar Goa. (Flow diagram of manufacturing process and Stages of solid waste generation of the industry are depicted shown in Appendix A5 and A6.

Seven days old cow-dung was procured from local farmer for the laboratory study and from a local dairy during the field trials in the industry.

B) Earthworms:

The earthworm inoculums surface feeder species *Eisenia fetida* and *Eudrilus eugeniae* procured from Indian Natural Organic Agriculture [INORA], Pune and University of Agricultural Science, Dharwad where as deep burrower species *Megascolex megascolex* is procured from local cashew plantation farm in Verna Goa. Rectangular plastic tanks of size 60 cm x 45 cm x 45 cm were used for maintaining the cultures of surface feeders, at the bottom of the tank dry leaves were spread to a thickness of 5cm above which 5cm thick layer of industry soil was spread, followed by 5cm layer of cow dung. This system was kept at moisture level of 70 % by sprinkling water every day. There is report available on moisture content to optimize the cocoon production by *Eisenia anderi* at 85% Geset *et al.,* (1992). The *Perionyx exavatus* in cattle manure highest juveniles and clitellite worms at moisture content 75 to 85%. Similarly Muyima *et al.,* (1994) reported a moisture range of 67 to 84% for *Dendrobaena veneta,* 85% is reported by Hallatt et al., (1992). The cow dung was applied to these beds as food substrates every alternate day. These boxes maintained the earthworm cultures.

2.1.2: Physicochemical characteristics of press mud and soil:

The physicochemical analysis were carried out according to the methods outlined in the Indian standard code of practice for soils, IS: 1948-1970, and agricultural methods by Iswaran (1980).

The moisture contents of the soil and compost samples were determined by the oven dry method. The loose bulk density was determined by using a standard core cutter of known volume.

Determination of Total solids, Organic carbon, Ash con tent, and Volatile solids was carried out using the method of oven drying and ashing (Iswaran, 1980).The detailed procedure is given in Appendix B.

pH was determined by using a digital pH meter.

The Nitrogen content was determined by Kjeldhal method, phosphorus by Vanadomolybdate method and potassium by flame photometry method (Appendix B)

2.1.4 Characterization of soil samples:

1. Collection of Soil Samples:

Soil Samples were collected from proposed vermi-processing plant site of Sanjeevani Sugar Factory Dayananad Nagar Goa. In order to collect soil samples, three different points were marked on the ground at each selected point, excavation to a depth of 60 cm and diameter of 60 cm was made. The excavated soil is thoroughly mixed and the representative sample was taken in a fresh plastic container. The three samples collected were used for further work.

Analysis of soil for particle size distribution, Identification of soil was done in accordance with IS: 2720- Indian Standard Methods of Test for soils (Part I, II, III, and IV).

pH test was carried out in accordance with IS Code (Part 2720) _1987 Using digital pH meter. 30 gms of soil were mixed with 100 ml of distilled water kept stirred continuously and the pH meter readings were taken after 30 minutes.

2.1.5 Total microbiological counts:

The total bacterial count in filter mud, soil samples collected from industry site determined by dilution and spread plate method using different Medias. Nutrient agar for bacteria, sabaroud's agar for fungi, and wickerhams agar for yeasts.

1g of filter mud or soil was suspended in 10 ml of normal saline. The suspension was vortexed and serial dilutions prepared in normal saline, one ml of each dilution was transferred using a sterile pipette on plate containing the required medium, and spread using the sterile glass rod spreader. The plates were incubated for 24 hrs for 7 days and the colonies of bacteria, fungi, yeasts formed were counted.

2.1.6 Optimization of soil cow dung proportion and selection of earthworm species.

The experimental set up for choosing the best proportion of soil +cow dung was a trial with four different proportions 1:6, 1:4, 1:3, 1:2. The selected proportion was added in different culture boxes of size 30 cm X 15 cm and in each 10 earthworms of *Eisenia fetida, Eudrilus eugeniae,* and *Megascolx megascolex* were added, the experimental set up was covered with gunny bag and an average of water content of 70 % was maintained for a period of six months (Plate 2.1). The observation of counting of number of earthworms was done at the end of 32 days, and soil aggregation properties were assessed after six months.

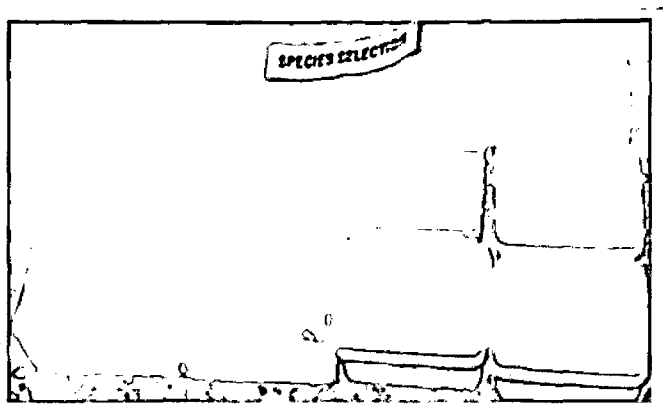
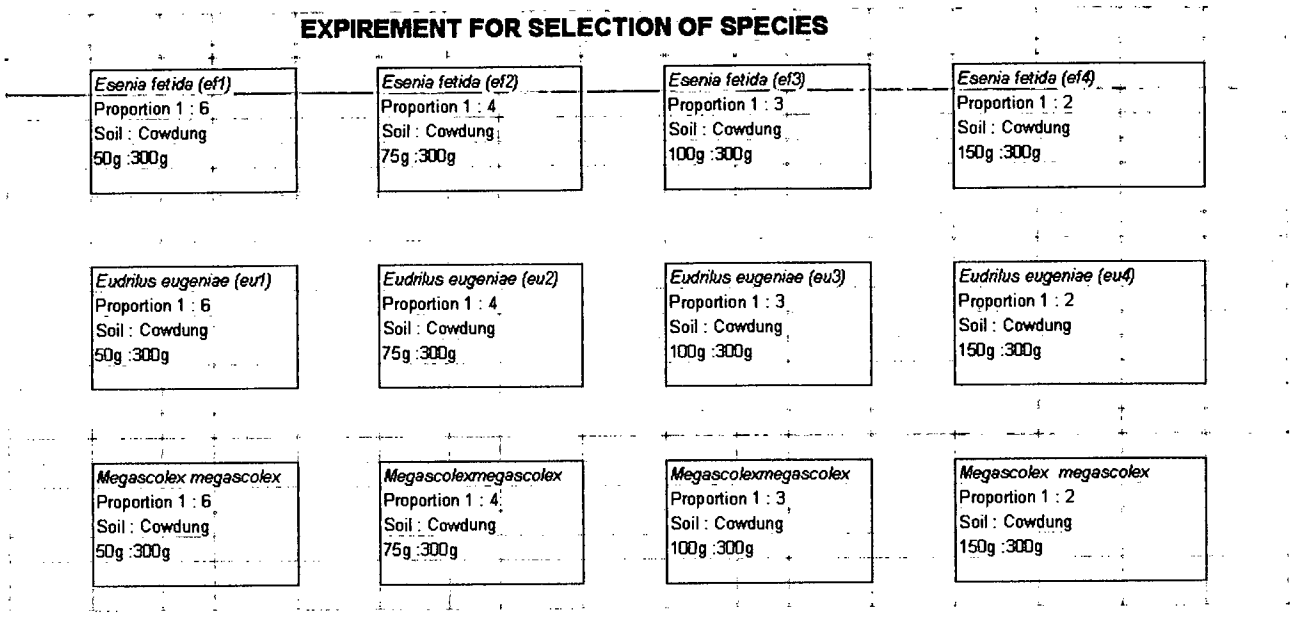


Plate 2.1: Optimization of soil cow-dung proportion and selection of earthworm species



Soil passed through 850μ IS Sieve was used in different proportions

Fig.2.1 Details of soil cow dung proportion and earthworm species.

2.3: Results and discussion:

The present disposal method of open dumping on the ground fallowed by the industry has created huge dump sites in the vicinity of the industry. The solid waste disposal methods are presented in Appendix A.

The waste materials characterized at source for the parameters as per procedures outlined in Appendix B and the results are presented in Table 2.1, 2.2.A visual of filter mud is presented in Plate 2.2.

2. Physicochemical characterization

Table 2.1 Physicochemical characterization of Press mud obtained from Sanjeevani sakhar kharkhana

Feel when touched	Soft
Appearance	Spongy
Texture	Bulky and Amorphous
Color	Blackish Brown
Storage	Openly dumped on ground
Loose Bulk Density	450 Kg/ m^3 [Variable]
Moisture Content	60 – 70% fresh sample
pH	8.6
Specific Gravity	0.5-0.7
Organic Carbon	20- 35%
Nitrogen [N]	1.2%
Phosphorus [P]	2.25%
Potassium [K]	0.8%

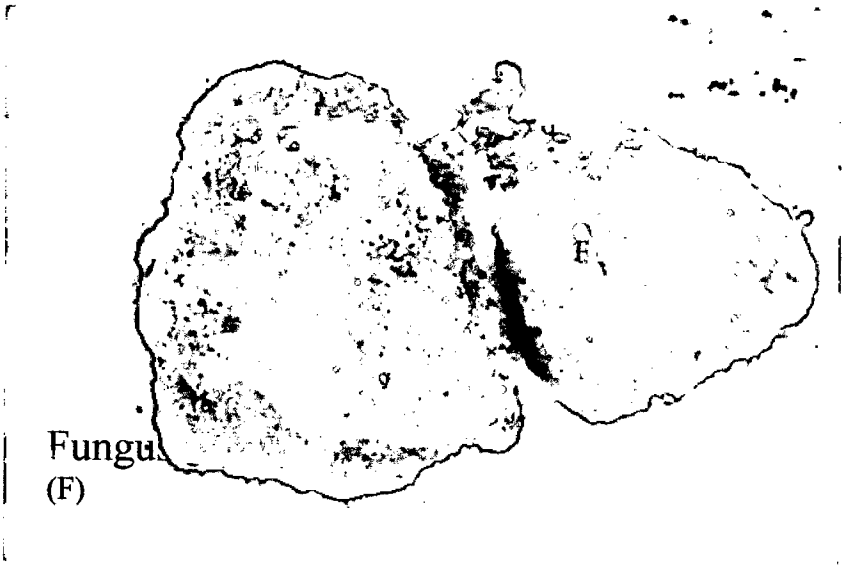


Plate 2.2: Fresh sample of press mud / filter mud

The filter mud openly stored on the ground was found to be soft, spongy ,with loose bulk density of 450 Kg/ m^3, and moisture content of 60-70%, with pH 8.6 , organic carbon was found to be ranging from 20-35%, indicating its suitability for use as a substrate for vermi-processing.

The waste pH was varying from 7.0 to 8.6. Therefore the need for any pretreatment and neutralization was not required and the waste could be directly fed to the laboratory and field trial vermi-beds. To support the idea of use of substrates with varying pH values several workers have reported a pH values of 5.0-9.0 by (Edwards and Lofty, 1977, Singh, 1997). The initial moisture was found to be varying between the range of 60- 70% with a temperature of around 100^0C. It was therefore important to have an initial open storage of 3-4 days before adding to vermi-beds to bring down the temperature.

However the vermi-processing of filter mud is reported by Bano (1987a) Parthasarathy (2006) and Singh (1997). The suitability of board mill sludge with pH 7.81 , organic carbon 42.06 % as a substrate for vermi-processing using *Aporrectodea caliginosa* and *Lumbricus terristris* is reported by Kevin et.al., (2005). The Initial pH of 6.5, N 1.06%, P 2.06%, K 1.35% is reported by Kitturmath (2002) for press mud obtained from M.K Hubli sugar factory Karnataka.

The use of surface feeder species for the treatment is reported by many workers Ghatenkar, (1995); Giraddi, (2002); Arora, (1997).

Therefore, the use of the indigenous species of earthworm *Megascoloex megascolex* the deep burrower variety was used for the study. This had enabled us to give the comparative study of the species and composts.

The specific gravity of the press-mud ranged from 0.5 -0.7 being light in weight and bulkiness it was posing problems to handle huge quantity.

The variation in the initial values N, P, and K are are attributed to the origin of the sugarcane arriving from different areas of cultivation and variations in nutrient values as reported by Kale, (1993); Giraddi and Tippannavar (2000).

The geotechnical properties of the raw material were determined and the results are presented in Table 2.2 which reveals that the press mud is porous in nature with 93% air voids. The bulk density suggests the large volume for lesser weight makes it more complicated to be handled and needs to be timely disposed, other wise growth of fungus (as depicted in Plate 2.1) makes it still difficult to handle. The water content recorded for this representative sample was 24.3%. A higher water contents and temperature were observed for fresh out falling samples. The characteristics presented in Table 2.1 and 2.2 together makes press muds an idle substrate for the vermi composting.

Table 2.2: Geotechnical properties of press mud

Parameter	Notation	Value
Specific Gravity	G	0.5
Water Content	w %	24.3
Voids ratio	e	1
Porosity	n	0.5
Bulk Density	$\sqrt{}$	0.32
Dry Density	$\sqrt{}_d$	0.25
Saturated density	$\sqrt{}_{sat}$	0.75
Degree of saturation	S_r	0.14
Air Content	a_c	0.86
% air voids	n_a %	93.9

2.5: Microbiological counts

The source of press mud being generated from an industry and collected from ground storage of the factory possibly would be having microorganisms associated with it. Especially the press mud is rich in organic carbon which supports the growth of microorganisms. It is therefore of interest to determine the total viable count of different groups of heterotrophic microorganisms such as fungi, yeast and bacteria as depicted in Table 2.4, It is interesting to note that the counts of bacteria were higher to the order of 2.4 X 10^9 as compared to fungi(3.6 x 10^8) and Yeasts (9x10^6). The presence of such high number of microorganisms reflects the easy biodegradability of the substrate. However the requirements of other growth nutrients such as nitrogen, and the temperature of the press mud would be the factor which restricts the growth of desired microorganisms. It is therefore intended to develop the process which would support an easy biodegradability of the solid waste as well as the growth of the desired microorganisms.

Similarly the count of the soil samples were also showed higher number of bacteria which would also contribute significantly to vermi- composting.

Other significant soil characteristics observed during the collection of soil samples that the presence of native earthworms which suggest the suitability of soil character for vermi-processing.

The cow dung obtained for the soil cow dung experimentation, were characterized for its total colony forming units and the results are presented in Table 2.4

Table2.3: Microbiological counts of press mud and industry soil

Microorganisms	Press mud	soil
Fungi Cfu/g [3 day observation]	3.6×10^8	11×10^5
Yeast Cfu/g [3 day observation]	9.0×10^6	6×10^4
Bacteria Cfu/g [24 hr observation]	2.4×10^9	1.28×10^7

Table 2.4: Fold increase/ decrease in micro organisms due to supplementation of cow dung.

Ingesta (cfu) (7 day old cow dung)	Egesta (cfu) Vermicompost	Fold increase after 30 days
200×10^7	377×10^7 *Eisenia fetida*	1.68
200×10^7	440×10^7 *Eisenia+Eudrilus*	2.23
200×10^7	490×10^7 *Eudrilus eugeniae*	2.45
200×10^7	490×10^7 *Megascolex megascolex*	2.05

2.6 Selection of earthworms for vermi-compost

Surface feeder *Eisenia fetida, Eudrilus eugeniae* and deep burrower *Megascolex megascolex* were procured and maintained in cow dung. These were then inoculated on different proportions of soil cow dung. It was interesting to note that the earthworms grew in all the proportions with highest being in 1:3 proportion for all the three species of (Fig 2.2, 2.3, 2.4). Significantly the weight and length of the organisms in different proportions showed marginal increase.

Based on the results shown graphically in the figures 2.2, 2.3, 2.4, the best proportion of soil + cow dung is 1:3 for culturing of earthworms as this proportion has shown highest number of earthworm hand sorted in all the three cases, *Eisenia fetida, Eudrilus eugeniae, Megascolex megascolex.* Whereas out of these three *Eeudrilus eugeniae* showed highest number of earthworms after 32 days as compared to *Megasclex megascolex* which was also found higher than *Eisenia fetida.*

As depicted in figure 2.5., 1:3 proportion has shown a higher number of worms recorded in each of the selected species of earthworm and the highest being 35 in case of *Eudrilus eugeniae* , 32 in case of *Megacolex megascolex* and 20 in case of *Eisenia fetida.*

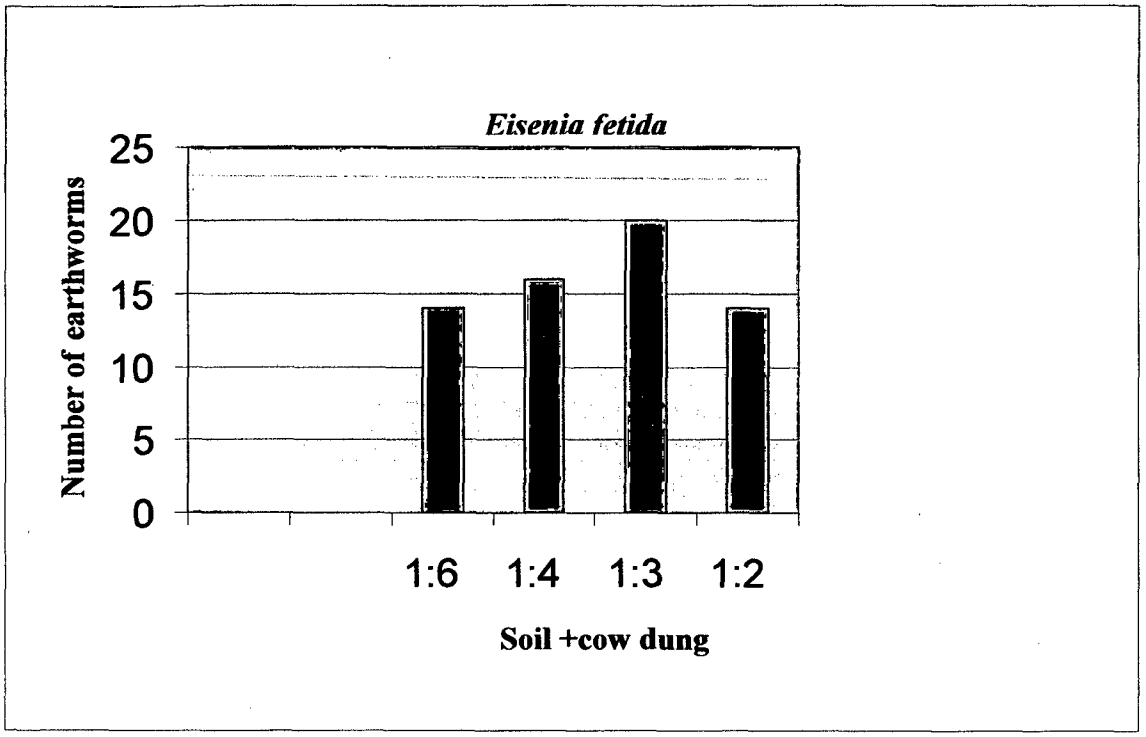


Fig. 2.2: Selection of best soil cow dung proportion for *Eisenia fetida*
Number of earthworms after 32 days

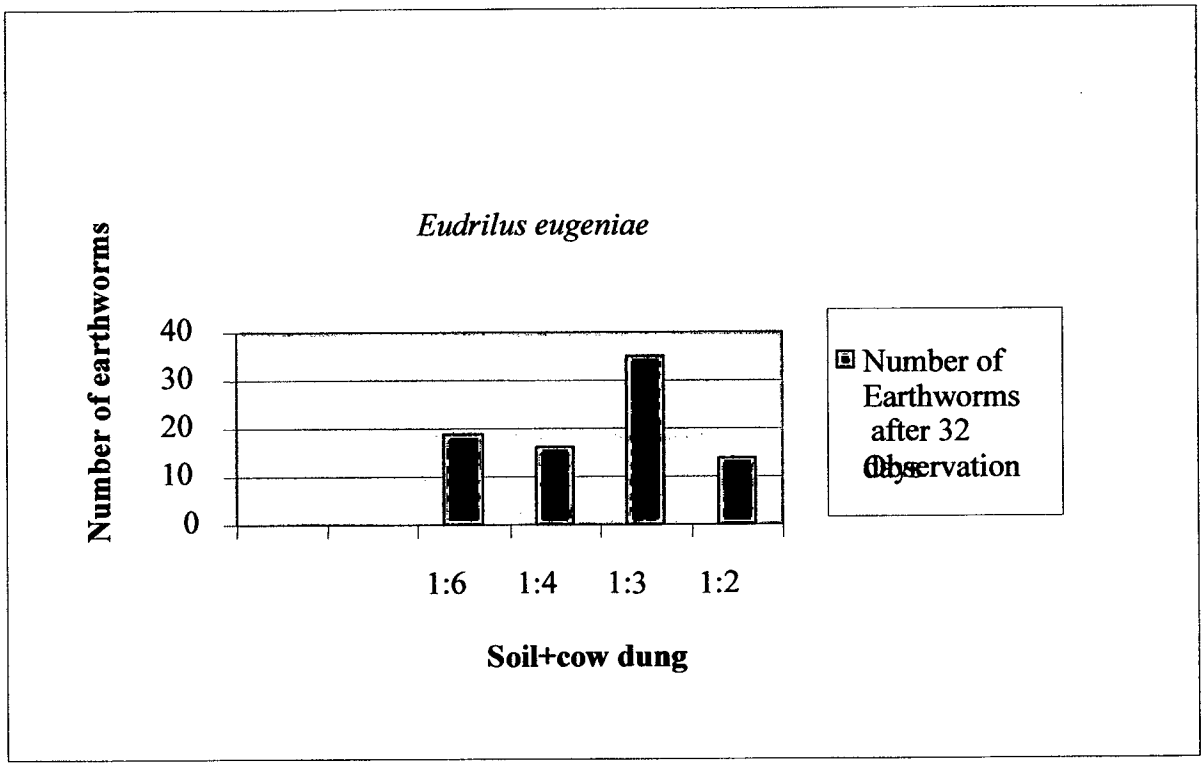


Figure 2.3: Selection of best soil cow dung proportion *for Eudrilus eugeniae*

Nunmber of earthworms after 32 days

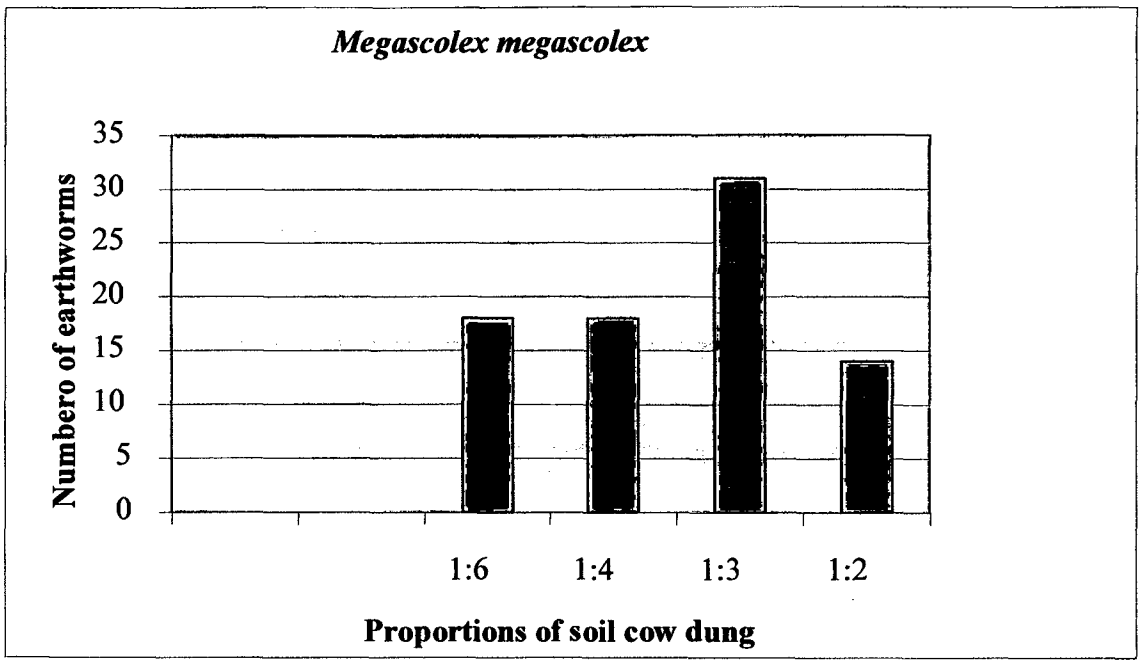


Fig. 2.4: Selection of best soil cow dung proportion for *Megascolex megascolex*
Number of earthworms after 32 days

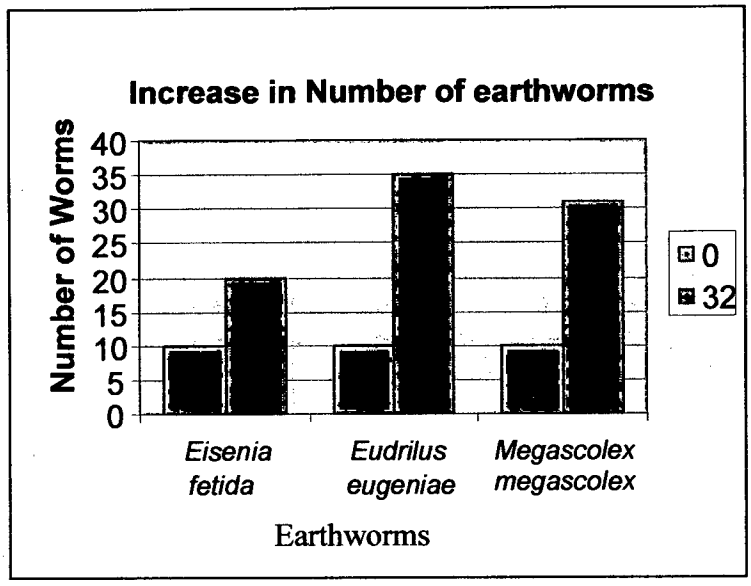


Fig. 2.5: Increase in number of earthworms for 1:3 proportion of soil + cow dung.

The pH values of the vermi-composts for 60 days are being observed to be marginally decreasing in the culture boxes *E. fetida,* 6.7 to 6.1; *E.eugeniae*, 6.7 to 6.0; and *M. megascolex*, 6.7 to 6.4. . However several workers have reported a decrease in pH and which was a significant parameter for the fecundity of earthworms in the pot experiments and field trials. Edwards and Lofty ,(1977) Doeksen,1964 ; Sahu,(2000); Barely(1961);Ranganathan V Christopher(1996);Raut et al.,(1997).

Percentage soil retained for vermi-compost with *Eisenia fetida,Eudrilus eugeniae and Megascolex megascolex* are placed in figures 2.6,2.7,2.8and the cumulative percentage retained on 850 micron sieve is presented in figure 2,9

it is evident that 50.06% of soil has been retained above 850 μ (Fig.2.9) which indicates the aggregation of soil particles with *Eisenia fetida,* 45.23 % of soil has been retained in case of *Eudrilus eugeniae* and 54.85 % has been retained in case of *Megascolex megascolex*(Fig.2.9). There fore it is evident that the aggregation is more in *Megascolex megascolex> Eisenia fetida > Eudrilus eugeniae* which is also depicted in the graph of particle size distribution as presented in figure 2.10.

Further the graph also clearly indicates the particle size distribution order, Press mud> industry soil > Vermicompost of *Megascolex megascolex*> Vermicompost of *Eisenia fetida* > Vermicompost of *Eudrilus eugeniae* (Fig.2.10).

The comparison of press mud particle size distribution to those of vermi-composts reveals that earthworms are responsible for grinding of the substrate, which makes the particle size distribution finer. The grinding capabilities of *Eudrilus eugeniae> Eisenia fetida> Megascolex megascolex*

The comparison of particle size distribution of soil and vermi-composts showed the grinding of soil for the initial period and later on especially for *Megascolex megasclex* it is aggregation, as the particle size shifts beyond the industry soil particle size distribution. The same is the case with *Eisenia fetida* and *Eudrilus eugeniae* but far later as compared to *Megascolex megascolex*. The *Megascolex megascolex* has highest aggregation capabilities as compared to other two species.

The soil type ascertained was SW, well graded (Punmia, 2000). The results of aggregation of soil by earthworm activity reported by using *Pheritima elongata* supports our findings Arora, (2002) Similar results on aggregation is being reported Stevenson,(1957) by the pigmented species *L terrstris , L rubellus, L costenus,D octaedra* and *B eiseni,* no aggregation in unpigmented species *D. cyaneum, O lactum, A.calignosa, A.longa* . The aggregation by deep burrower (Singh, 1997) and earthworms been reported Nijhawan (1952); Kale, (1993).

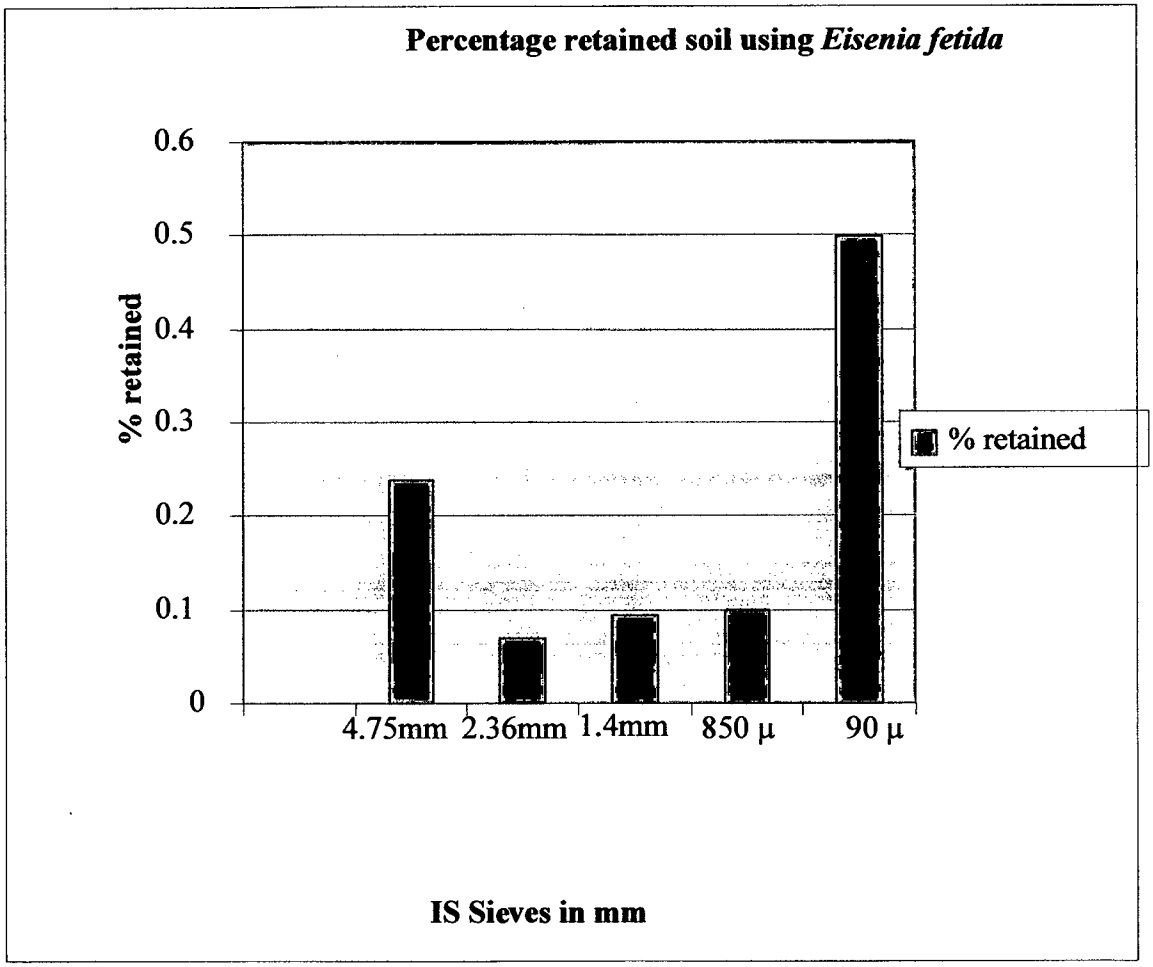


Fig. 2.6: Percentage soil-manure retained for vermi-compost with *Eisenia fetida*

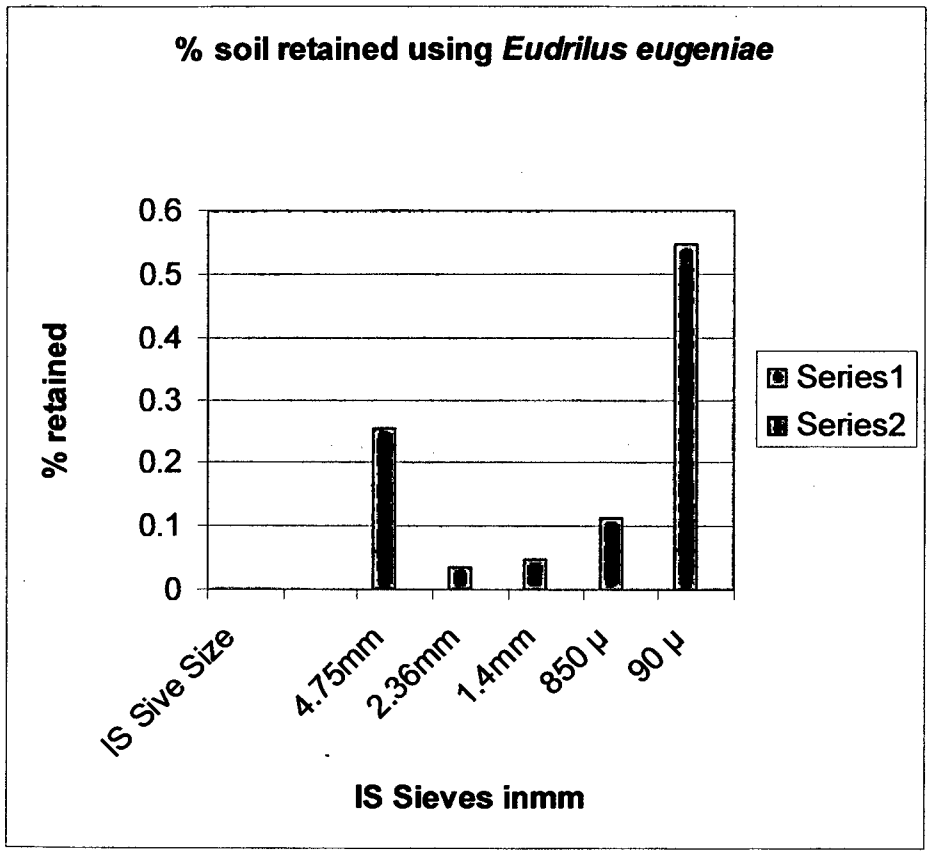


Fig. 2.7:- Percentage soil retained for vermicompost with *Eudrilus eugeniae*

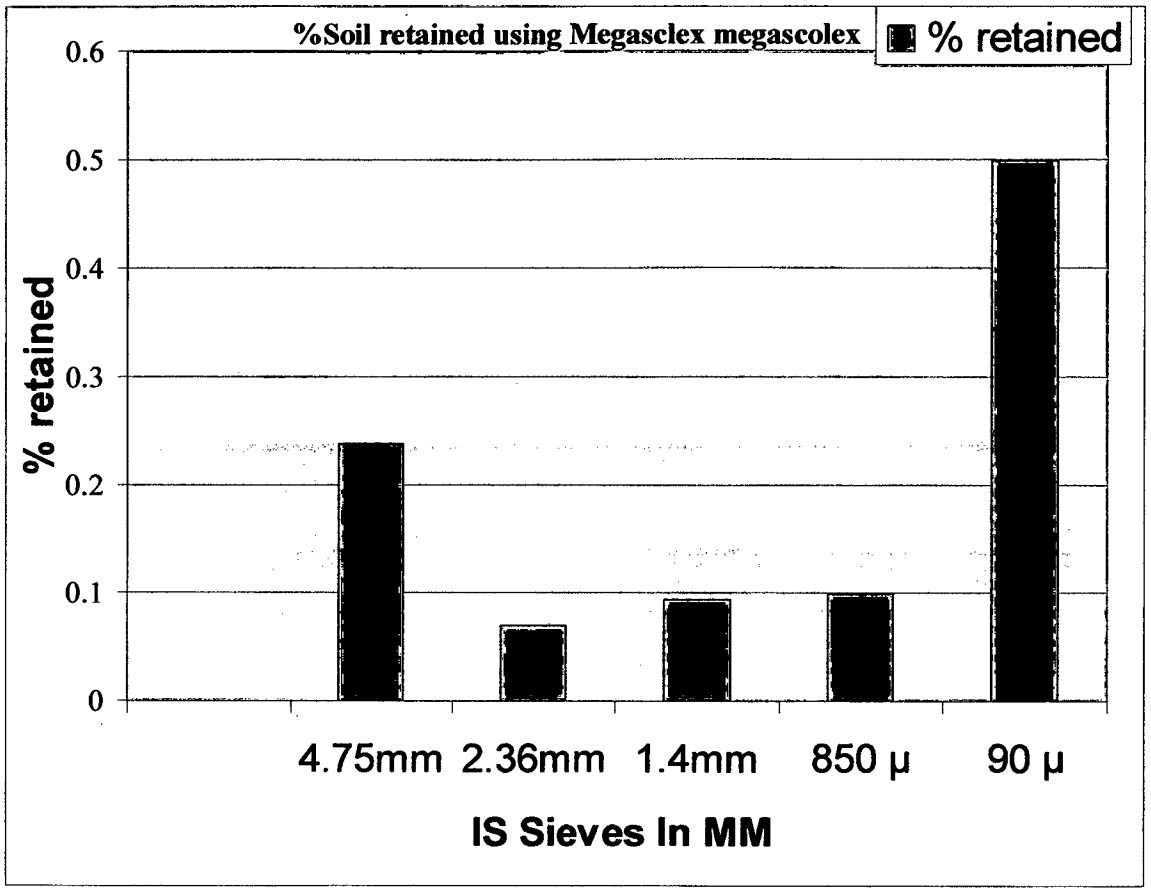


Fig. 2.8: Percentage soil retained for vermi-compost with *Megascolex megascolex*

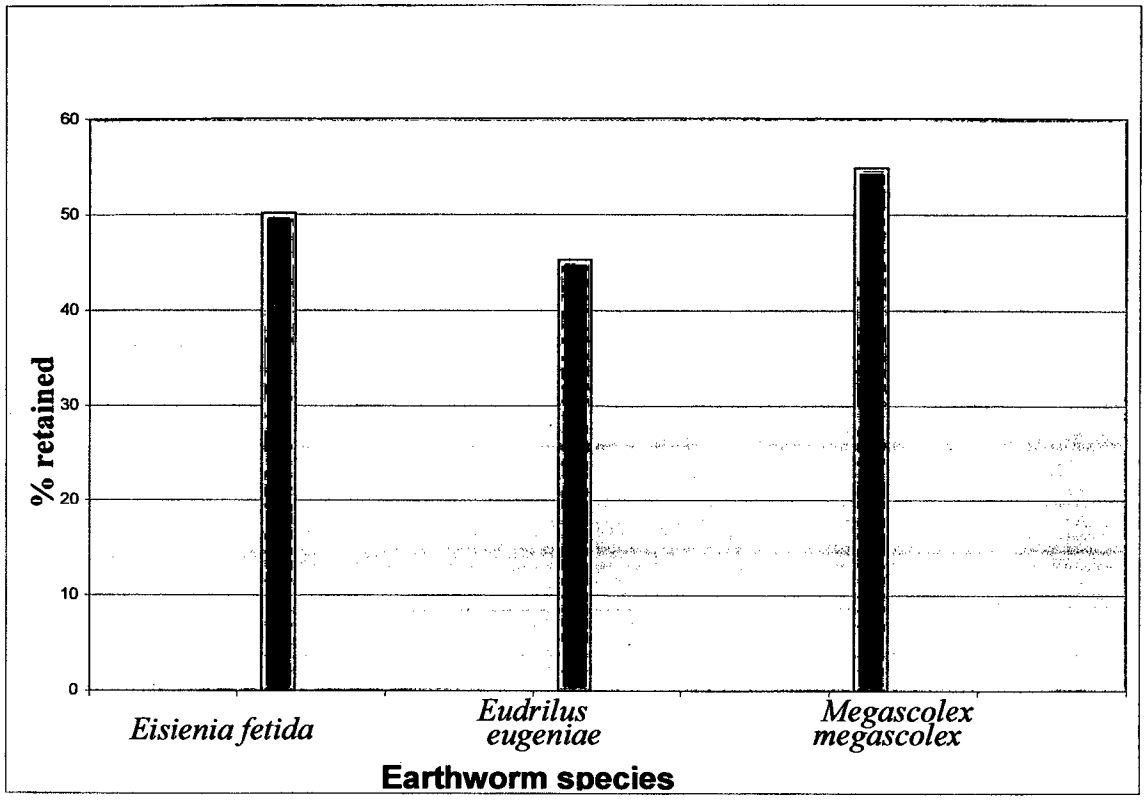


Fig. 2.9: Cumulative % of vermi-composts retained on 850μ Sieve size.

Table 2.5: Cumulative percent finer for soil, press mud and vermi-composts

IS Sieve Size	Soil Cumulative % Finer (N)	Press mud Cumulative % Finer (N)	*Eisenia fetida*	*Eudrilus eugeniae*	*Megascolex megascolex*
4.75 mm	83.50%	64%	76.2%	74.40%	69.1%
2.36 mm	66.50%	50%	69.24%	70.83%	53.82%
1.40 mm	52.00%	38%	59.84%	66.07%	49.77%
1.18 mm	46.00%	32.5%	----	------	----
850 µ	39.00%	20.5%	49.94%	54.77%	45.15%
600 µ	27.00%	13.5%	----	-----	-----
425 µ	11.00%	12%	-----	----	----
300 µ	9.50%	4.5%	-----	----	-----
150 µ	1.50%	1.5%	-----	----	-----
90 µ	0.50%	0	0	0	0
70 µ	0	0	0	0	0

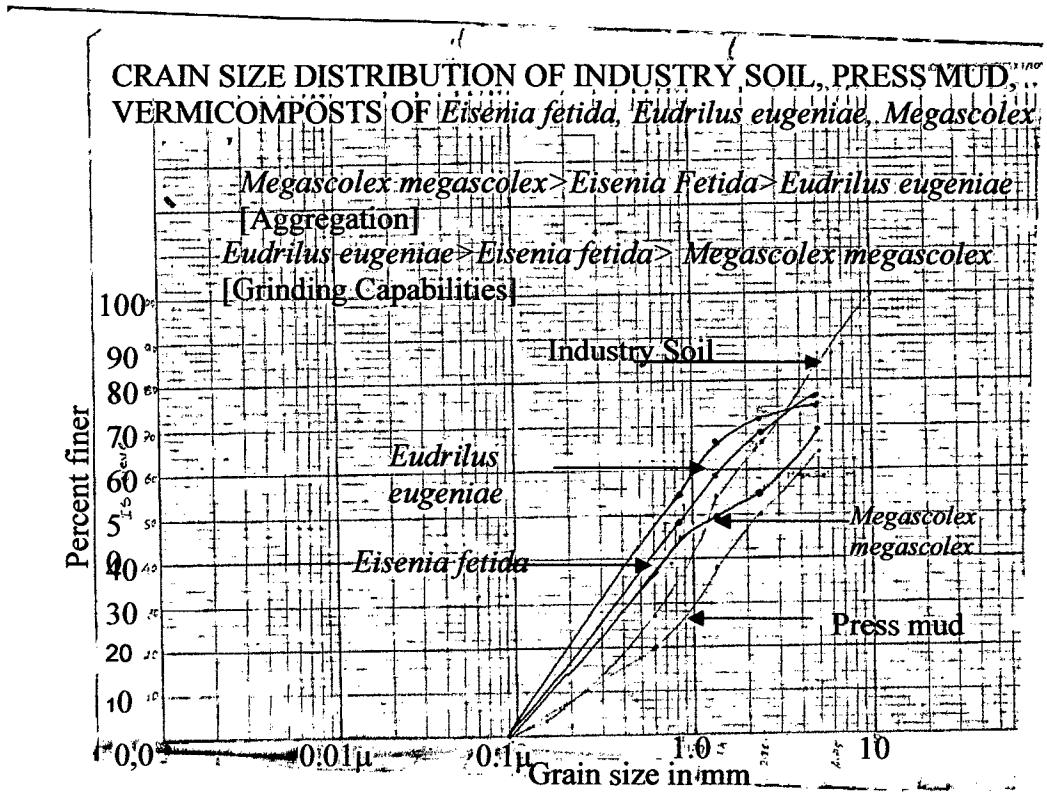


Fig. 2.10: Grain size Distribution curves for industry soil, press mud, and vermi-composts prepared from *Eisenia fetida, Eudrilus eugeniae, Megascolex megascolex.*

2.8 The significant points of study

- The filter mud had shown an organic carbon varying from 17 to 35 %, along with varying specific gravity of 0.5 to 0.7.
- The soil cow dung proportion showed the best results of earthworm production in 1: 3 soil Cow dung proportion.
- The order of highest number of earthworms recorded in soil cow dung proportion is *Eudrilus eugeniae* > *Megascolex megascolex* > *Eisenia fetida.*
- The industrial soil designated as SW Well graded sand is suitable for vermi-processing without any amendments in it.
- The particle size distribution of filter mud is higher than that of the industry soil.
- The substrate preference (Cow dung-soil) for grinding capabilities is *Eudrilus eugeniae*> *Eisenia fetida*> *Megascolex megascolex* .
- The order of aggregation of vermi-compost of 1:3 soil cow-dung is *Megascolex megascolex*>*Eisinea fetida*>*Eudrilus eugeniae.*
- The aggregation is more in the soil with 90μ sieve size.
- The fold increase due to supplementation of cow dung, varied from 1.68,2,23, 2.45, 2.05 times in case of *Eisenia fetida*, Co culture of *Eisenia* and *Eudrilus eugenae*, *Eudrilus eugeniae*, *Megascolex megascolex* respectively

The results obtained on the physico chemical characteristics of the raw materials has revealed that the press mud is having an organic carbon of 20-35 % and temperature 100^0C needs to be maintained in open storage prior to subjecting for vermiprocessing.

The industrial soil designated as SW (well Graded) showed native earthworms during soil sampling is suitable for vermi-processing without any soil amendments along with optimum proportion of 1:3. Three earthworm inoculums were used surface feeder *Eisenia fetida* and *Eudrilus eugenae* including a native worm *Megascolex megascolex*, deep burrower. The results have shown substrate preference of *Eudrilus eugeniae*> *Eisenia fetida*> *Megascolex megascolex* have the aggregation of vermicomposts of 1:3 proportion as *Megascolex megascolecx*> *Eisenia fetida*> *Eudrilus eugeniae*.

It was therefore, envisaged to study further the composting of press mud with the optimized soil cow dung proportion in the laboratory as well as field trials. The experimental lay out and data on composts obtained including the isolation of microorganisms their identification, Enzyme activities are presented in the fallowing chapter.

CHAPTER III

Laboratory and field trials for vermi-composting of press mud and characteristics of composts

Chapter 3.0: Laboratory and field trials for vermi-composting of press mud and characteristics of composts

3.1 Introduction:

The sugar industries generating filter mud has become a cause of concern in the present day contents due to the problems faced by its haphazard disposal. The industries have a disjointed approach to take up the waste management as an integral part of industrial process. The need of appropriate technologies with pin pointing experimentation is of great importance in arriving at standard procedures to be followed to adopt the waste management practice. Therefore, in the present chapter the filter mud as a substrate subjected to three varieties of worms with the optimized soil cow dung proportion. The laboratory as well as field trials was undertaken keeping in view bringing sustainability in vermi-composting.

3.2 Materials and methods:

A) Preparation of culture box vermibeds for laboratory studies

Glass culture box of size 42 cm x 42 cm x 20 cm were fed with brick bats for 1 cm thick, there after soil and cow dung in 1:3 proportion (wet weight basis) was laid to give a thickness of 5 cm and maintained at 70% water content. Hundred worms of each variety were introduced in the culture box.

The filter mud was fed to the vermi beds and the observations for, juvenile predominance is recorded. (Plate 3.1)

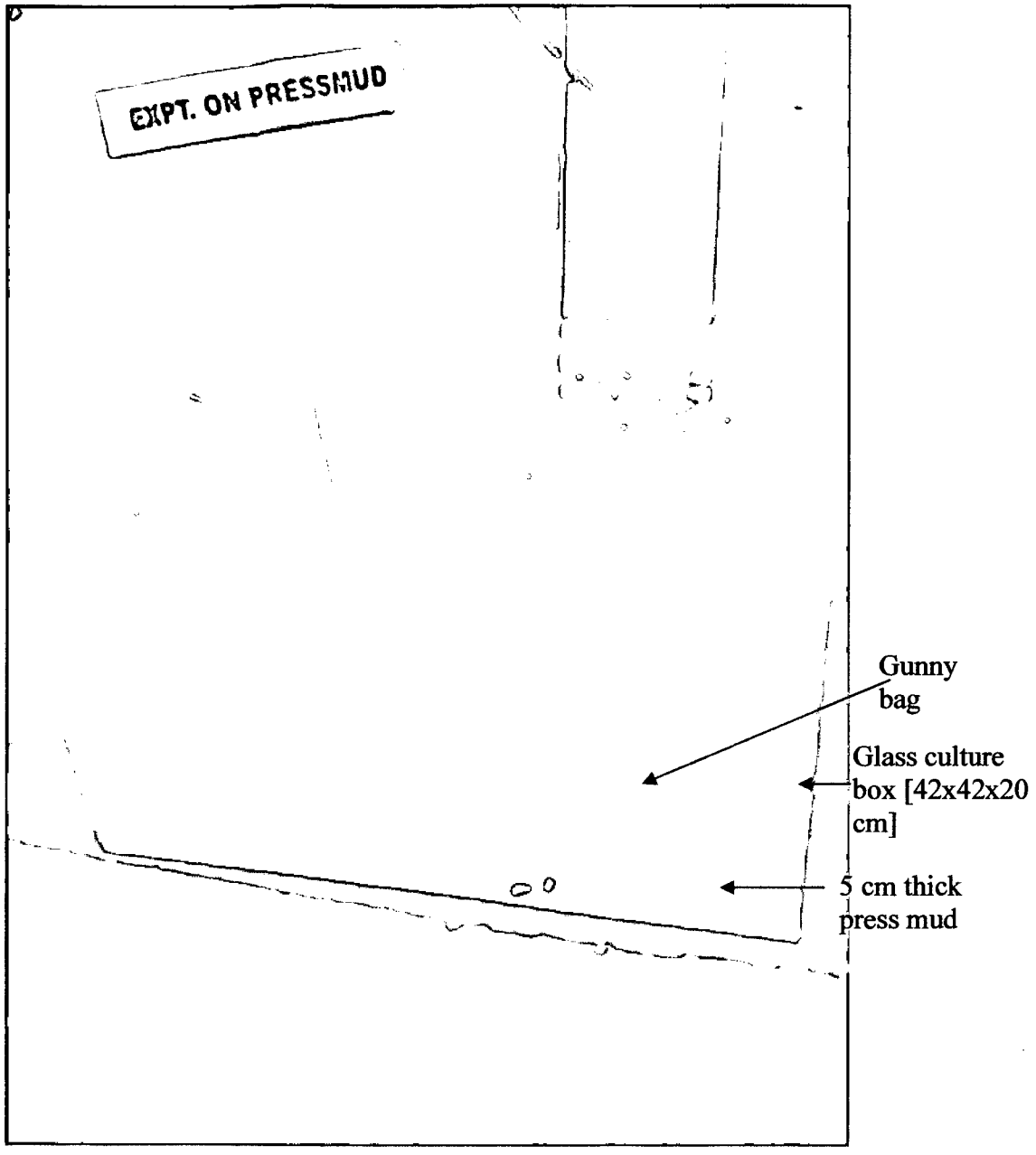


Plate 3.1: Laboratory biodegradation culture box

B) Vermi beds field trial at the Sanjeevani sugar factory site:

For deep borrower species, the beds were prepared on a tank of 1m x 1m x 0.6 m height. Initially 2.5 cm thick brickbats were spread evenly on this cow dung and about seven days old (easily biodegradable substrate) having neutral pH was spread in the tank. Inoculums, at the rate of 100 earthworms per tank were introduced. This was covered with cow-dung of 5 cm thick.

For surface feeder species a layer of bedding material (grass) was laid to a thickness of about 5 cm at the bottom of beds (1x1x 0.6m dimension). Above this a cow-dung layer of 5 cm thick (7 days old) was evenly spread. There after the hundred worms were introduced in to the beds. The worms developed within 2 to 3 weeks and the beds were used for further experimental purposes. The beds were covered with paddy and jute bags. A wire mesh and jute bag on top of the tank was kept to minimize evaporation. The moisture level was maintained at 60-70 %. The plates of vermi-composting of filter mud field trials were presented in plate 3.2 to 3.5.

Feeding details of these beds were further loaded with uniform thickness of 5cm for vermi-processing.

3.3 Analysis of composts

A periodical measurement of juvenile predominance, pH, and temperature was carried out for lab and field trial samples during the period of 45 days. The moisture level of 60-70% was maintained in the vermibeds,throughout theexperiment.

A) Physico chemical and Geotechnical characteristics: The compost samples were analyzed for total solids, organic carbon, nutrient value, and water holding capacity, geotechnical properties using standard methods as detailed in Appendix B.

C) Microbiological analysis: Total viable count of bacteria, fungi, and yeasts in the composts were determined by spread plate method on the respective media, plates were incubated for 24 to 72 hrs and the colony forming units were counted.

3.4 Results and discussion:

3.4.1 Vermicompost of filter mud:

The press mud laid with uniform thickness was regularly monitored for changes with regards to visual observations for biodegradation.

The biodegradation of filter mud was observed at regular intervals in the laboratory and field trials the results presented in Table 3.1and 3.2. The pH maintained in the beds was nearly neutral 6.7- 6.8 and the moisture level 50-70%. The conversion of filter mud to vermi-compost recorded for laboratory set up showed a time of 45 days for *Eisenia fetida,* 40 days for *Eudrilus eugenae*, co culture of *Eisenia fetida* and *Eudrilus eugeniae* , *Megascolex megascolex.* The conversion of filter mud to vermi-compost recorded for field set up showed a similar time of 40 days for *Eisenia fetida, Eudrilus eugenae*, co culture of *Eisenia fetida* and *Eudrilus eugeniae, and* 35 days for *Megascolex megascolex*

It is interesting to note that the bioconversion of filter mud with *Eisenia fetida* has taken more than 45 days and the rest of the species showed complete conversion at 40 days. Uniform appearance of cast everywhere was observed in the bed with odor completely removed. The order of increase in number of earthworms by hand sorting showed the number of *Eudrilus eugeniae and Megascolex megascolex*> that of *Eisenia fetida* and *Eudrilus eugeniae and Eisenia fetida* for both field and labarotary trials. The highest number recorded for *Megascolex megascolex* (490) for field trial and fallowed by *Eudrilus eugeniae* (390, 380) for field and laboratory trials.

There are few reports on biodegradation of filter mud. Jambekar (1992) reported biodegradation of industrial waste filter mud using *Eisenia fetida*, *Eudrilus eugeniae*. and *Perionyx arboricla* and suggested efficient use of these species. Where as Mala *et al.*, (1998) adopted *Limpitto mauritii* for her study in press mud. The report of Giraddi and Tippanavar (2000) showed a bioconversion time of 85 days, which is almost the double of our findings for filter mud bioconversion using *Eudrilus eugeniae*. The difference could be due to the rate and thickness of organic loading. The report of Singh (1997) fully justifies our bioconversion time varying from 35 to 40 days using deep burrower species *Pheritima elongata.*

Table 3.1: Biodegradation study of filter mud:

Parameter	*Esienia fetida*	*Eudrilus eugeniae*	*E fetida + E eugeniae*	*Megascolex megascolex*
Number	100	100	50+50	100
Soil + Cow dung Praportion	1:3	1:3	1:3	1:3
Av. Length cm	6.8	6.8	6.8	8-10
Av. Weight gm	80-90	85-90	85-95	85-100
pH	6.8	6.8	6.8	6.7
Moisture %	60-70	60-70	60-70	60-70
Bio conversion Time [Days]	45	40	40	40
Increase in Number	2.9 times	3.8 times	3.10 times	3.8 times

Table 3.2: Field trial of filter mud biodegradation:

	Tpes of species			
Parameters	*Esenia fetida*	*Eudrilus euginiae*	*Co culture of E fetida and E eugeniae*	*Megascolex megascolex*
No. of worms	100	100	100	100
Soil+cowdung Proportion	1:3	1:3	1:3	1:3
Average Length	6-8 cms	6-8 cms	6-8 cms	8-10 cms
Average Weight	80-90 gms	85-95 gms	80-95 gms	85-100 gms
pH	6.7	6.7	6.7	6.7
Moisture	60-70	60-70	60-70	60-70
Time required	45 days	40 days	40 days	35 days
No. of worms	290	390	310	490
Average Length	10-12	10-12	10-12	15-20

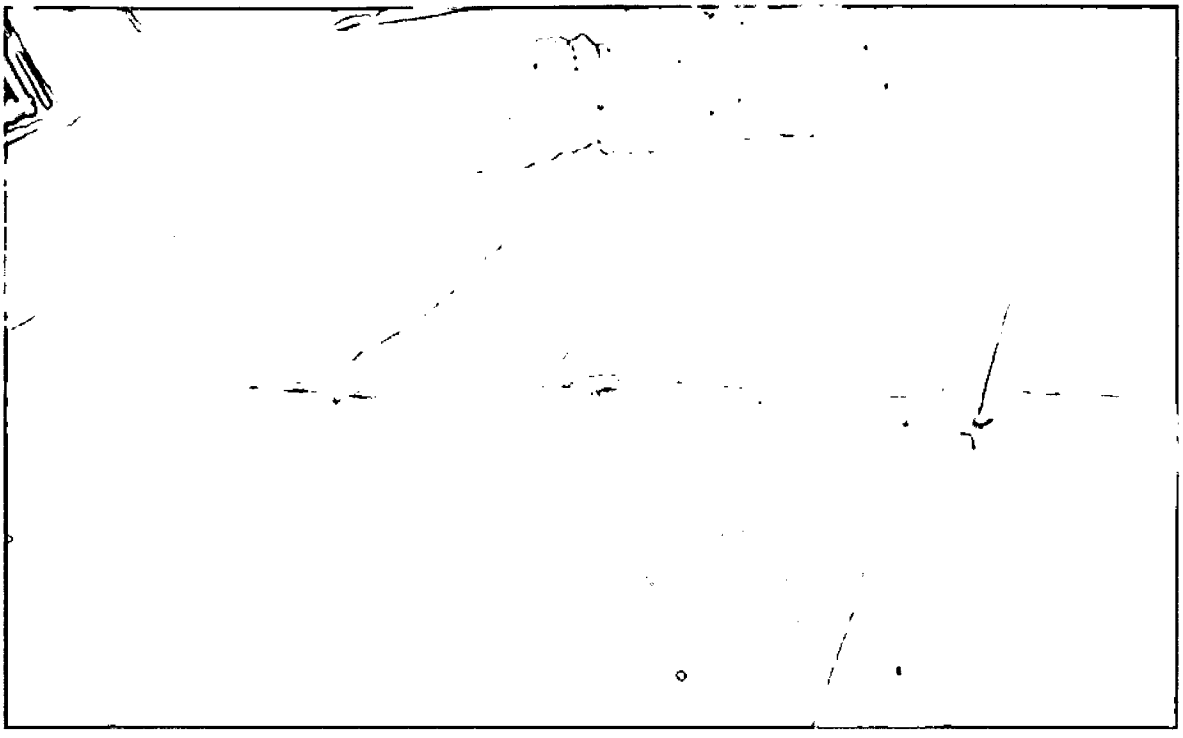


Plate 3.2: Field trial vermin bed covered with wire mesh and jute bags.

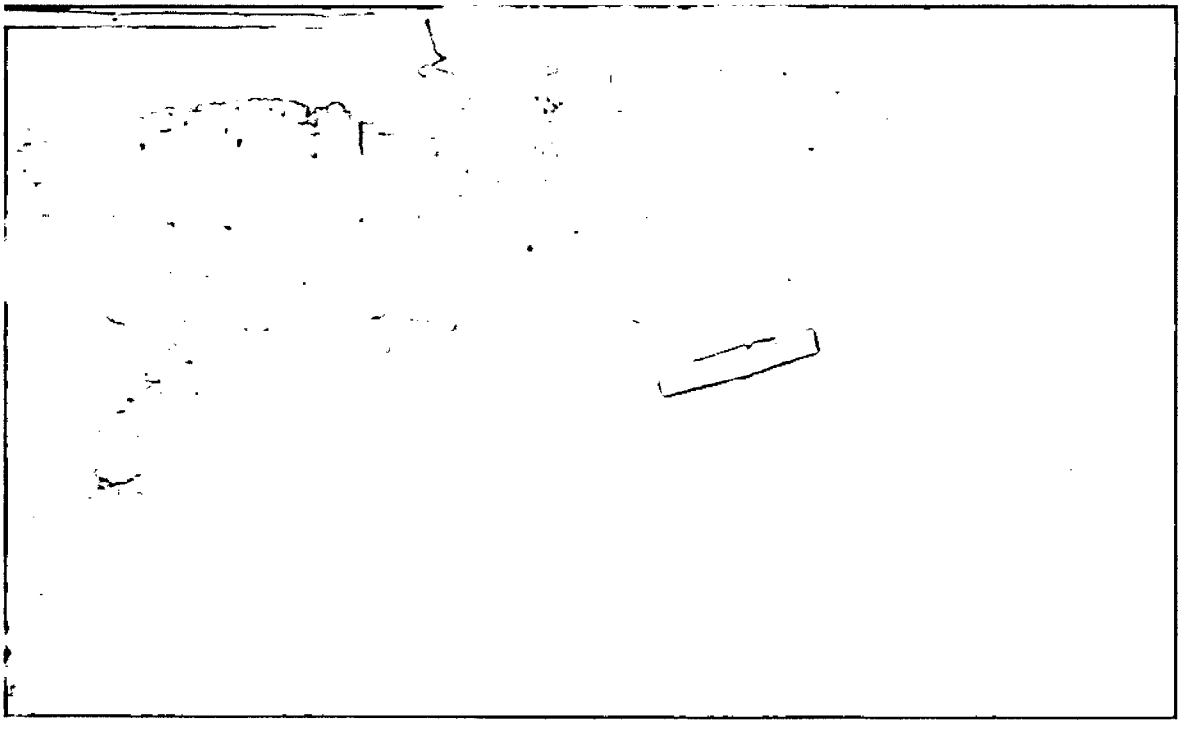


Plate 3.3: Field trials bed covered with wet jute bags with deep burrower species

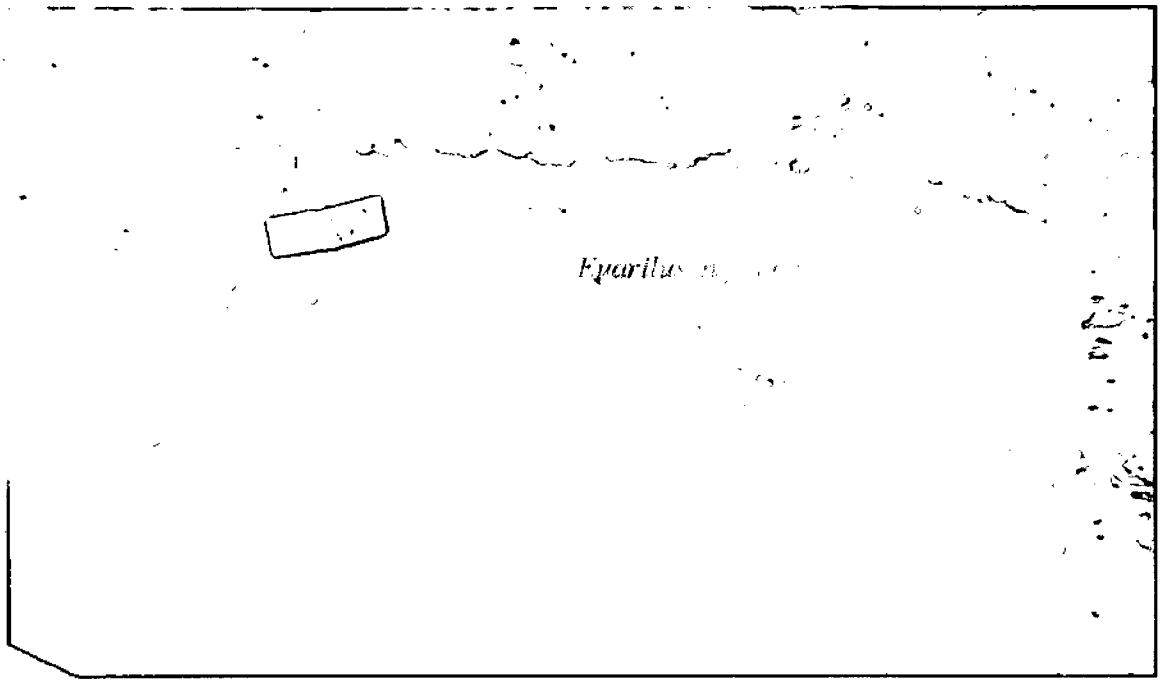


Plate 3.4: Field trials showing bed covered with jute bags with surface feeder

Plate 3.5: Field trials showing ready vermi-compost

3.4.2 Juvenile predominance:

The energy demand of small immature juvenile earthworms is greater than that of large non-clitellate earthworms. Juveniles have accordingly been reported to consume more food (Geeta and Reddy, 1997; Edwards and Bater, 1992; Edwards *et.al,* 1998).

Hatchlings and juveniles increase in biomass faster, process waste by body weight than adults. Juvenile predominance has therefore been taken as a criterion for assessment of substrate preference. Observations were madder on the surface of the beds along with removal of compost up to full depth and hand sorting juveniles was resorted for recording juvenile predominance.

The physical observations with regards to juvenile predominance for laboratory and field trials presented in Table 3.3 revealed that at zero day the earthworms added produced juveniles. The 20 days observation showed the highest juveniles in *Megascolex megascolex as* compared with the other two. While at 30^{th} day *Megascolex megascolex* still showed higher number of juveniles where as the *Eudrilus eugeniae* produced more juveniles as compared to *Eisenia fetida.* Juveniles are the indicators of energy transformation and hence biodegradation indicating the species preferences of substrates.

The plates 3.6 and 3.7 show the vermi-composts in polythene bags immediately taken after adding to the bags and after keeping the bags in refrigerator for 15 minutes respectively. The bags after 15 minutes showed juveniles move from the compost to radial outward. The plate 3.6 and 3.7 also show the movement of juveniles clearly with order of juveniles greater with *Megasclex megascolex* > *Eudrilus eugeniae*> *Eisenia fetida.* This along with observations personally made together the results of juvenile predominance are gauged and recorded.

The juvenile predominance of the field trials also showed similar results as that of laboratory studies. The order of juvenile predominance, which is a measure of substrate preference, remains the same. *Megascolex. megascolex* > *Eudrlus eugeniae* > *Eisenia fetida.* The co culture produced the same number of juveniles as that of *Eudrius eugeniae* alone.

The bags again kept for continuous observations at every 2 day intervals. The good earthy smell indicates or no smell of the original material indicates the compost is stable and be harvested. This was used as one of the indicators of production of stable compost and bioconversion time recording (Rodale, 1967; Giraddi and Tippanavar,2000).

Table 3.3: Visual observations for juvenile predominance:

Species	Observations in days					
	0^{th} day		20^{th} day		40^{th} day	
	Lab	Field	Lab	Field	Lab	Field
Eisenia fetida	------	-----	x	x	x x x	xxx
Eudrilus eugenae	-------	----	xx	xx	xxxx	xxxx
Eisenia fetida + *Eudrilus eugenae*	-------	-----	x x	xx	x x x x	xxxx
Megascolex megascolex	-----	------	x x x	xxx	x x x x x	xxxxxx

-Nil, x = Poor, x x = Good ,x x x = Very good, x x x x = Excellent

Eisenia foetida

Eudrilus eugeniae

5 gm vermicompost placed in Polythene bags

Megascolex megascolex

Plate 3.6: Fresh compost sealed in polythene bags

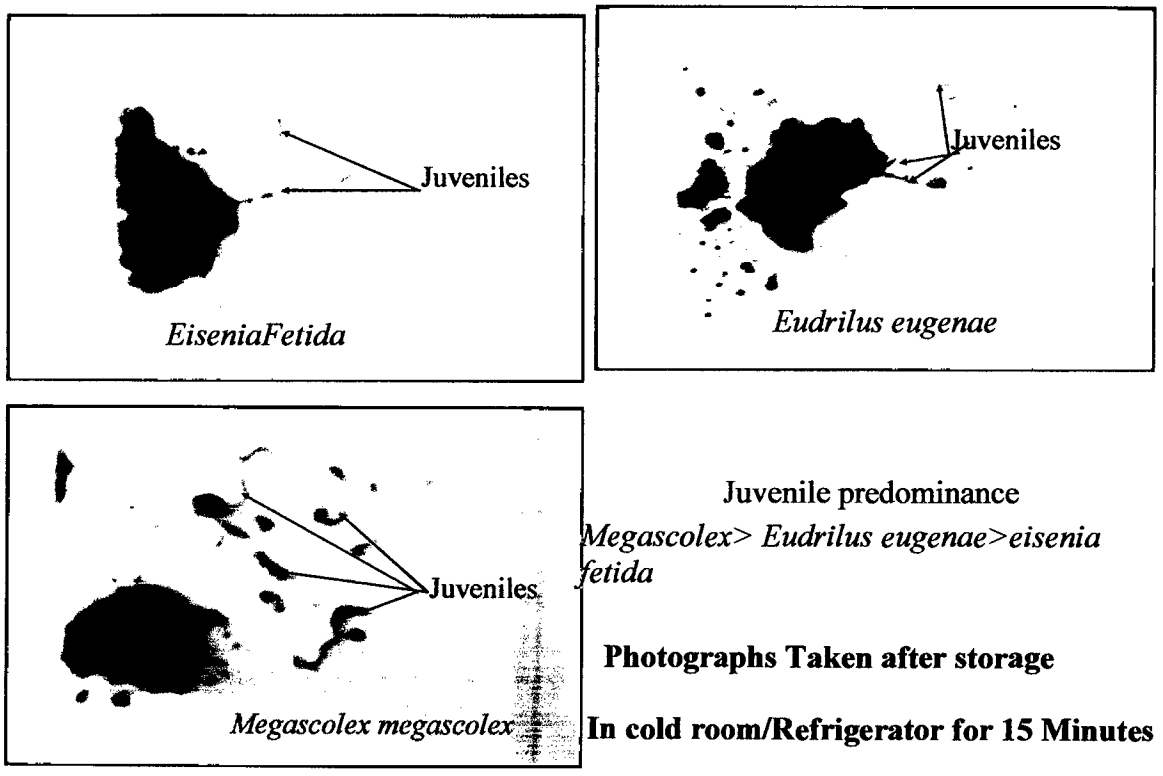


Plate 3.7: Juveniles seen moving away

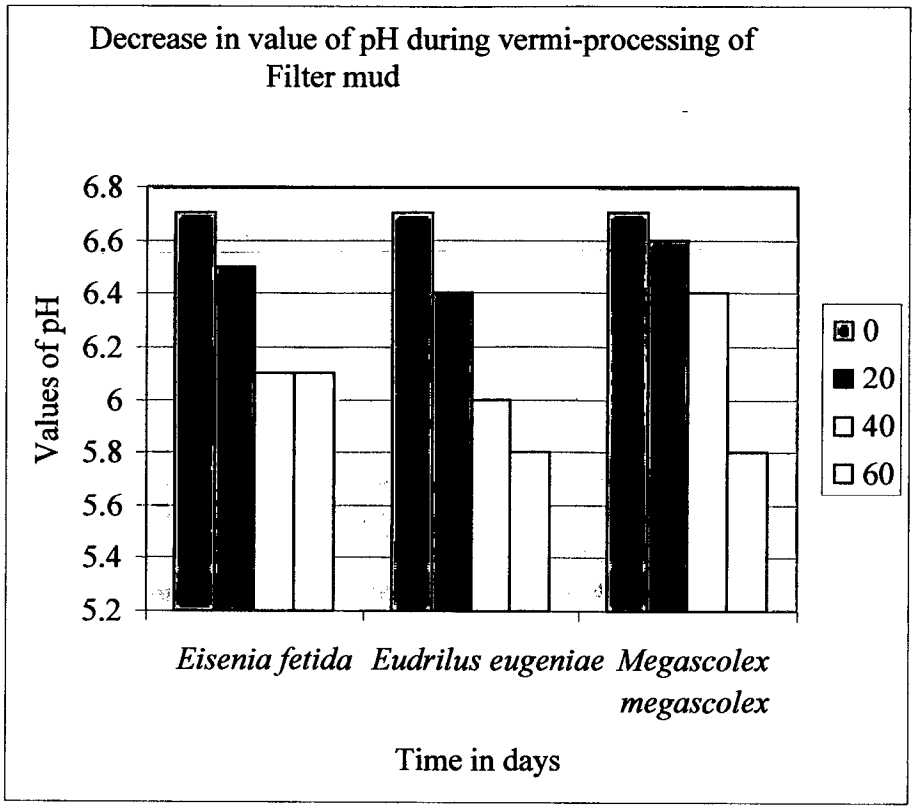


Fig. 3.1: Variation in pH during Field trials.

3.4.3. Variation in pH during vermi-processing:

The decrease in pH values of the vermi-composts for 60 days is being represented graphically in figure 6.0. The soil cow dung proportion chosen was 1:3 for all the three cases. The reduction in pH values from 6.7 to 6.1, 6.7 to 6.0, and 6.7 to 6.4 in the case of composts prepared from *Eisenia fetida, Eudrilus eugeniae, and Megascolex megascolex.* A decrease in pH from 9.0 to 8.3 of alkaline soils in pot experiment on vermiculture is reported by Barely (1961). Earthworms are sensitive to the hydrogen ion concentration, so it is observed that pH is a significant factor that limits the distribution, number of earthworms and species of earthworm in a particular type of soil.

These findings are almost similar to the results obtained during the soil cow dung proportion study (Edwards and Lofty, 1977; Petrov, 1946; Richardson, 1938; Edwards, 1985; Singh, 1997).

. *A calignosa is* reported to be in diapose with a pH of 6.4 and less Doeksen, 1964; Edwards and Lofty, (1977). For increasing the productivity of bioconversion a pH range of 5.5 to 9.0 is being suggested for Indian soils(Edwards,1985;Munnoli,2000;Gajalakshmi 2004; Ranganathan,1996).The decrease in pH from 7.7 to 8.8, 6.5,7.0 is reported in the vermi compost's prepared from leaf, grass, straw and water hyacinth; Leaf litter and home garbage respectively Sahu,(2000). The decrease in pH will also helps in deciding whether the soil of the industry needs any amendments or not before setting up of the vermi-composting unit.

Makeschin,(1991) report says liming and gypsum fertilization in acid forest soils increased the earthworm activity due to increased pH, Such application are found to be of great help in amending the soil suitably for waste land development.

Where as *Dendrobaena madeirensis, Dendrobaena octaedra, Dendrobaena pygmae, Eisenia eisenia* and *Allolobophora oliveirae* preffered acid organic soils, with low calcium and magnesium contents and high aluminum content, as reported by Briones et al., (1992). There fore soil pH is a factor to be noticed for the successful setting up of vermin-compost units. It is therefore evident that the growth and survival of earthworms is adversely affected in extreme acidic and alkaline conditions. For majority of the species a neutral pH of soil is found to be suitable for maximizing the efficiency in waste management.

3.4.5 Vermi-compost samples:

The visuals on vermi-composts of filter mud obtained from the field trials are presented in Plate 3.13. The visuals appear to have higher size particles in case of *Megacolex megascolex* > *Eudrilus eugeniae*> *Eisenia fetida.*

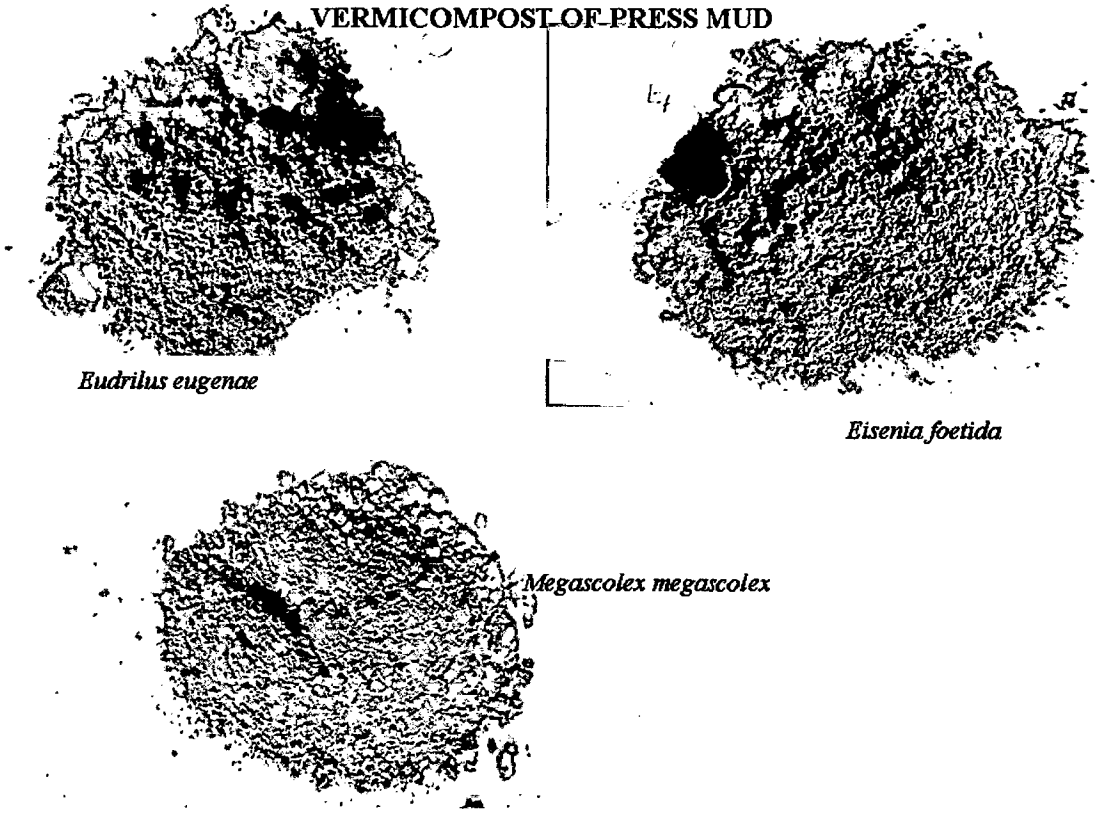


Plate 3.8: Vermi-composts samples

3.4.6 Total solids and organic carbon:

The comparison of decrease in organic carbon in ingesta and egesta presented in Fig 3.5 The variations in total solids for all the three cases of *Eisenia fetida, Eudrilus eugeniae, Megascolex megascolex* have shown increasing trend (Figs 3.2, 3.3, 3.4) depicting conversion of organic matter to hums like substances also indicates the utilization of substrate by the earthworms. The increase was found to be 51.85%, 23.65%, 13.94 % in case of *Eisenia foetida, Eudrius eugeniae, and Megascolex megascolex* respectively. A marginal increase in total solids during vermin processing of industrial wastes is reported by Singh, (1997), where as vermi-composting of potato waste had shown 2.25 times increase in total solids Munnoli, (2000).

The organic carbon has shown a decreasing trend in vermi compost samples of all the three earthworm species depicting continuous utilization of substrates with release of carbon dioxide and final conversion of wastes to composts. The decrease inorganic carbon and increase in total solids as seen with composting using three species confirm the efficiency of selected species of worms. The decrease in organic carbon 56.25%, 49.79%, 47.9% clearly shows the superiority of *Eudrilus eugeniae* as compared with other earthworms. Therefore the order of grading the vermicomposts based on decrease in organic carbon is *Eudrilus eugeniae* > *Megascolex megascolex* > *Eisenia fetida.*

It is evident that the extent of biodegradation with *Eudrilus eugeniae* and *Eisenia fetida* is achieved in 40 days where as the same trend is achieved in 35 days with *Megascolex megacolex.*

The marked selectivity of earthworms towards substrate preference is been reported by Ramachandran (1997) and influence on worm population and cocoon production of filter mud when used in combination with cow dung has been reported by Giraddi (2002).

The food processing waste in general have been reported to be the best substrates for vermi-processing Piccone et al., (1986); Singh (1997).

A similar decrease in organic carbon of 41 to 28 % being reported by Morgan and Morgan (1992) in case of organic wastes with *Lumbricus terristris* and *Approctodea longa.*

A decrease of 28 to 5.5 % in case of ingesta and egesta by singh, (1997) by using *Pheritima elongate* species. Studies on potato and distillery sludge waste reported a decrease of 87.07% and 81.8%; 39.43% and 32.12 % in ingesta and egesta when treated with *Pheritima elongata.* (Munnoli, 2000, Singh, 1997)

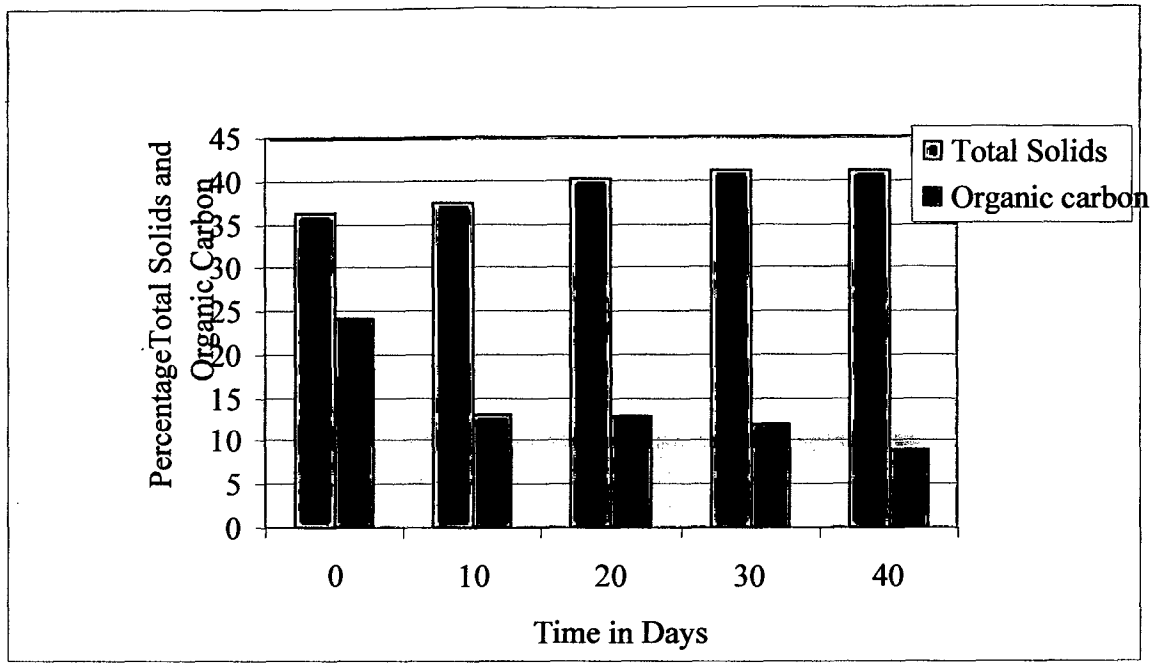


Fig. 3.2: Total solids and organic carbon during vermi-processing by *Megascolex megascolex.*

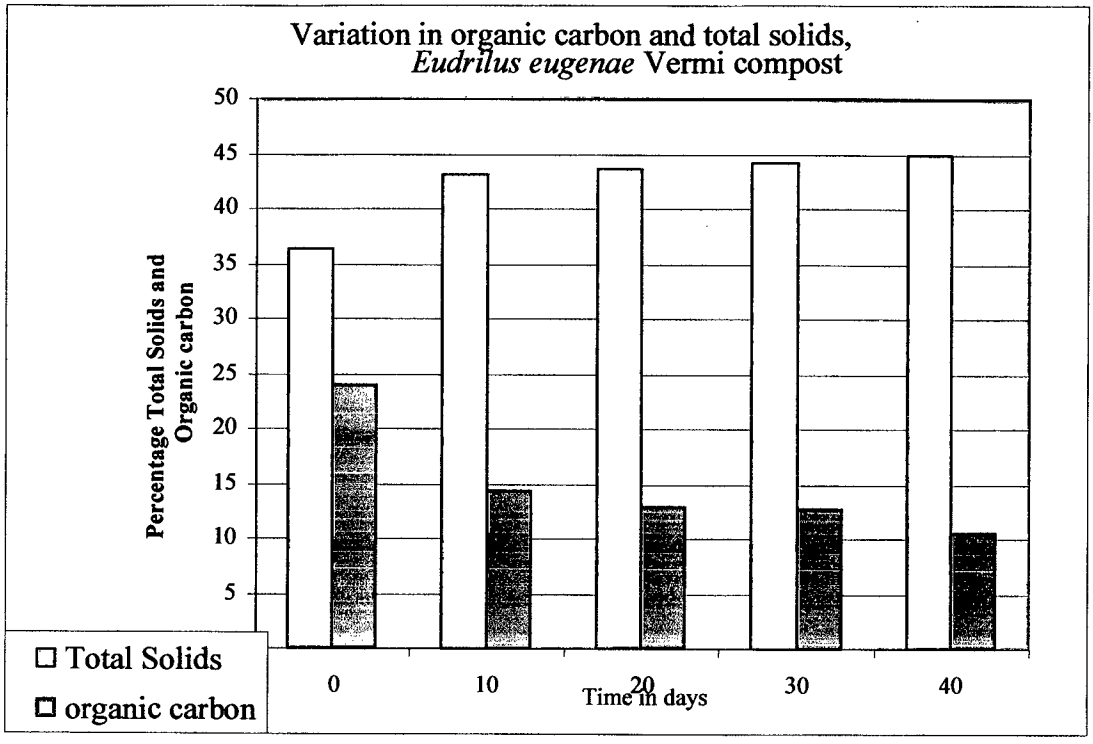


Fig. 3.3: Total solids and organic carbon during vermi-processing by *Eudrilus eugenae*

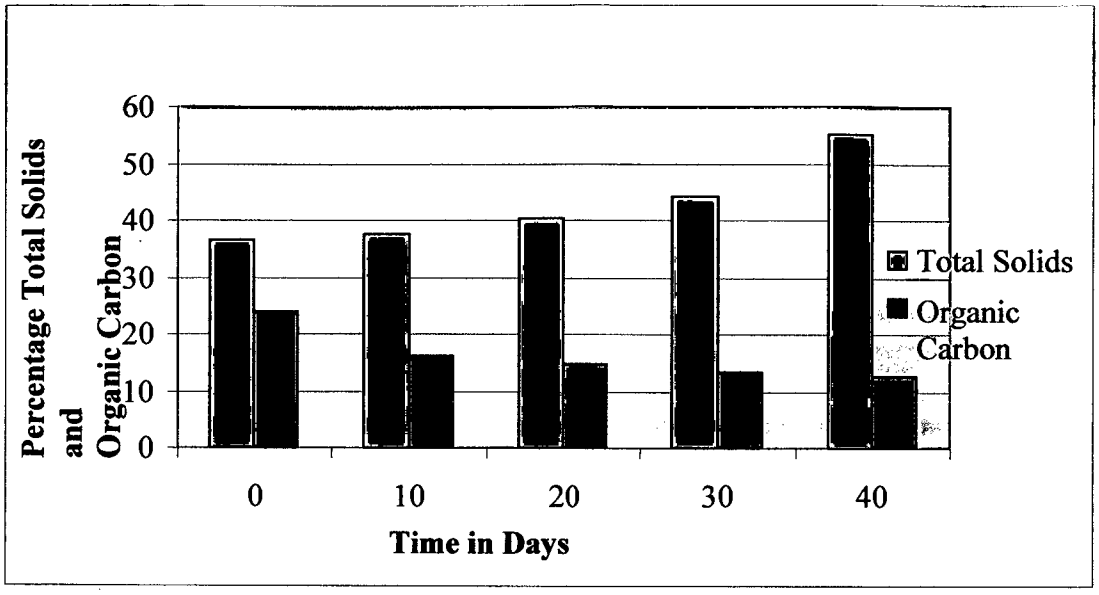


Fig. 3.4: Total solids and organic carbon during vermi-processing by *Eisenia fetida*

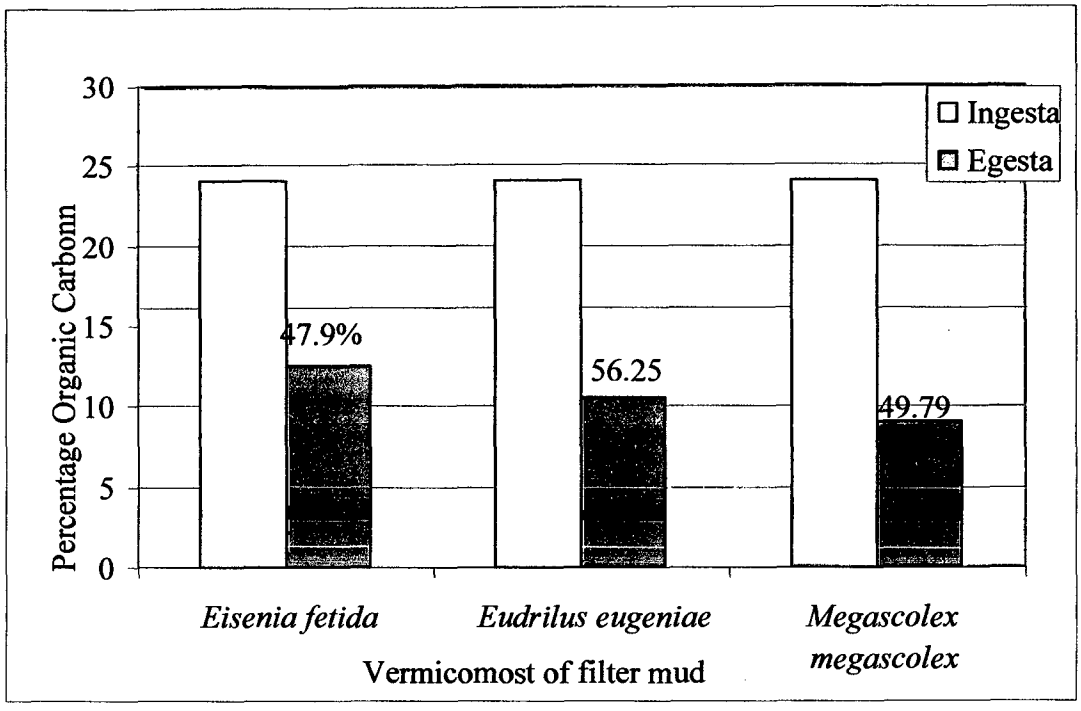


Fig. 3.5: Decrease in organic carbon in Ingesta and Egesta.

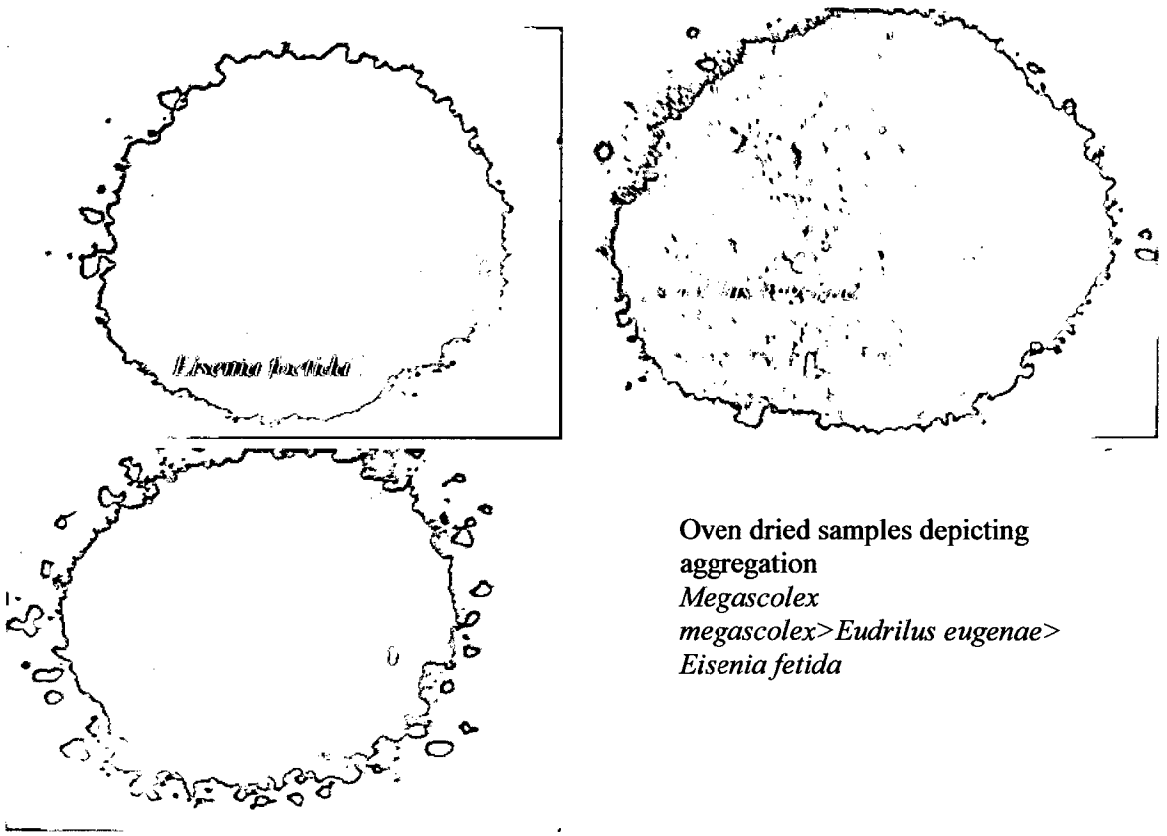
3.4.7: Particle size distribution:

The particle size distribution for industry soil and oven dried vermin composts of press mud is presented in a standard graph in Table 3.4 & Fig.3.6 which shows that the particle size of the filter mud is much higher than the industry soil. Our experimental study regards to species selection using cow dung as substrate with industry soil < 850 μ had shown

Both aggregation and fragmentation (grinding) activity of earthworms. The soil under went bio-changes and about 50.06% with *Eisenia fetida*, 45.23 % *Eudrilus euegniae,and* 54.85 % in case of *Megascolex megascolex* aggregation is achieved. It concludes that aggregation is more in *Megascolex megascolex> Eisenia fetida > Eudrilus eugeniae.* This was represented graphically in Fig. 2.10.Indicating vermi-composts of cow dung alone showed an order of particle size distribution ie., Filter mud > Industry soil> *Megascolex megascolex> Eisenia fetida > Eudrilus eugeniae.*

The same could be compared with the vermi-compost of filter mud the particle size distribution which almost remains the same as both the graphs are showing identical distribution. One interesting thing is that *Megascolex megascolex* vermi-compost is leading in aggregation as compared with the other two vermi-composts as there is a particle size distribution shown which shifts beyond the particle size distribution of filter mud. This had not happened in case of vermin compost of cow dung. This clearly depicts the functional role of deep burrower earthworms in building the soil. This is of interest for harnessing this property to develop degraded lands.

In totality the aggregation order is *Megascolex megascolex>Eisinea fetida>Eudrilus eugeniae* which is supported by the aggregation from visuals presented in plate 3.9



Oven dried samples depicting aggregation *Megascolex megascolex>Eudrilus eugenae> Eisenia fetida*

Plate 3.9: Oven dried samples of vermi-composts.

Table 3.4: Cumulative percentage finer of vermi-composts

IS Sieve Size/Particle size(mm)	*Eisenia fetida* Cumulative %Finer	*Eudrilus eugeniae* Cumulative %Finer	*Megasclex megascolex* Cumulative %Finer
4.75	81.2	81.82	95.46
2.36	67.54	69.73	58.65
1.18	52.45	54.01	44.11
850 μ	46.17	49.29	26.84
600 μ	37.75	40.93	21.39
300 μ	18.04	20.66	15.94
90 μ	3.66	4.57	2.31
70 μ	1.72	0	0

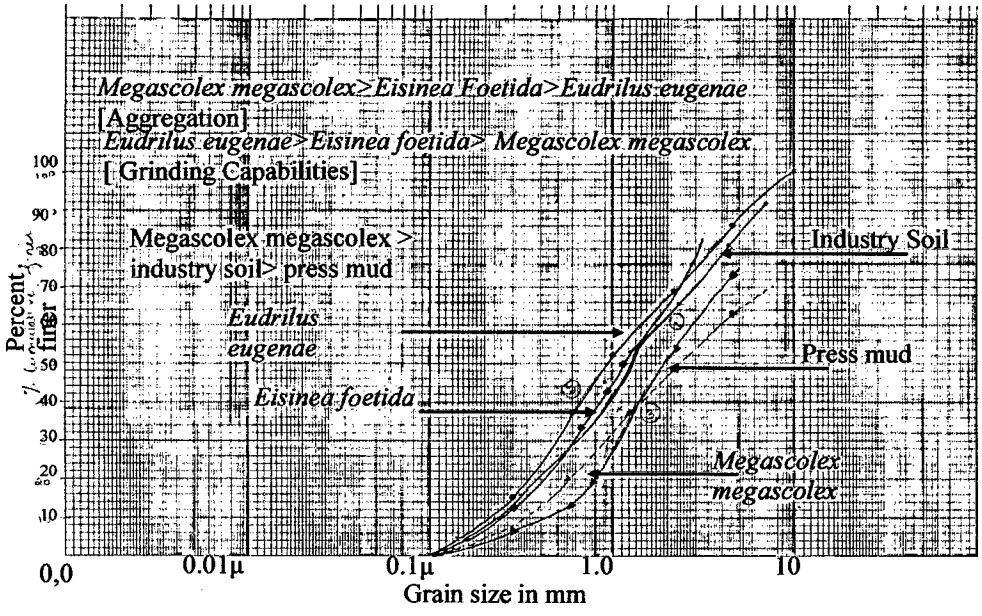
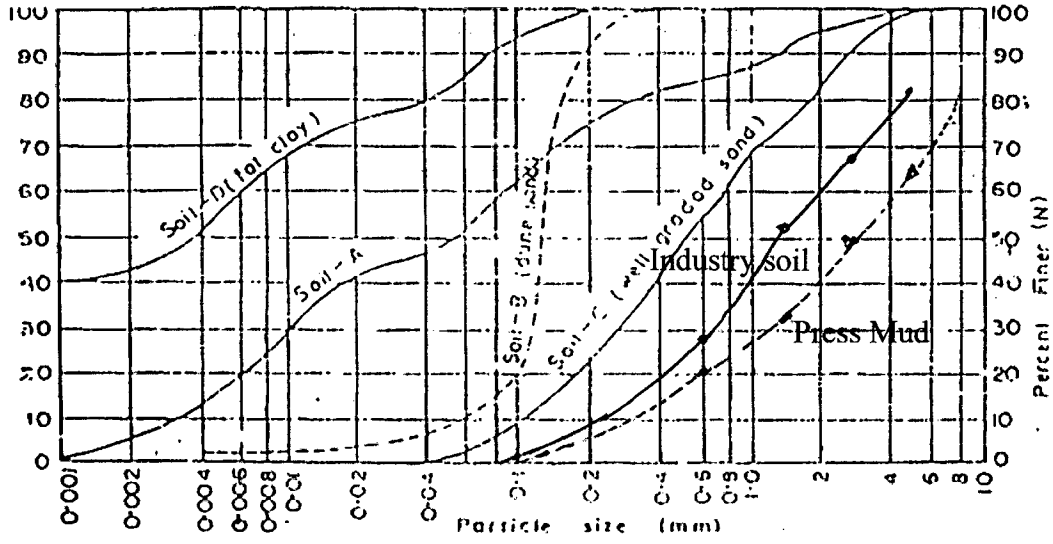


Fig. 3.6: Comparison of particle size distribution of vermi-composts with industry soil and press mud.



(Punmia, 2001)

Fig. 3.7: Standard particle distribution curves for soil.

The comparison of press mud particle size distribution to those of vermi-composts reveals that earthworms are responsible for grinding of the substrate, which makes the particle size distribution finer. The grinding capabilities of *Eudrilus eugeniae*> *Eisenia fetida*> *Megascolex megascolex.*

The soil type is SW, well graded (Fig 3.7). The vermi composts have shown the same pattern as shown by the selected species of earthworms, with regards to grinding of soil, more in surface feeder where as aggregation is more in deep burrower and *vice versa.* Where as the industry soil and vermi- composts have shown smaller size particles indicating reduction in particle size at the start of the vermi processing i.e. grinding of the substrates. But once the vermin beds have stabilized aggregation takes place markedly as shown on the graph by *Megascolex megascolex* with initial grinding and followed by aggregation.

Both the grinding and aggregation are the natural soil processes in a soil eco system. The role of earthworms in building the soil is been reported by Guild (1948); Edwards and Lofty, (1977)

The results of aggregation of soil by earthworm activity reported by using *Pheritima elongata* supports our findings (Munnoli, 2000; Singh, 1997). Similar results on aggregation is being reported by Sevenson, (1957) by the pigmented species *L terrstris* , *L rubellus, L costenus,D octaedra* and *B eiseni,* There is no aggregation in unpigmented species *D. cyaneum, O lactum, A.calignosa, A.longa* . The aggregation by deep burrower earthworms has been reported by Kale, (1993) and Singh, (1997).

3.4.8: Nutrient in filter mud vermi composts:

The results of the nutrient values obtained and their variations in nutrients are presented in Fig 3.8.

A maximum nitrogen is obtained in vermin compost with *Megascolex megascolex*. The fold increase in Nitrogen is 2.07, 2.21, and 2.48 in case of *Eisenia fetida, Eudrilus eugeniae* and *Megasclex megascolex.*

The variation in phosphorus is of increasing order 1.11, 1.20 times in case of *Eudrilus eugenae* and *Megascolex megascolex* respectively where as it has decreased by 0.75 times in case of *Eisenia fetida.* The variations in potassium are of continuous decreasing order 0.46, 0.7 and 0.54 times in case of *Eisenia fetida, Eudrilus eugeniae* and *Megasclex megascolex.*

The studies on enrichment of soil report's the increase in N,P,K by using *Octochaetona serrata* when treated in experimental pots with organic wastes like potato, Tomato, mixed vegetable and cow dung (Prabhakar *et al.,* 2006). Lunt and Jacobson (1944) established that worm casts are enriched in N, P, K, Ca and Mg. A similar report on increase in nitrogen and phosphorus in sericulture waste was reported by Gunthilingaraj (1996).

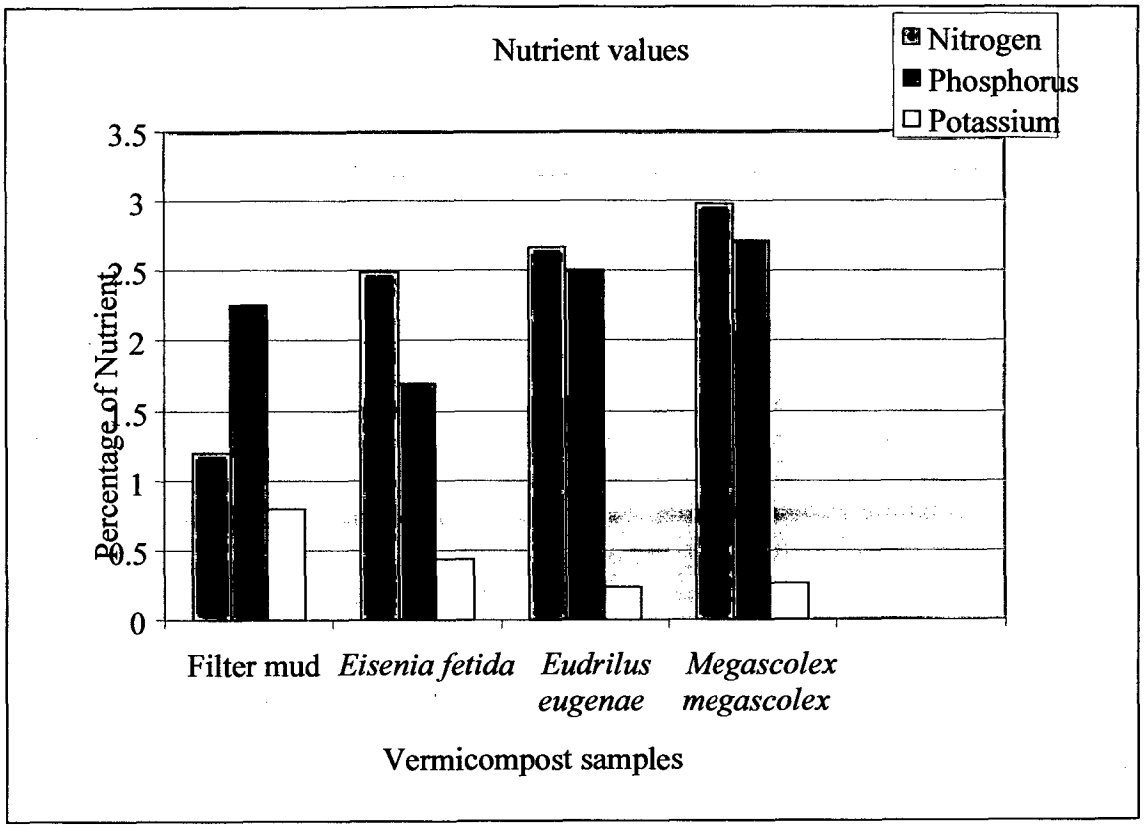


Fig. 3.8: N, P, K Values of filter mud and vermi composts

3.4.9 Geotechnical properties:

The geotechnical properties worked out for all the three composts with soil cow dung as control are presented in Table 3.5.

The increase in water contents of the vermi composts measured at the same time is due the nature of the composts and the soluble aggregates, retention of water on the skin of the earthworm etc., anf varied from 225%, 301% and 323% for *E fetida, E eugenae and M Megascolex* respectively.

The order of water contents of vermicomposts is found to be *Megascolex megascolex> Eudrilus eugenae >Eisenia fetida.* All other geotechnical properties of the composts make it better for holding moisture hygroscopic ally within the compost solids.

The moisture content of the vermin beds has to be maintained depending upon the initial moisture contents of the substrates. For instance the moisture content of filter mud 60-70% is less than that of potato waste which ranges up to 90-94%, therefore additional moister has to be given in case of filter mud as compare to the water required for the treatment of potato waste.

The parameters dry density and bulk density, and their decrease indicates amorphous nature of compost, lesser is the dry densities greater is the softness of the compost. In the present situation vermicompost of M*egascolex megascolex* is having least dry density as compared to other composts. Therefore the order of amorphous nature is, vermicompost of M*egascolex megascolex* is > *Eudrilus eugeniae >Eisenia fetida* > Cow dung +soil (1:3) respectively.

Table 3.5: Geo Technical parameters of Vermi-composts of press mud

Parameter	**Soil + Cow dung**	***Eisenia fetida***	***Eudrilus eugeniae***	***Megascolex megascolex***
Water Content	1	2.25	3.01	3.23
Voids Ratio (e)	1.43	5.09	6.81	7.16
Porosity (n)	0.58	0.84	0.87	0.88
Bulk Density (√)	0.97	0.72	0.64	0.60
Dry Density (√d)	0.48	0.22	0.16	0.14
Saturate density √sat)	1.07	1.05	1.05	1.01
Degree of Saturation (Sr)	0.82	0.59	0.55	0.52
Air Content (ac)	0.12	0.41	0.45	0.48
Percentage Air Voids (na)	10%	34 %	40%	42%

The percent saturation is highest in control, (Soil + cow dung 1:3), and there is no measurable difference in the degree of saturation with vermicomposts prepared from *Eisenia fetida, Eudrilus eugeniae* and *Megascolex megascolex.*

Higher the air content more will be the space for air circulation and survival of aerobic microorganisms. The air content of vermicompost of *Megascolex megascolex* > *Eudrilus eugeniae* > *Eisenia fetida* > cow dung +soil (1:3) The *Megascolex megascolex* is a deep burrower which has larger length and movement in the soil at greater depth , leaves large holes behind its movement, this could be the reason as to have higher air content.

The air voids of *Megascolex megascolex* >*Eudrilus eugeniae*> *Eeisenia fetida* > cow dung +soil (1:3). Another measure to demonstrate the aerobic nature of vermicomosting process, which ultimately is responsible for removal of odor of substrates.

According to Edwards and Lofty, (1977); Tewatia, (2007) organic matter plays a major role in maintaining soil quality. It improves soil quality and productivity by enhancing their granulation, porosity, water holding capacity nutrient supply rate and activity of soil biota. Also improves soil texture, structure and proliferate useful microorganisms (Sengar,2006) .It is a well known fact that endogenic anecgic earthworms influence soil macro porosity and aggregation Lee(1991).

The beneficial effects of the ubiquitous earthworm have been clearly reported. Earthworm activities influence rates of soil turnover, mineralization and humification of soil organic matter and improvements in consistency of soil texture with concomitant increases in porosity, (Sengar and Salini Gupta 2006) infiltration and soil-water retention characteristics

have also been measured for worm-worked soils (Kale, 1993; Nijhawan S D and Kanwar, 1952; Kolher, 1995).

3.6.11 Water holding capacity:

The results of the compaction experiment using vermin-composts presented in Fig 3.9. The press mud soil combination showed optimum dry density above 1.6 and corresponding water content 34%, which almost resembles a compaction curve for soil. The experiment further revealed that when compacted by adding vermicomposts the density observed for the first set of reading 0.86, 0.8 for *Eisenia fetida, Eudrilus eugeniae* and *Megascolex megascolex* respectively. The experiment further carried out with addition of vermicomposts showed a continuous decreaseing trend in dry density, in other words they showed increasing water voids. The water holding recorded for *Megascolex megascolex* is about 200% whereas the for *Eudrilus eugeniae* and *Eisenia fetida* it is 170% (Stockdill and Lossens,1996).

The increased water holding capacity of the earthworm composts is due to the presence of soluble colloidal particles, and mucus of earthworm which is a good absorbing agent. Added to this the water holding and movement of water is due to the increased voids and porosity of composts during vermi processing. Similar increase in water holding is being reported Kavian (1996). A 30% decrease in bulk density is being reported (Kolher1995), which intern helps in water holding capacity. Soluble aggregates are responsible for increased water holding capacity (Edwards and Lofty, 1977).

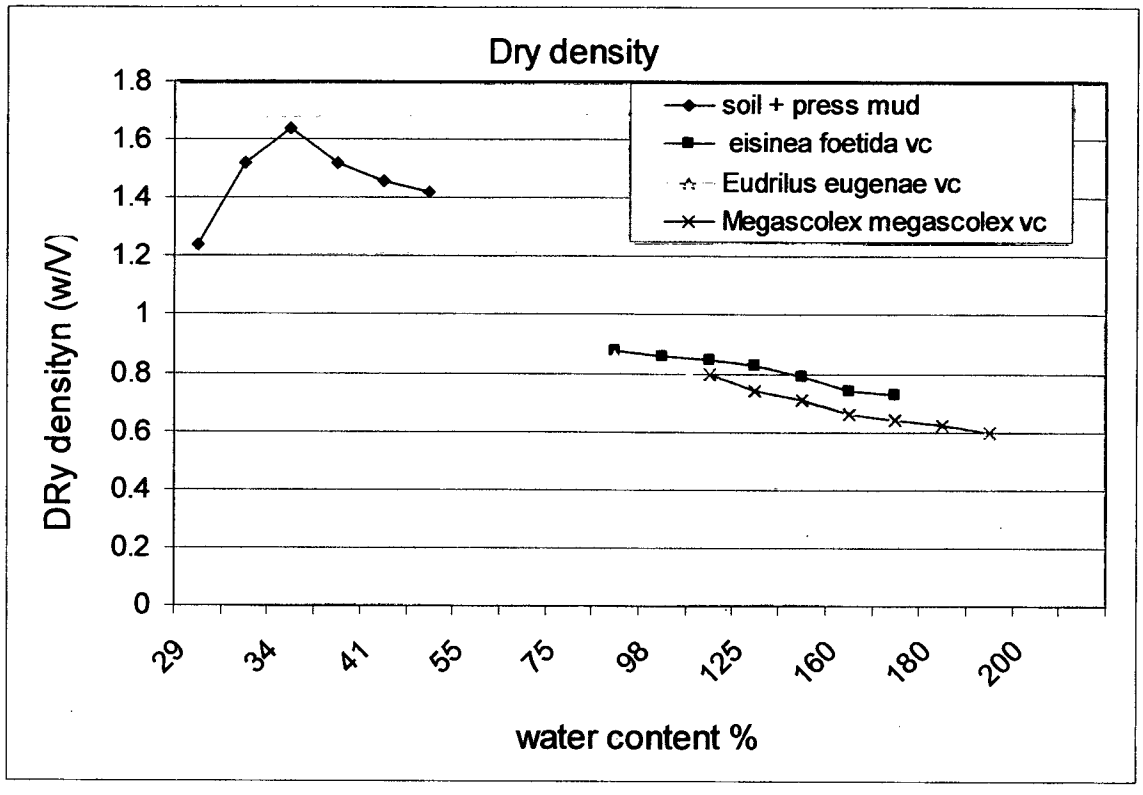


Fig. 3.9: Compaction of industry soil using filter mud and vermi-composts.

The various advantages mentioned above will definitely suggest us to advocate the use of this technology especially for industries generating organic wastes.

3.7 Bacterial count of vermi composts:

The total microbial populations are determined in the ejected cast of press mud harvested (by using *Eisenia fetida, Eudrilus eugeniae* and *Megascolex megascolex*) zero day, and 40^{th} days and of the industrial soil samples collected are determined. The results presented in Table 3.6. A visual showing dilution of the vermicomposts is placed in plate 3.10. The comparison of colony forming units is placed in Fig.3.10.

There is a remarkable increase in the bacterial count of the vermi composts of the press mud as compared to the industry soil sample and zero day sample (Table 3.6, Fig 3.10). The increase is 16, 6.4; 42.7, 17.1; 41.6, 16.6 times in case of vermi-compost with *Eisenia fetida, Eudrilus eugeniae,* and *Megascolex megascolex*, as compared to industry soil and zero day sample respectively. The size of microbial population in worms casts mainly depends on the type and quality of ingested soil and substrate (Edwards and Bohlen, 1996).

Table 3.6: bacterial count of industry soil and vermi -composts per gram of the sample.

Industry soil	zero day sample	Vermi-composts 40 day sample	Fold increase as compared to industry soil	Fold increase As compared to Zero day sample
$180x10^6$	$450x10^6$	$2900 x10^6$ *Eisenia fetida*	$16x10^6$	$6.4x10^6$
$180x10^6$	$450x10^6$	$7700 x 10^6$ *Eudrilus eugeniae*	$42.7x10^6$	$17.1x10^6$
$180x10^6$	$450x10^6$	$7500x 10^6$ *Megascolex megascolex*	$41.6x10^6$	$16.6x10^6$

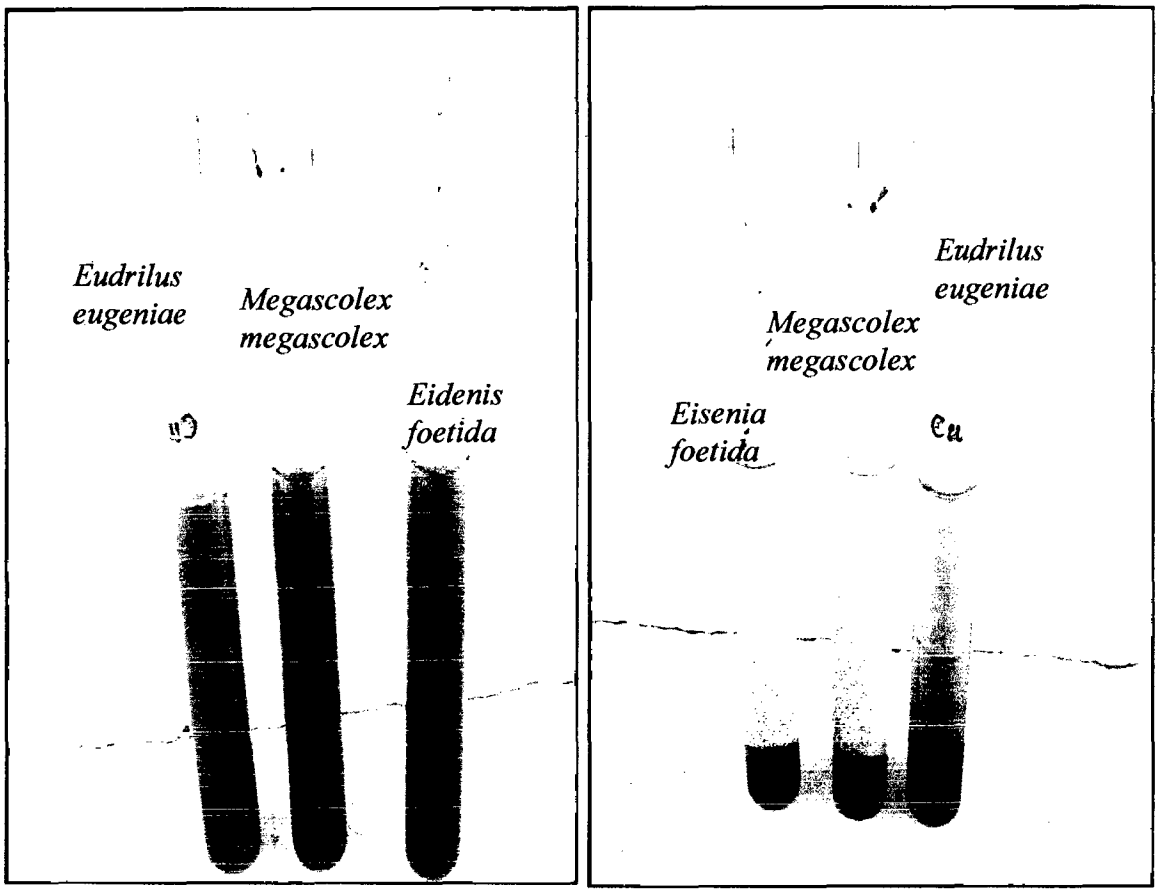


Plate 3.10: Dilutions of vermi- compost samples.

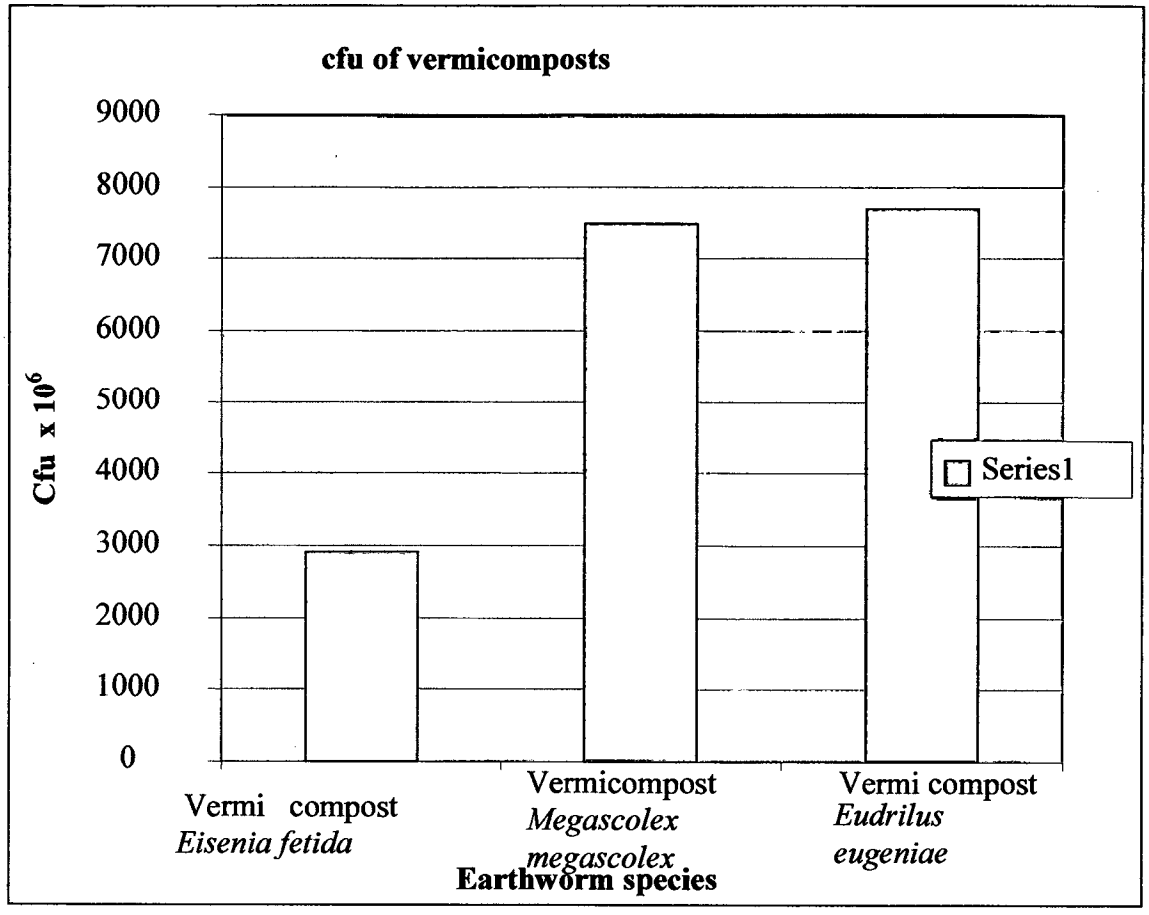


Fig. 3.10: CFU for vermi compost of press mud with selected species of earthworms

As the cow dung was used in the experiment of bioconversion of press mud, the additional micro flora the cow dung is giving to the vermi composting system table 2.4 (Chapter 2) presents the data on fold increase due to supplementation of cow dung. The fold increase varies from 1.68, 2, 23, 2.45, 2.05 times in case of *Eisenia fetida*, Coculture of *Eisenia* and *Edrlus eugenae*, *Eudruilus eugenae*, *Megascolex megascolex* respectively.

Treatment of filter mud using *Pheritima elongata, Eudrilus eugeniae, Esienia fetida h*ad significantly enhanced the micro and macro nutrients (Singh1997; Parthasarathi and Ranganathan, 2000).

During the vermi-composting process organic wastes pass through the gut of earthworms. The wastes undergo physical, chemical and biochemical changes. The combined effect of microbial, earthworm and soil processes simultaneously taking place along with the micro flora of the waste itself is responsible for biodegradation of organic wastes. The role of these processes is to release of nutrient to the plants. The worm casts, compared to soil without worms /organic wastes have shown increased microbial/enzyme/micro and macronutrients. (Jambekar.1992; Bano,et.al 1987; Parthasarathi,2006).The fold increase of 2.42,1.15,1.17 times in fungi, actionmycetes, and bacteria is reported by Bhattacharrya (2000) in vermi- compost as compared with the traditional compost.

The analysis of gut content of earthworms has revealed the occurrence of different kinds of symbiotic micro fungi, bacteria, protozoa's etc. Maximum numbers of micro fungal species are found in the fore gut, gradually decreasing in number in the mid gut and hind gut with a minimum number of freshly laid casts (Dash *et.al,* 1985).

Whereas Parle (1959) has reported 13.76, 572.96 times increase of actinomycetes and 700.8, 927.78 times of bacteria in the mid gut and Hindgut as compared with fore gut actinomycetes and bacteria respectively.

A significant higher number of microbes were observed in experimental plots treated with vermi-compost and nitrogen fixing bacteria after harvest of the crop. Higher microbial load was also observed in paddy, applied with vermi-compost. (Kale *et al.*, 1992).

The effect of vermi-compost on microbial population in soil environment is reported to be best with vermi-compost prepared out of the combination of leaf litter, straw, grass and water hyacinth(VC_1) as compared to vermi-compost of leaf litter (VC_2), Home garbage(VC_3) and partially decomposed cow dung VC_4 when applied at the rate of 5%(w/w). The 2.16,1.83,1.71,1.69 increase in bacteria,1.49,1.30,1.52,1.40 in actinomycetes, 2.89,2.76,2.38,2.47 in fungi for VC_1,VC_2,VC_3,VC_4 respectively,(Sahu, *et.al.*, 2000).

Teotia (1950) reported 3.4 to 5.4 time's bacterial increase as compared to surrounding soil. Where as Atlavinyte and Lugauskas, (1971) reported a five fold increase in microorganisms in earthworm introduced pot tests. Ghilarov (1963) has reported in his study the number of microorganisms in earthworm casts and soil , and, the fold increase is 1.64,1.35,1.97 times in three different fields namely oak forest, Rye field and Grass field respectively. The moisture content of vermi-compost becomes an essential environmental condition for survival of the beneficial micro organisms, Irrespective whether earthworms continue to live or not. The decrease in moisture content will bring

down the colony forming units and Organic carbon, (Scheu, 1987; Parthasarathi and Rang Nathan, 2001;Parthasarathi,2006).

The study on microbes in the gut of earthworm revealed the increase in number of bacteria, actinomycetes as compared to those in soil, follow exponential law (Parle, 1959; Meenatachi, 2007). The above literature survey showed that the increase in microorganisms in the gut and vermi-casts of earthworms contain higher number of microorganisms is taken as the one of the measure to evaluate the vermi-composts. It also enables us to say that earthworms are important in inoculating the soil and their casts are the foci for dissemination of soil microorganisms, which will elevate the overall fertility of the soil (Tewatia, 2007).

Microbial biomass was found to be more in vermi-casts as compared with the surrounding soil. Similar results have been reported by Singh, 1997 and Munnoli, (2002). The remarkable increase in total bacterial count is being reported by Allevi, *et.al.* 1987., Kavian *et.al.*,(1996). The greater microbial population and activity of microbes is believed to be caused by selective food substrate consumption (Scheu, 1987; Lavelle and Martin, 1992; Pederson and Henderiksen, 1993, Parhthsarathi, 2006). Morgan and Morgan, (1992), report remarkable significance of selectivity of substrates. Our results are also inline with the report made by Ghabbour, (1966)

In the present study, the oreder of higher number of microbes is Eudrilus *eugeniae* > *Mehgascolex megascolex* > *Eisenia fetida* for press mud vermi-cast. Our study showed the decrease in population of fungi and increase in the population of total microbes (fungi+bacteria+actinomycetes). This is in full conformity of the results reported on press

mud vermi-casts as the hot spots of fungi. (Parthasarathi and Ranganathan,1998).

Therefore, such a biomass rich in microbes will enrich the soil and supply balanced plant nutrients. Bhawalakar, (1989) also agrees upon this in his studies, that the wastes bio-processed by earthworms in to plant nutrients and enhance the growth of bacteria, actinomycetes in surrounding soil.

The total microbial population of filter mud vermicomposting taken at start, 20, and 40 days for all the three species of earthworms. The population of fungi, yeasts, bacteria are being experimentally found and presented in Table 3.7. These findings are subjected to Analysis of variance (ANOVA) as detailed by Indrajit Singh, (1990) and shows significant at $p<0.05$.Table 3.8.

Table: 3.7 Microbial population of vermi-casts using *Eisenia fetida, Eudrilus eugeniae , Megascolex megascolex.*

Moisture of vermi-composts 70%							
Microbial population	Press mud	*Eisenia fetida*		*Eudrilus eugeniae*		*Megascolex megascolex*	
		20 day	40 day	20 days	40 days	20 days	40 days
Fungi x 10^4 g^{-1}	108	17	11	37	24	55	30
Yeasts x10^4g^{-1}	35	65	85	110	138	86	56
Bacteria x$10g^{-1}$	70	1700	2900	5400	7700	5100	7500
Total x 10^6g^{-1} (Fungi+ yeasts+Bacteria)	71.73	1700.82	2900.96	5401.47	7701.62	5101.41	7500.86

The fold decrease in fungi, 9.81; 4.5; 3.6, yeast, 2.43; 3.94; 1.6; and increase in bacteria 41.4; 110; 107 for vermicomposts of *Eisenia fetida, Eudrilus euegeniae*, and *Megasclex megascolex* respectively

Table 3.8ANOVA ($p<0.05$)

Sum of squares	df	Sum of squares	Mean sum of squares	F ratio
Between	7-1=6	169346424	28224404	
within	20-7=13	33693783	2591829.462	10.88

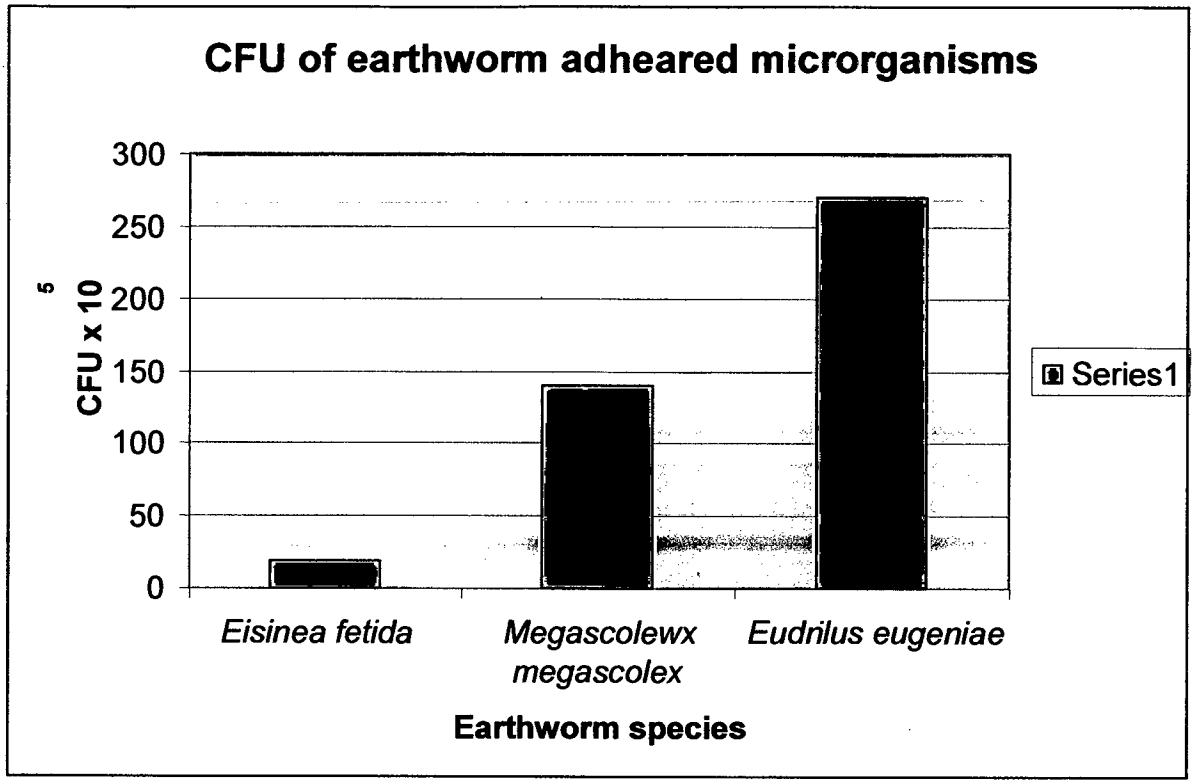


Fig. 3.11: Earthworm adheared microorganisms of selected earthworm Species (cfu)

3.7.1 Earthworm organisms of selected earthworm species.

The comparison of population of earthworm organisms presented in figure 3.11 has revealed the highest number of earthworm skin adhered organisms in Eudrilus *eugeniae* as compared to *Megascolex megascolex* which is also higher in number as compared to *Eisenia fetida.* The trends of increasing skin organisms observed here, is same with the total microbial population for these species.

The microorganisms of skin (surface of the earthworm body) and cast of *Apporrectodea caligniosa, Lumbricus rubellus* in comparison with surrounding soil showed the increase in nitrogenase activity indicating the presence of N_2 - fixing bacteria, due to the increase activity of burrowing and feeding activity of earthworms (Bhattachaya ,2000;Smick and Pizl, 1989).

An increase of 10-20 times in microbial activity is being reported with worm ingested soil and organic matter (Sonia Thomas, 2002). The significant increase in the bacterial count during vermi-processing is reported by Kavian et.al. (1998) Alllevi et.al.,(1987), Bhattachrya, (2000) has reported increase in microbial content of vermi compost as compared to traditional composts.

The materials ingested by earthworms undergo biochemical changes in the digestive track and in the ejected cast, the plant nutrients and growth substances are rendered in plant assimeable form. The fertility is also attributed to enzymatic activity and microbial activity that associated with earthworms. (Kale et.al.1987)

Atlavinyte and Lugauskes (1971) have reported earthworm increases the number of microorganisms in soil as much as five times. Earthworms are therefore important in inoculating the soil with microorganisms and their casts are foci for dissemination of soil microbes.

The data presented in the Table 3.8 indicates that there is a decrease in the fungi, increase in bacteria and yeasts in all the species of earthworms ($p<0.05$). The decrease in fungi could be attributed to the type of substrate, which is acted upon, by the soil and the earthworm intestine micro flora. The bacterial digestive enzyme activities like protease, cellulose, amylase, chitinase and lipase that continue to disintegrate organic matter. The enzyme activities are the processes of microorganisms; the greater the population of the vermi cast indicates the role played by them in organic matter decomposition. The increase in bacteria is due to consumption and selectivity food substrates. There is a marginal increase in yeasts in case of *Megascolex megascolex.*

Therefore, the increase in number of microorganisms during the process of vermi-composting is a greater significance of synergy of microorganisms and earthworms.

Such biomasses when added to the wastelands, the nutrient and bacterial mass will be added to the soil, leading to recovery of the wastelands. Which is a need of the day?

The role of microbes in the industrial applications is a known fact, for production of bread, cheese, beers, wines, antibiotics, vitamins etc. The use of earthworm and microorganisms in the field of organic waste management of industries remains as a new area yet to be accepted and adopted by the industries.

3.8: Significant points of study:

- The higher decrease in organic carbon is obtained in case of vermicompost of press mud with *Megascolex megascolex>Eudrilus eugenae> Eisenia foetida* respectively.
- The higher aggregation of soil finer than 850 micron is 54.85 %, 50.26 %, 45.23 % in case of *Megascolex megascolex >Eisemia foetida > Eudrilus eugenae respectively.*
- The order of aggregation in case of composts is also the same
- Grinding Capabilities is in the order of *Eudrilus eugenae>Eisinea fetida> Megascolex megascolex.*
- The press mud has shown larger size particle size distribution than the industry soil
- Larger size particle size distribution is shown by *Megascolex megascolex,*
- The *Megascolex meascolex* vermi-comopsost has shown its superiority in all the Geotechncal properties as compared to *Eudrilus eugenae* and *Eisenia fetida,*
- The higher nutrient values of N, P, K is shown by *Megascolex megascolex.*
- There is a remarkable increase in the bacterial count of the vermi composts of the press mud as compared to the industry soil sample and zero day samples. The fold increase is 16, 6.4; 42.7, 17.1; 41.6, 16.6 times in case of vermi-compost with *Eisenia fetida, Eudrilus eugenae,* and *Megascolex megascolex*, as compared to industry soil and zero day sample respectively

The vermi compost of press mud undertaken in the laboratory and field trials showed successful degradation of press mud. The compost obtained as a product was also of higher nutrient value showing sufficient moisture holding capacity. The variation in the compost obtained from the soil and cow dung 1:3 and with press mud , The vermi-composts of press mud showed better performance in the all the geotechnical properties as compared to the compost obtained with 1:3 soil cow dung. It reflects that the press mud is a substrate, which is sufficiently biodegradable, and could support the growth of bacteria. It was therefore envisaged that the composting process would also involve the activities of bacteria associated with the earthworms and those present in the composts. The studies on such bacteria were important to understand the effect of microorganisms on the composting process and the final product obtained. To understand this bacterial isolates from the composts need to be made available as inoculums along with earthworm. The bacteria were therefore isolated from the composts of press mud, Their biodegradable ability and characteristics was undertaken and the consortium of the potential isolates was prepared for use as inoculums in vermi-composts. These results presented in the next chapter.

CHAPTER IV

Isolation, purification, Identification and Characterization of vermi-compost microorganisms (Bacteria)

Chapter 4.0: Isolation, purification, Identification and characterization of vermi-compost microorganisms (bacteria)

4.1 Introduction: In the previous chapter, it is evident from the viable counts (cfu) of vermi-composts obtained form field and laboratory trials harbor a vide variety of heterotrophic microorganisms like bacteria, yeast, fungi etc. These organisms play an important role in nutrient turn over, formation of humus, which is based on degrading abilities. Nutrient cycling is one of the important basic processes which regulates soil fertility through weathering of soil minerals and decomposition of organic matters by microorganisms bacteria, fungi etc., along with soil animals (Marinissen and Ruiter,1993). Symbiotic relation between microorganisms and earthworms exist in breaking down and fragmentation of organic matter progressively (Bhawalkar and Bhawalkar,1992).

The decreasing trends in fungi and yeasts and increasing trend in bacteria seems to be of greater significance in the vermi-composting system indicating the major supportive role-played will be of bacteria. Therefore, it as envisaged that the work of isolation of bacteria could be of great help in understanding the role-played in bioconversion. But it was interesting to note here that the vermi-compost derived from the worms and the bacteria adhered to skin of the worms together will give better understanding of the microbes present in the vermi-composting system. Kale *et al.,* (1989, 1992) reported the increase in bacteria in worm and higher number of N fixers even though the total population was unaffected. Whereas Bano *et al.,* (1987) reported that vermicompost obtained from culturing of *Eudrilus eugenae* showed large number of

humus forming bacteria, fungi, actnomycetes and N fixers. Similarly worm soils have been reported to have shown increased microbial populations (Edwards and Loft, 1977; Tiwari, 1989; Singh, 1997).

4.2 Materials and Methods:

The vermi-composts obtained from the field trials was further utilized for plating out and the three species of earthworm's viz. *Eisenia fetida, Eudrilus eugenae, Megascolex megascolex* were picked up from the respective vermicompoting units of the field trials

4.2.1 Isolation of vermi-compost and earthworm microorganisms (Bacteria)

The respective vermi-composts were serially diluted and spread plated on nutrient agar plates. Following a incubation of 18-24 hrs the plates were observed for the growth of organisms(bacteria)and the colony characteristics viz. size, shape, consistency, color, opacity, margin, elevation were recorded. Further the plates with count between 30 -300 were preserved for isolations. These selected organisms were purified mechanically by streaking on respective media, the growth for well developed colonies were picked up and was designated with a alphanumerical number and transferred aseptically to slants in duplicate for further study.

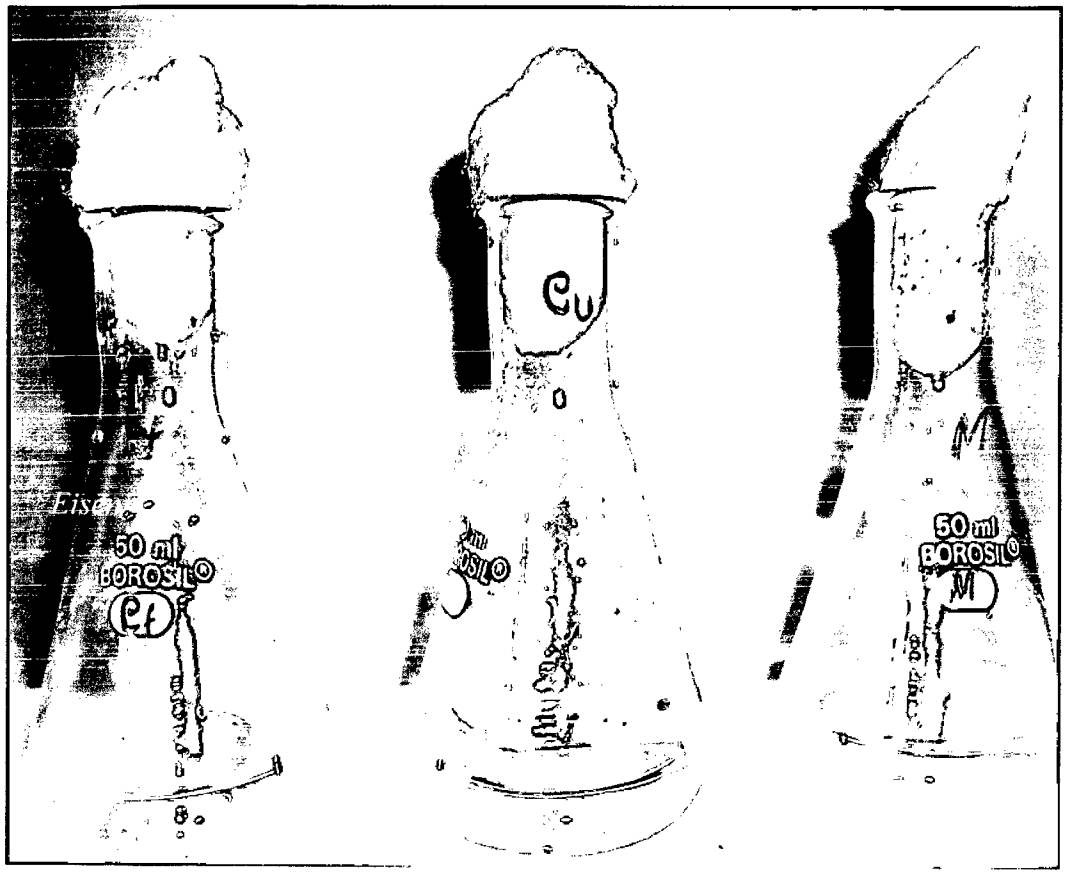


Plate 4.1: Earthworm in saline for isolation of skin microorganisms

An individual of selected species of earthworms namely *Eudrilus eugenae, Eisenia fetida,* and *Megascolex megascolex,* was picked up and washed in distilled water, transferred aseptically to a conical flask containing 10 ml normal saline(Plate 4.1). The conical flasks was kept on shaker for 10 minutes, the earthworm removed from the conical flasks and replaced them back in respective composts. This saline was serially diluted and was spread plated on nutrient agar plates for determining the population of earthworms. The same plates were incubated and the colonies formed were counted. The colony characteristic's of the individual colony was studied and the isolates purified on respective media and preserved.

4.2.2 Maintenance of microorganisms

The isolates were maintained on nutrient agar slants / plates and designated with alphanumerical number and maintained through out the experiment. The cultures maintained in duplicate one for regular experimentation and the other for stock, which was sub cultured periodically. The day to day experimentation was carried out with cultures maintained on plates.

4.2.3 Purification of microorganisms

The colony's showing discrete natures were picked up and streaked on respective agar plates for purification. Following the incubation, single colony developed at the end of streaks was picked up on a nutrient agar slant and plates, sealed with paraffin tape and preserved in the cold room at 4^0C. The isolates were periodically checked for purity. The sub culturing carried out at every month.

3.3.5: Morphological characteristics

Grams staining was carried out using the strains Crystal violet, Grams iodine, Counter stain safranin and endo-spore staining and using malachite green as per the standard procedure given in Appendix C.

4.2.4: Identification tests:

4.2.5: Biochemical tests for identification of microorganisms:

The below mentioned biochemical tests were carried out as per the procedures given in the Laboratory manual in General Microbiology By Kannan,(1996);Satish Gupte,(1998). The list of biochemical tests performed is given below.

i)Hughlifeson test for aerobic and anaerobic, ii)Triple sugar Iron Test iii)Hydrogen sulfide and motility iv)Motility v)Sugar fermentation vi)Urea's test vii)Methyl Red Viii)Vogas pauskar ix)Catalase x)Oxidase xi)Geletin Liquefaction xii)Citrate utilization xiii)Indole production xiv) Deaminase Phenylanine Deamanise Test xvi)Nitrate reduction xvii)Maltonate utilize. The details of media compositions for each tests presented in Appendix C (1 to 27). Tests were performed on fresh cultures in the exponential stage; controls were kept for each test for noting the test result the test results presented in section 4.3.2.

4.2.6: Enzyme activities:

The Amylase, protease, cellulose, lipase geletinase, and chitinase activities were determined by spot inoculating on respective agar medias and incubated for 24 hrs. The colonies formed were checked for enzyme activity by flooding the plates with respective reagents. The detailed procedures are presented in appendix C.

4.2.7: Preparation of consortia

A) Consortium of Vermi-compost Microorganisms

The consortium of the vermi-compost microorganisms prepared by transferring aseptically a loop full of pure cultures in 6ml normal saline. The O.D was adjusted until it attained a value of 1.0. Two ml of this preparation was transferred to 100 ml of nutrient broth aseptically and kept on a shaker for 24 hrs. The growth of cultures is noticed buy the turbidity of the broth. The nutrient broth is centrifuged for 10 minutes with at 7000 rpm. The pallet farmed at the bottom is transferred to the 20 ml of saline and the preparation is kept in cold room. The viable count found by serial dilution and spread plate method. Required volume of 40ml of the inoculums is prepared fresh and added to the experimental culture box

B) Consortium of cellulose degrading microorganisms.

The major constituents of filter mud are cellulose 25%: hemi Cellulose 27%. The micro organisms isolated from skin and vermi compost showing maximum cellulose enzyme activity. The organisms were designated as Peu9, Peu14, Sef4, Sef5, Sme12, and Sme16. A single colony of each is picked up in a 6ml saline aseptically the OD was adjusted to

0.5. Three ml of this was transferred to 100ml of nutrient broth and kept on a shaker for 24 hrs. The cultured nutrient broth was centrifuged at 7000 rpm and the pellet was taken in 20 ml of saline and preserved in conical flasks. The viable count was measured by dilution and spread plate method

4.3 Results and discussion:

4.3.1 Bacterial Isolates:

Bacterial isolates obtained from the vermi-composts of filter mud from field trials using *Eisenia fetida, Eudrikus eugenae, Megasclex megascolex* and the bacteria isolated from skin of *Eisenia fetida, Eudrikus eugeniae, and Megasclex megascolex* are presented in Table 4.1. The plates 4.2 to 4.5 present the isolated single colony on nutrient agar. Sneh Goyal (2005) has reported the increase activity of microbes during composting process.

The number of isolates obtained from vermi-composts shows *Eudrilus eugenae>Megascolex megascolex>Eisenia fetida.* Where as the skin organisms showed number of isolate order as *Eisenia fetida>Megascolex megascolex>Eudrilus eugenae.* The order of highest number of microorganisms in vermicomposts and skin of earthworm species as bacteria> fungi> yeasts.

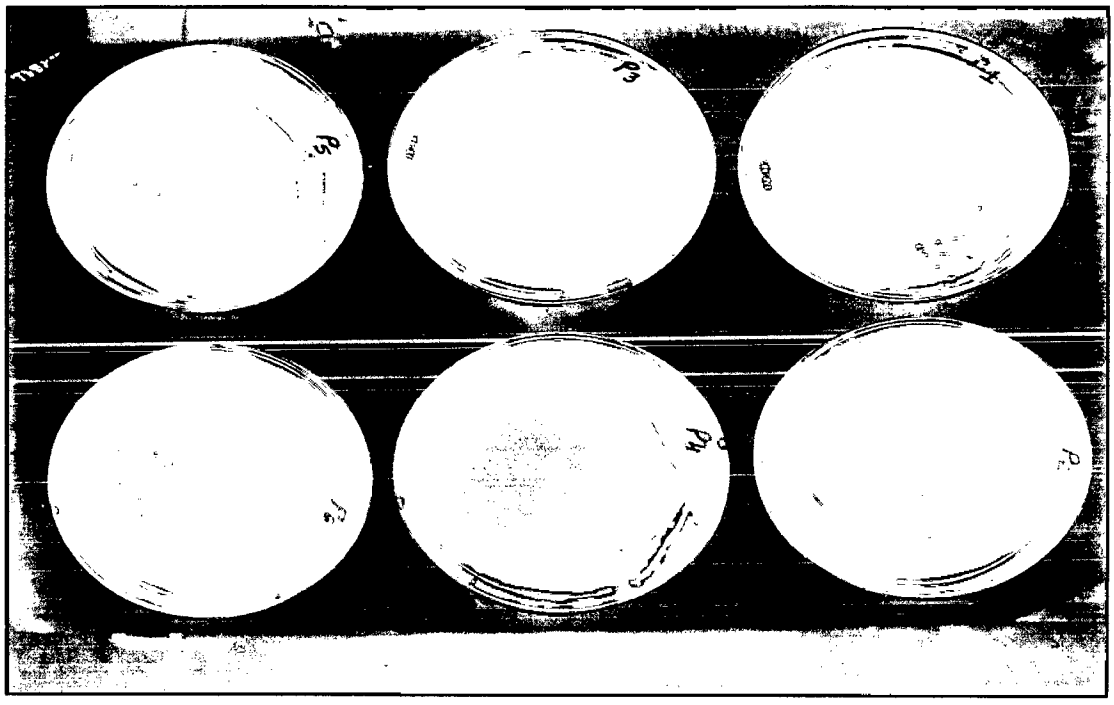


Plate 4.2: Purification of microorganisms

Plate 4.3: Purification of microorganisms

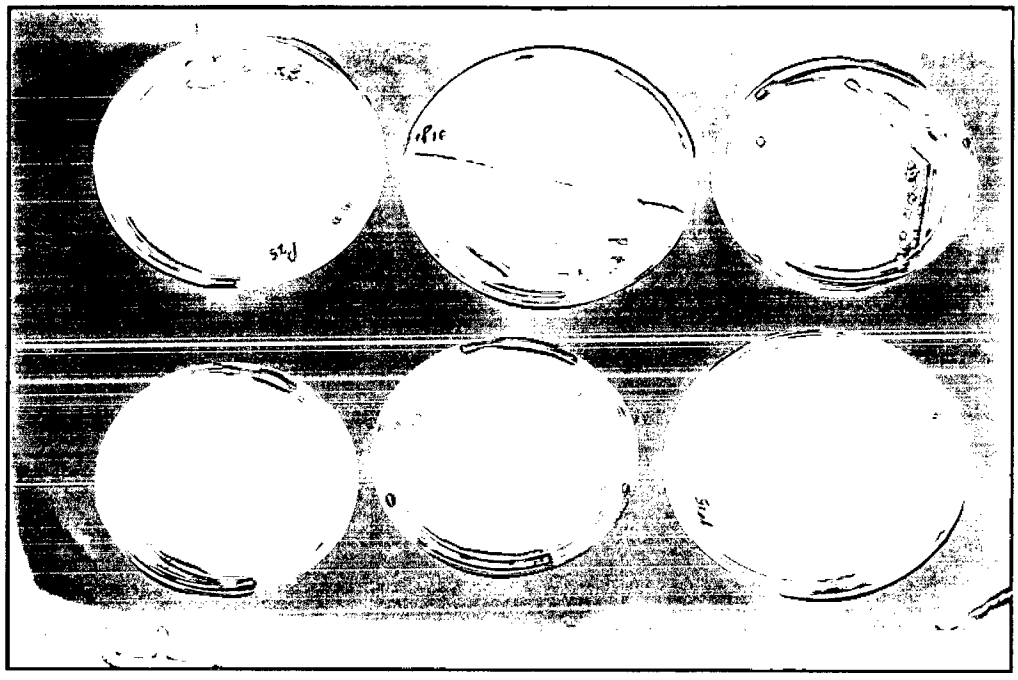


Plate: 4.4 Purification of microorganisms

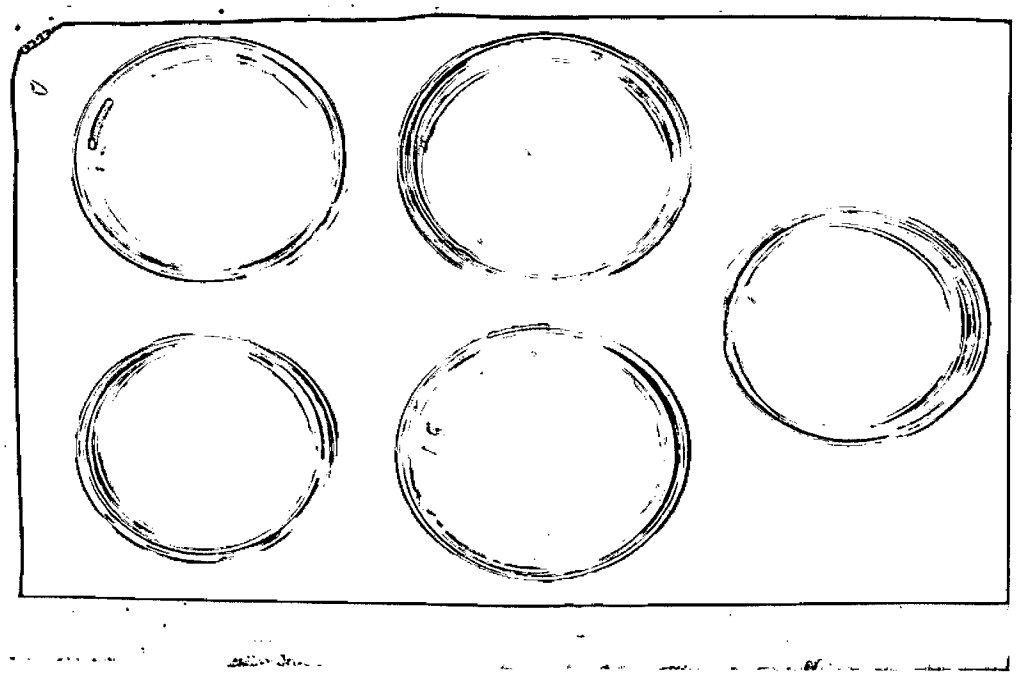


Plate 4.5: Purification of microorganisms

Table 4.1 Microbes in vermi-compost system and isolates.

Inputs	**Process** **Earthworm activity**	**out puts**
Press mud Fungi 108 x 10^4 Yeasts 35 x 10^4 Bacteria 7 x 10^4	**Skin Organisms** **[CFU/ml/g]** *Eisenia fetida 19 x 10^5* ***7 Isolates*** *Eudrilus eugenae27 x 10^5* ***3 Isolates*** *Megascolex megascolex* *21 x 10^5* ***6 Isolates*** **Soil processes** Fungi 11 x 10^5 Yeast 6 x 10^4 Bacteria 1.28 x 10^7	**Vermicasts CFU/ml/g** *Eisenia fetida* Fungi 11 x 10^4 Yeast 85 x 10^4 Bacteria 29 x 10^8 **6 Isolates** *Eudrilus eugenae* Fungi 24 x 10^4 Yeast 138 x 10^4 Bacteria 77 x 10^8 **23 Isolates** *Megascolex megascolex* *Fungi 30 x 10^4* *Yeast 56 x 10^4* *Bacteria 82 x 10^7* ***8 Isolates***
0 Isolates	**Total 16 Isolates**	**37 isolates**

4.3.2 Identification of microorganisms:

The purified microorganisms subjected to various biochemical tests are presented in Table 4.2, 4.3 and 4.4. The isolated organisms subjected to Grams staining and endo-spore staining. The results of these tests revealed that the out of the 37 isolates 35 isolates were gram positive. Out of these 35 isolates 7 were non sporulating and the remaining 28 sporulating. Following the biochemical tests the comparison of organisms with the Bergy's Manuel for determinative bacteriology revealed the occurrence of organisms belonging to *Bacillus*. The identified bacteria presented in Table 4.2, 4.3, and 4.4.

Fifty organisms isolated from alimentary canal of the *Lumbricus terristris* were same as those from surrounding soil Basslik, (1913).The same was confirmed for three different species of earthworms Parele, (1963). Zhdannikova,(1961) reported The comparison microbial composition of two species of earthworms guts namely *Octolasium lacteum* and *Lumbricus rubillus* and surrounding soil , the density of microorganisms observed is the same in gut and surrounding soil. There were more spore forming bacteria and actinomycetes and few fluorescing bacteria in the gut of *O.lectium* than in the surrounding soil.

Where as the *L rubellus* showed 10 times more microorganisms in cast than surrounding soil. The casts of these two species contain more fungi, actinomycetes, butyric acid forming bacteria of *Clostridium* type, and cellulose decomposing bacteria.

He also reported the microorganisms in soil differed from those in casts, the spore forming bacteria and actinomycetes were predominant in worm casts. The number of

Bascillus idosus and *Bascillus cerus* were greate in casts than in soil. Ponomavera, (1953) also stated that there is increase in number of actinomycetes, pigmented bacteria and other bacteria of *biscillus cerus* group. Pranamalik, 2007 has reported application of lime (5 g/kg) and inoculation of microorganisms increased the nutrient content in vermi-compost and also phosphatases and urease activities. *Bacillus polymyxa*, the free-living N-fixer, increased N-content of vermi-compost significantly.

Table 4.2: Filter mud vermi-compost microorganisms (Bacteria)

Designation	Morphology / Gram Staining	Endo spore staining	Motility	Hydrogen Sulphide	Aerobic	Anaerobic	Facultative	Acid butt Alkaline slant(TSI)	Acid Butt-Acid SlantTriple sugar Iron	Alkaline Butt Alkaline Slant	Bubble in Butt	H2S Production	NO2Nitrate reduction	NO3	Indole	Methyl red	Vagous pausksr	Ureas	De amyne pholyamyline	Sucrose	Fructrose	Catalse	Maltonate	Oxidase	Geletin liqifaction	Tentative Identification
Peu1	+ve Rods	spo	+	-	+	-	+	+	-	-	+	-	+	-	-	+	+	+	-	+	+	-	-		+	*B.anthracicis*
Peu2	+ve Rods	Nspo	+	-	+	-	+	-	+	-	-	-	-	+	-	+	+	+	-	-	+	+	-	+	+	****
Peu3	+ve Rods	Nspo	+	-	+	+	+	-	+	-	+	-	+	-	-	+	+	+	-	+	+	+	-	+	+wp	****
Peu4	+ve Rods	Spo	+	-	+	-	+	+	-	-	-	-	+	-	-	+	+	-	-	+	+	+	-	+	+wp	*B.pantothenticus*
Peu5	+ve Rods	Spo	+	-	+	-	+	+	-	-	+	-	+	-	+	+	+	+	-	+	+	+	-	+	+	*B.licheniformis*
Peu6	+ve Rods	Spo	+	-	+	-	+	-	+	-	+	-	-	+	-	-	+	+	-	+	+	+	-	+	+	*B.alvei*
Peu7	-cocci	Nspo	+	-	+	-	+	+	-	-	+	-	+	-	-	+	+	-	-	+	+	+	-	+	+wp	****
Peu8	+ve Rods	Spo	+	-	+	-	+	-	+	-	+	-	-	+	-	-	+	-	-	+	+	+	-	+	+wp	B.mycoides
Peu9	+ve Rods	Spo	+	-	+	+	+	-	+	-	+	-	-	+	-	-	+	-	+	+	+	+	-	+	+	*B.marinus*
Peu10	+ve Rods	Spo	+	-	+	-	+	+	-	-	+	-	-	+	-	+	+	-	-	+	+	+	-	+	+	*B.brivis*
Peu11	+Cocci	Spo	+	-	+	-	+	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+	-	+	+	*B.mycoides*
Peu12	+ve Rods		+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	ND	ND	+	-	+	+	*B.alcalophilus*
Peu13	+ve Rods	Spo	+	-	+	-	+	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+	-	+	+	*B.subitus*
Peu14	+ve Rods	Spo	+	-	+	-	+	-	-	+	+	-	-	+	-	+	+	+	-	+	+	+	-	+	+	*B.mycoides*
Peu15	+ve Rods	Spo	+	-	+		+	-	+	-	+	-	-	+	-	-	+	+	-	+	+	+	-	+	+	*B.polymyxa*
Peu16	+ve Rods	Spo	+	-	+	+-	+	+	-	-	+	-	+	-	-	+	+	-	-	+	+	+	-	+	+	*B.baldius*
Peu17	+ve Rods	Spo	+	-	+	-	+	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+	-	+	+	*B thuringiensis*
Peu18	+Cocci	spo	+	-	+	+	-	-	+	-	+	-	-	+	-	+	+	-	-	+	+	-	-	+	+	*B.larve*
Peu19	+Cocci	Spo	+	-	+	+	-	-	-	+	+	-	-	+	-	+	+	+	-	+	+	-	-		+	*B.polymyxa*

Table 4.3: Filter mud vermi-compost microorganisms (Bacteria)

Designation	Morphology / Gram Staining	Endo spore staining	Motility	Hydrogen Sulphide	Aerobic	Anaerobic	Facultative	Acid butt Alkaline slant{TSI}	Acid Butt-Acid SlantTriple sugar Iron	Alkaline Butt Alkaline Slant	Bubble in Butt	H2S Production	NO2Nitrate reduction	NO3	Indole	Methyl red	Vagous pauskar	Ureas	De amyne pholyamyline	Sucrose	Fructrose	Catalse	Maltonate	Oxidase	Geletin liqifaction	Tentative Identification
Peu20	-Rods	Spo	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	-	-	+	+	+	-	+	+wp	*B.alvei*
Peu21	+ve Rods	Spo	+	-	+	-	+	-	+	-	+	-	+	-	-	-	+	-	-	+	+	+	-	+	+	*B.pumilus*
Peu22	+ve Rods	Spo	+	-	+	+	-	-	-	+	+	-	+	-	-	+	+	+	-	+	+	-	-	+	+	B.pumilus
Peu23	+ve Rods	Nspo	+	-	+	+	-	-	+	-	+	-	-	+	-	-	+	+	-	+	+	-		-	+	****
Pme24	+ve Rods	Spo	+	-	+	-	+	+	-	-	+	-	+	-	-	--	+	+	-	+	+	+	-	-	+	*B.schlegeliu*
Pme25	+ve Rods	----	+	-	+	+	-	-	-	+	+	-	-	+	-	-	+	-	-	+	+	+	--	+	+	*B.schlegeliu*
Pme26	+ve Rods	-----	+	-	+	+	-	-	-	+	+	-	+	-	-	+	+	-	-	+	+	+	-	+	+	*B.cogulans*
Pme27	+ve Rods	spo	+	-	+	-	+	+	-	-	+	-	+	-	-	-	+	-	-	+	+	-	-	+	+	*B.licheniformis*
Pme28	+ve Rods	Spo	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	-	-	+	+	-	-	+	+	*B.licheniformis*
Pme29	+ve Rods	Nspo	+	-	+	-	+	-	+	-	+	-	-	+	-	+	+	-	-	+	+	+	-	+	+	****
Pme30	+ve Rods	Spo	+	-	+	+	+	-	+	-	+	-	+	-	-	+	+	-	-	+	+	-	-	+	+	*B.plymyxa*
Pme31	+ve Rods	Spo	+	-	+	-	+	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+		+	+	****
Pef32	+ve Rods	Nspo	+	-	+	-	+	-	+	-	+	-	+	-	-	+	+	+	-	+	+	+	-	+	+	*B.alvei*
Pef33	+ve Rods		+	-	+	+	-	-	-	+	+		+	-	-	+	+	-	-	+	+	-	-	+	+	*B.coagulans*
Pef34	+ve Rods	Spo	+	-	+	-	+	-	+	-	+		+	-	-	+	+	+	-	+	+	-	-	+	+	*B.laterosporus*
Pef35	+ve Rods	Spo	+	-	+	-	+	-	+	-	+		+	-	-	+	+	+	-	+	+	+	-	+	+	*B.larve*
Pef36	+ve Rods	Nspo		-	+	+	-	-	+	-	-		-	-	-	+	+	+	-	+	+	-	-	+	++	****
Pef37	+ve Rods	Spo	+	-		-	+	+	+	+	+		-	-	-	+	+	-	+	-	+	-	-	+	+	*B.pumilus*

Table 4.4 Identification of earthworm microorganisms (Bacteria)

Designation	Morphology / Gram Staining	Endo spore staining	Motility	Hydrogen Sulphide	Aerobic	Anaerobic	Facultative	Acid butt Alkaline slant(TSI)	Acid Butt-Acid SlantTriple sugar Iron	Alkaline Butt Alkaline Slant	Bubble in Butt	H2S Production	NO2Nitrate reduction	NO3	Indole	Methyl red	Vagous pausksr	Ureas	De amyne pholyamyline	Sucrose	Fructrose	Catalse	Oxidase	Maltonate	Geletin liqifaction	Citrate utilisation	Tentative Identification
Sef1	–ve Coco Bac	S	+	-	+	+	+	-	+	-	+	+	+	-	-	+	+	-	-	+	+	+	+	-	+	+	***
Sef2	+ Bacilli	NS	+	-	+	+	+	-	+	-	+	-	-	nd	+	+	+	+	-	+	+	+	+	-	+	+	****
Sef3	-Short rods	S	+	-	+	+	+	-	+	-	+	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	****
Sef4	+ Bacilli	S	+	-	+	+	+	-	+	-	+	-	-	+	-+	+	+	+	-	+	+	+	+	-	+	+	*B.sphaericus*
Sef5	+ Bacilli	S	+	-	+	+	+	-	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	+	*B.megaterium*
Sef6	- coco bacilli	S	+	-	+	+	+	-	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	+	*B.licheniformis*
Sef7	+ Bacilli	S	+	-	+	+	+	-	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	+	*B.macerans*
Sef8	+ Short rods	S	+	-	+	+	+	-	-	+	+	-	-	+	-	+	+	+	-	+	+	+	-	-	+	-	*B.popilliae*
Seu9	Gram -Ve	S	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+	+	+	++	+	+	-	+	+	***
Seu10	+ Bacilli	S	+	-	+	+	+	-	+	-	+	-	+	-	-	-	-	+	-	+	+	+	++	-	+	+	*B.marinus*
Seu11	+ Short rods	S	+	-	+	+	+	-	-	+	+	-	-	+	-	-	-	+	-	+	+	+	-	-	+	-	*B.alcalophilus*
Seu12	+ short rods	S	+	-	+	+	+	-	+	-	+	+	-	+	-	-	-	-	-	+	+	+	+	-	+	+	*B.thuringiensis*
Sme13	- Coco bacili	S	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	***
Sme14	- Coco bacilli	S	+	-	+	-	-	-	-	+	+	-	+	-	+	+	+	+	-	+	+	+	++	-	+	+	***
Sme15	+ Bacilli	S	+	-	+		+	-	-	+	+	-	-	+	-	+	+	-	-	+	+	+	+	-	+	+	*B.subitus*

4.3.3 Enzyme activities:

The test results of the enzyme activities of the earthworm microrganisms presented in table 4.5 and the comparison of enzyme activities presented in figure 4.1

The enzyme activity results reveal that the earthworm organisms of *Eisenia fetida* are showing all the enzyme activities. The skin organisms of *Eudrilus eugenae* show all the activities except for geletinase and the same is true with *Megascolex megascolex* out one of the organism designated Sme 6 shows geletinase. Therefore, the *Eisenia fetida* takes the top list in producing enzyme activities. Based on the total number of isolates in each species and number of organisms showing enzyme activities it evident that 77%, 75%, 62.5%, of Eisenia *fetida, Megascolex megascolex* are showing selected enzyme activities respectively. Therefore the order f preference of earthworm species based n the enzyme activity of earthworm microorganisms (bacteria) is *Eisenia fetida*> *Megascolex megacolex* > *Eudrilus eugeniae.*

Table 4.5 Zone of clearance of enzyme activity for the earthworm adheared bacterial islates

	Clear zone dia in mm					
Culture Designation	Amylase	Protease	Cellulose	Lipase	Chitinase	Geletinase
Sef1	20	5		3	4	-
Sef2	6	5	3	12	2	13
Sef3	2	3	8	5	-	8
Sef4	8	20	6	-	2	-
Sef5	2	4	20	5	2	8
Sef6	12	4	4	7	8	6
Sef7	5	2	3	3	-	-
Sef8	-	5	-	-	4	-
Seu9	3	2	4	2	2	-
Seu10	-	4	-	-	4	-
Seu11	12	3	-	2	-	-
Seu12	6	10	8	2	8	-
Sme13	4	6	2	5	8	-
Sme14	8	-	4	4	12	-
Sme15	3	-	2	3	12	-
Sme16	12	-	10	8	6	15

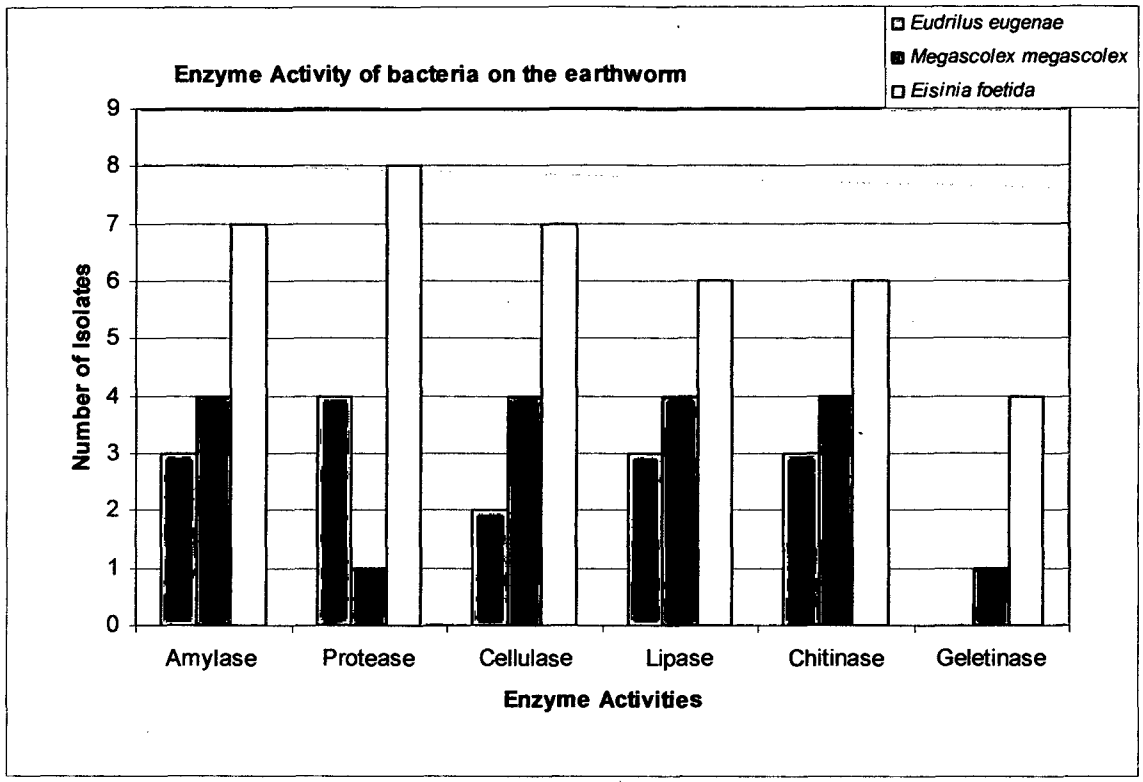


Fig. 4.1 Comparison of enzyme activity of bacteria on the earthworm

Table: 4.6 Zone of clearance for enzyme activities of the bacterial isolates of press mud vermi-compost.

Culture designation	Amylase	Protease	Cellulase	Lipase	Chitinase	Geletinase
Peu1	-	4	4	8	10	13
Peu2	6	4	4	10	8	7
Peu3	-	3	-	10	8	-
Peu4	10	10	10	10	12	4
Peu5	8	25	25	-	8	14
Peu6	-	4	4	-	8	10
Peu7	8	-	3	6	12	6
Peu8	4	5	5	-	-	8
Peu9	8	10	10	10	-	10
Peu10	-		4	12	10	7
Peu11	8	4	6	8	10	8
Peu12	14	6	8	4	12	-
Peu13	8	4	4	13	12	14
Peu14	6	6	6	8	6	9
Peu15	8	6	6	4	12	8
Peu16	6	6	6	2	12	10
Peu17	8	6	6	-	12	8
Peu18	-	6	6	4	14	6
Peu19	6	6	-	-	-	18
Peu20	-	-	-	-	-	-
Peu21	-	-	-	-	-	-
Peu22	-	-	-	-	8	-
Peu23	-	-	-	-	8	-
Pme24	20	-	-	-	8	14
Pme25	-	4	4	-	8	16
Pme26	-	6	4	2	6	-
Pme27	-	-	-	-	-	-
Pme28	-	-	-	3	-	8
Pme29	12	6	6	-	8	14
Pme30	-	-	-	8	8	16
Pme31	10	-+	-	6	8	16
Pef32	-	-	-	-	8	14
Pef33	-	10	10	4	6	-
Pef34	-	-	-	4	6	8
Pef35	10	10	10	6	8	8
Pef36	-	10	10	-	-	6
Pef37	6	10	10	10	8	8

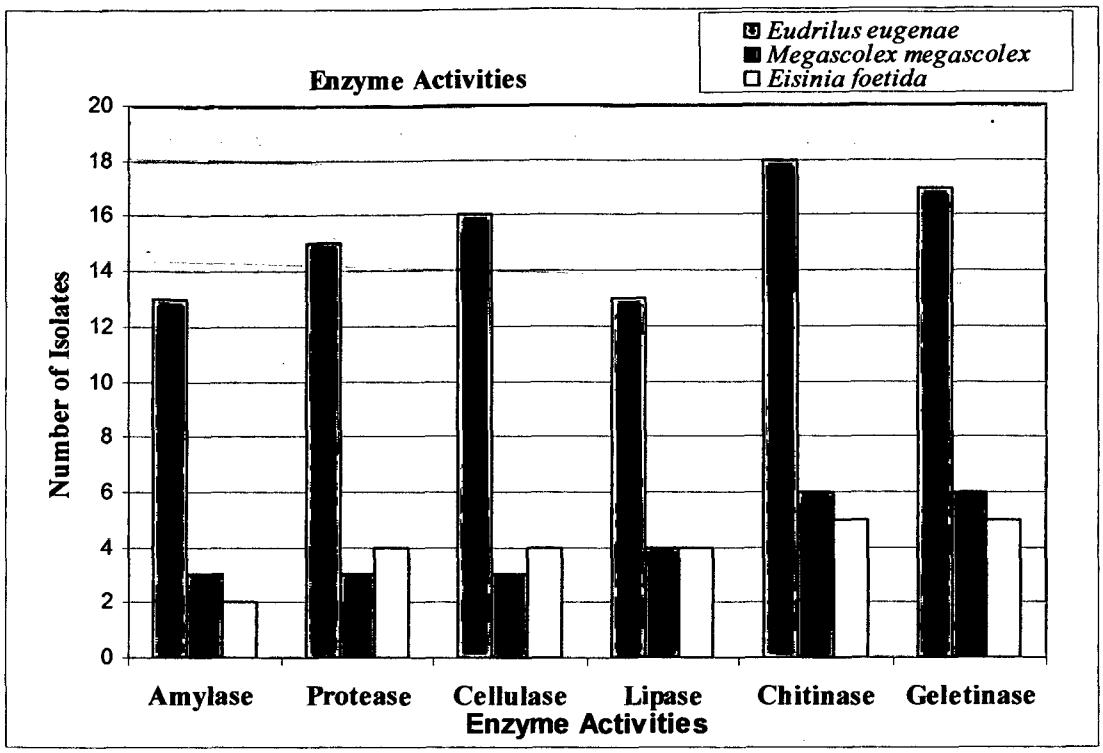


Fig. 4.2 Comparison of enzyme activity of vermi-compost microorganisms (Bacteria)

Based on the number of isolates showing the enzyme activity(Table 4.6) it is evident that the *Eudrilus eugenae* stand as superior as compared to other two and the order of preference is given as *Eudrilus eugeniae>Megascolex megascolex> Eisenia fetida.*

Based on the individual enzyme activities recorded by each organisms the species of earthworms rank showing enzyme activity as 68.11%,66.66%,52% for *Eudrilus eugeniae, Eisenia fetida> Megascolex megascolex.* The comparison of enzyme activity is presented in figure 4.2. The visuals enzyme activities presented in plates 4.6, 4.7, 4.8, 4.9, and 4.10.

The presence of active enzymes amylase, cellulase, protease, chitinase, lipase and lichenase in earthworm gut signifies the digestive ability of the eatrtheworms. These enzymes reported to operate in a stable pH range of 6.3 to 7.3 through out the length of the intestine which encourages the growth of bacterial colonies as reported by Senapati (1993).

Our experiment has demonstrated that the earthworm *Eisenia fetida* has shown its cellulase enzyme activity in skin and vermi-compost organisms. The results are in accordance with the findings of Whiston, (1998) that *Eisenia fetida* has shown the carboxymethyl cellulose activity. The report of Trivedy, (2002) indicates the vermi castes also contain enzymes such as protease, amylases, lipases, cellulases, chitinase has 10 to 20 times higher microbial activity as compared to the soil and organic matter of worm ingest. The occurrence of lichenase in the digestive track of invertebrates, the earthworm species *Helodrilus caliginosus* showed lichenase and enzyme activity, Jowell and Lewis, (1917). Our findings go parellel to the findings of the Tracy (1951) for the presence of the amylase, protease, cellulase, and chitinase, lipase produced by the earthworms and

possibly the cellulose and chitinase by gut microorganisms, whereas the report of Ross and Cairns,(1982) supports our findings of presence of enzyme activities in earthworm casts and an increase phosphatase, dehydrogenase, protease and nitrigenase enzyme activity due to earthworm influence Weiss and Trespendorfer,(1993). Sneh Goyal, (2005) Changes in activities of cellulase, xylanase and protease, and microbial population were determined during composting of different organic wastes such as mixture of sugarcane trash and cattle dung, press mud, poultry waste and water hyacinth biomass. The activities of cellulase, xylanase and protease were maximum between 30 and 60 days of composting in various wastes. *Eisenia fetida,Perionyx excavatus, E. eugeniae* were used to study on the biodegradation of some community wastes and they were found to secrete enzymes such as proteases, lipases, amylases, cellulases and chitinases which bring about rapid biochemical conversion of the cellulosic and the proteinaceous materials in the variety of organic wastes. *E. eugeniae* was found to have higher feeding, growth and biodegradation capacity compared to other two species and within 5 to 6 weeks, 95–100 percent degradation of all cellulosic materials was achieved as reported by Sinha, (2002).

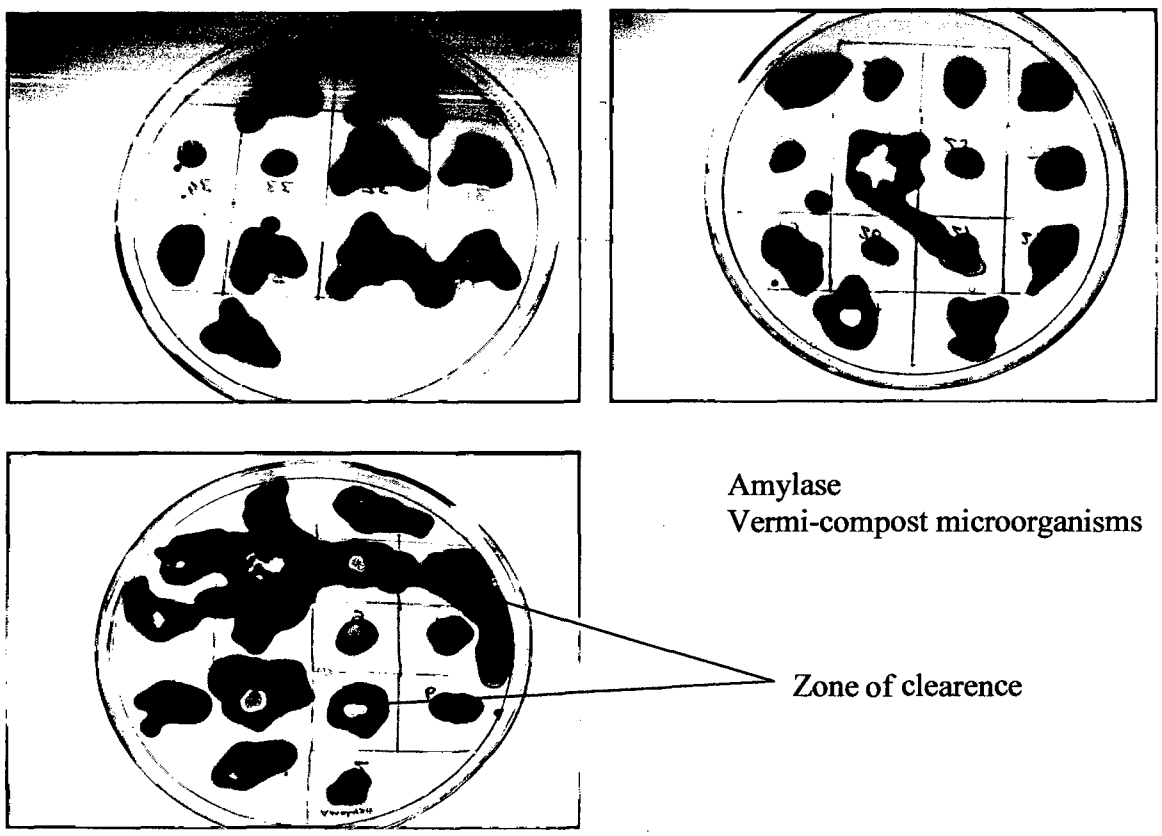


Plate 4.6: Amylase enzyme activity

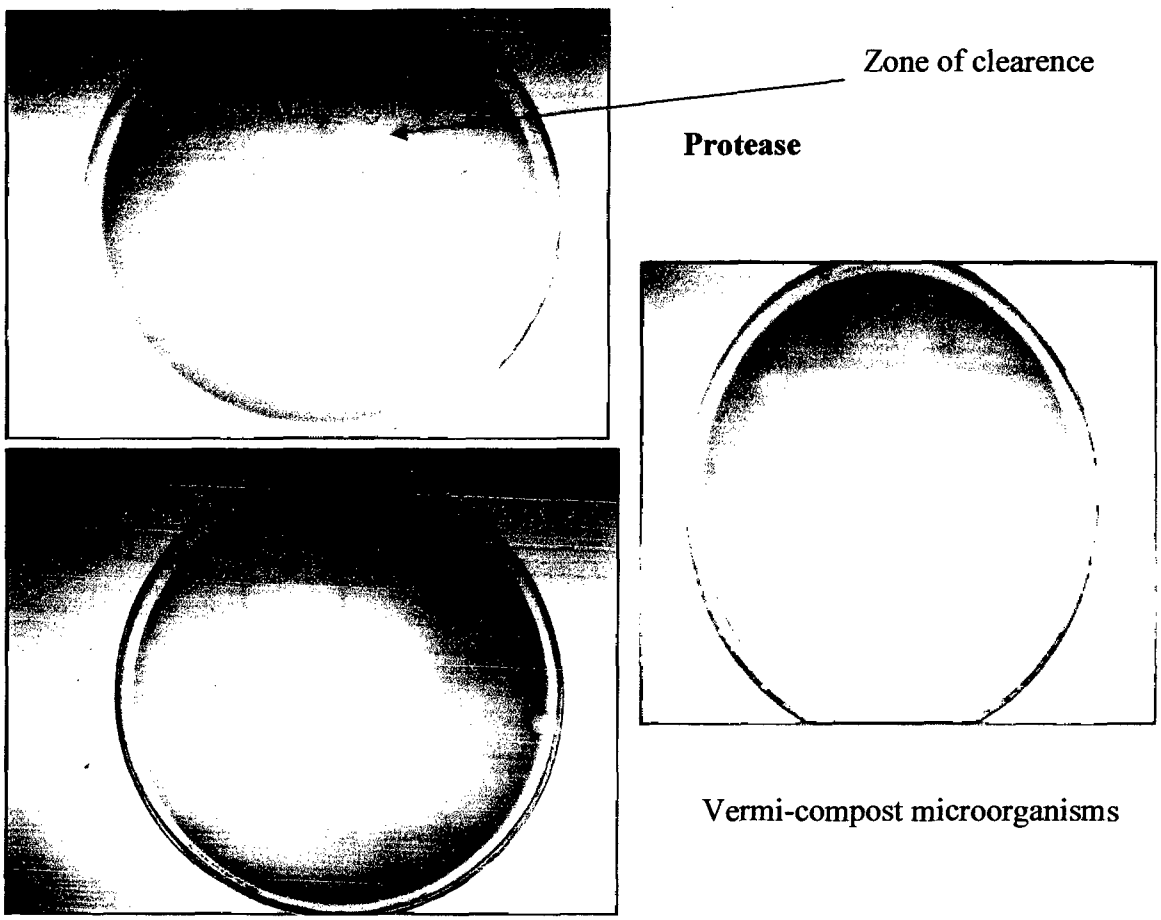


Plate 4.7: Protease enzyme activity:

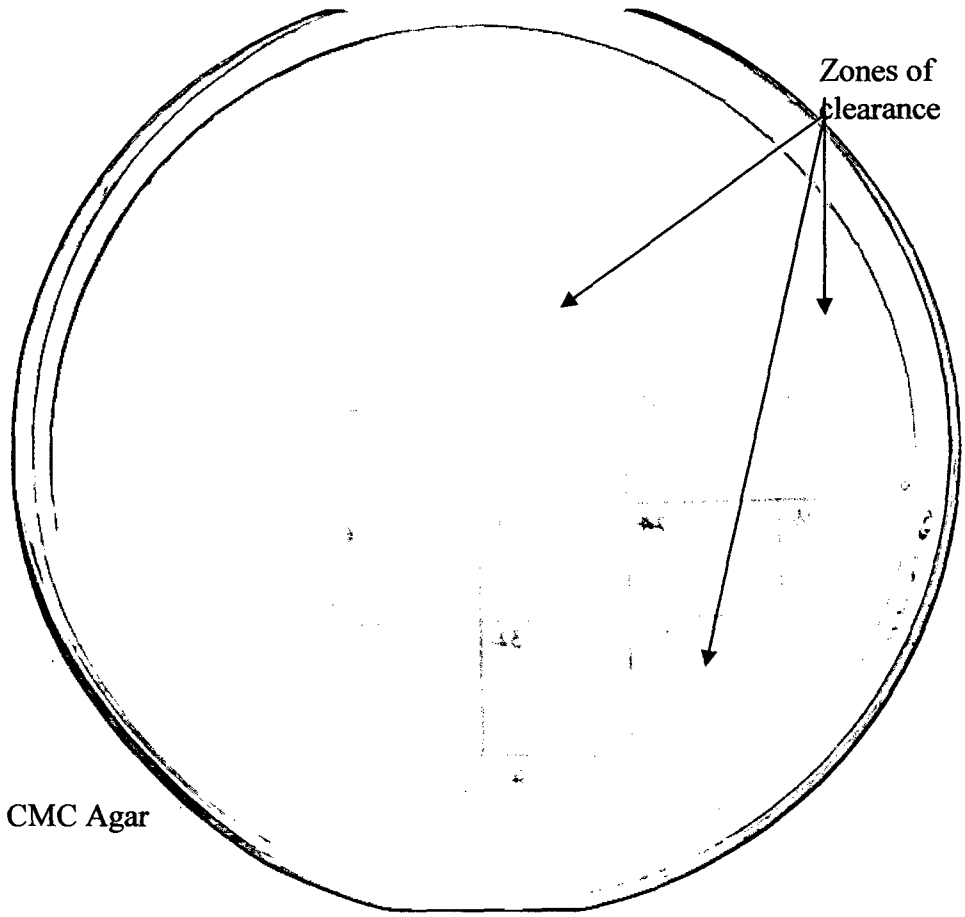
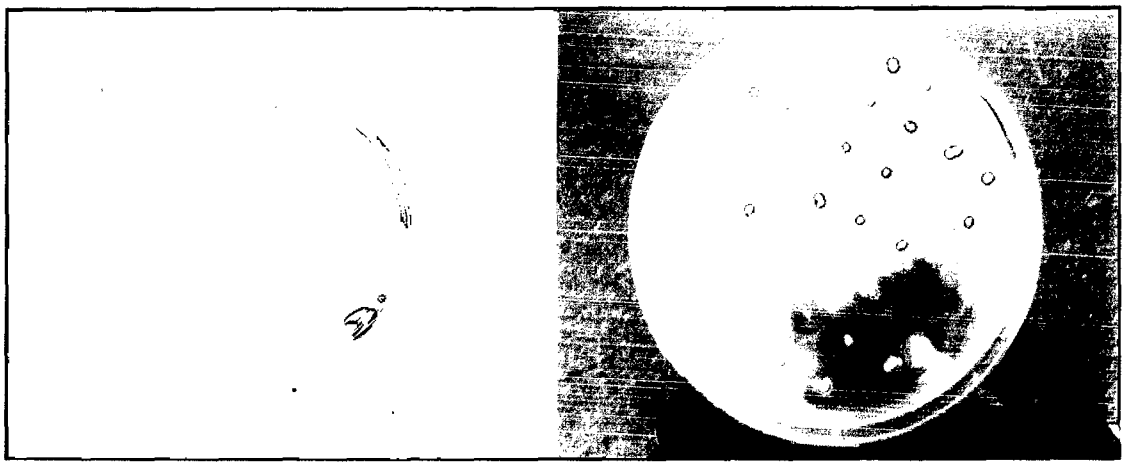


Plate 4.8: Cellulase enzyme activity (Vermi compost microorganism)



Control

Plates flooded with Mercury Chloride
Zones of clearance

Plate 4.9: Geletinase Enzyme activity

Chitinase
Vermicompost
microorganisms

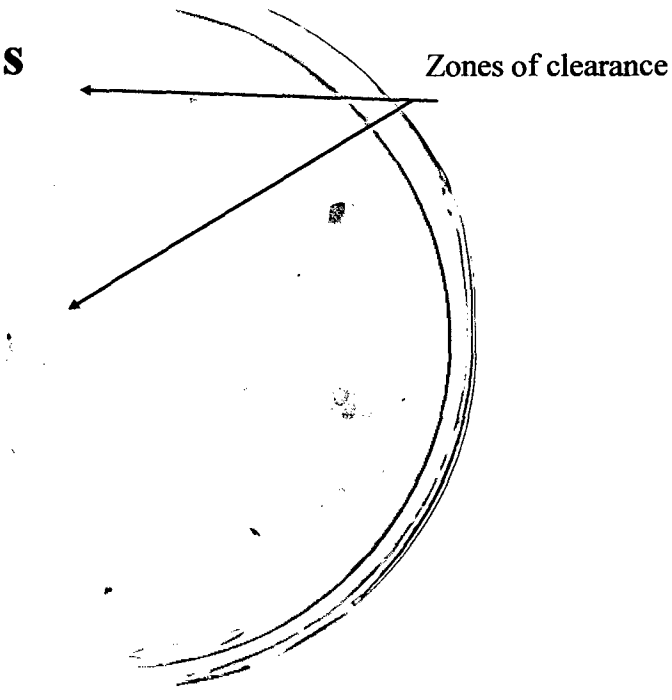


Plate 4.10: Chitinase activity

4.3.4 Consortiums

Consortium of all the compost bacteria was prepared and maintained having a of viable count 168 x 10^9 /ml. The viable count of cellulose degrading organisms measured 270 **x** 10^6 cfu/ml.

Consortium of cellulose degrading microorganisms presented in Table 4.4

Table 4.7: Consortium of cellulose degrading organisms

Designation	Tentative identification
Peu9	***B.marinus***
Peu14	***B.mycoides***
Peu16	***B.baldius***
Sef4	***B.sphaericus***
Sef5	***B.megaterium***
Sme12	***B.thuringiensis***
Sme16	***B.larve***

3.8: Significant points of study:

- Following the biochemical tests as per Bergy's Manuel for determinative bacteriology revealed the occurrence of organisms belonging to *Bacillus* group.

- The order of preference of earthworm species based on the enzyme activity of earthworm organisms is *Eisenia fetida> Megascolex megacolex > Eudrilus eugenae.*

- Based on the number of isolates showing the enzyme activity it is evident that the *Eudrilus eugenae* stand as superior as compared to other two and the order of preference is given as *Eudrilus eugenae>Megascolex megascolex> Eisenia fetida.*

- Based on the individual enzyme activities recorded by each organisms the species of earthworms rank showing enzyme activity as 68.11%,66.66%,52% for *Eudrilus eugenae, Eisenia fetida> Megascolex megascolex.*

The study of isolation of bacteria and there tests on utilization of various carbon sources indicates their degradability and the capability in reducing the carbon contents of the substrates. In addition to strengthen this bacteria subjected to various enzyme activities which showed there specific degradability capabilities. As such, these properties were utilized to prepare consortium of cellulose degrading organisms. Therefore it is of importance to set up experiments on the chosen substrate press mud first with earthworms, and with earthworms and inoculums and further with inoculums only. This will give a better understanding of the role of microbes in vermi-composting system as a whole and the role of bacteria in composting press mud along with aggregation of soil. These experiments and results compiled and presented in the next chapter.

CHAPTER V

Role of microbes in biodegradation of Press mud and soil aggregation

Chapter V: Role of bacteria in biodegradation of press mud And soil aggregation

5.0 Introduction: The vermi-composting system studied for the treatment of filter mud in the earlier chapters by using earthworm species *Eisenia fetida, Eudrilus eugeniae, Megascolex megascolex* revealed the symbiotic role of bacteria. The bacteria predominated by increase in the number of isolates with varied enzyme activities utilizing the substrates. It was therefore envisaged to further study the role of bacteria on the bio degradation of press mud. As the major constituents of press mud are cellulosic compounds derived from plant materials with organic matter, organic carbon up 24-to 36% it was felt that effect of the cellulose degrading bacteria on degradation of press mud could be assessed and optimized.

Further the study on treatment of filter mud using the isolated organisms with varied enzyme activities is of importance in increasing the efficiency of bioconversion yielding bio-fertilizers at the faster rate. In order to study the effect of bacteria on the efficiency of vermin compost system a bacterial consortium was prepared using the bacteria isolated from the vermin-composts. The effect of this consortium on vermin- composting was also studied and the geotechnical properties of the compost along with their role on soil aggregation was determined.

5.1 Materials and methods:

Studies to demonstrate the role of bacteria in composting of industrial waste (Press mud) using consortium of bacterial isolates

5.1.1 Preparation of bacterial consortium

The micro organisms isolated from earthworms and vermi compost designated as Peu9, Peu14, peu16, Sef4, Sef5, Sme12, Sme16, showing good growth on cellulose medium were used to prepare the consortium of cellulose degrading bacteria. The selected isolates were grown on nutrient agar plates for 24 hrs, loop full of each was picked up from Petri plate and suspended in one test-tube containing normal saline and the absorbance adjusted to 1.0 at 480 nm. This suspension of bacteria was further used as inoculum for preparing the consortium.

One hundred of nutrient broth in conical flask was inoculated with 2 ml of above suspension and incubated on shaker for 24 hrs. The culture broth was centrifuged at 7000 rpm for 10 minutes and the pellet obtained was suspended in 20 ml of normal saline. This consortium designated as **(BC)** was used as inoculum for the press mud.

Bacterial cultures 37 in number isolated from vermin composts were grown on nutrient agar plates for 24 hrs, and used as inoculum for preparing the consortium as above.This consortium designated as **(BM)** was used as inoculum for the vermi beds.

5.1.2 Effect of consortium of cellulose degrading bacteria on press mud

A) Preliminary study:

Ten g of press mud was taken in Petri Plates and 20 ml of the consortium (BC) comprising of cellulose degrading bacteria (240 X 10^7 /ml cfu) was added, and the compost was analyzed after 20 days.

B) **Optimization of inoculum for composting**

Ten g of filter mud was taken in Petri Plates and inoculated with the consortium of cellulose degrading organism (**BC**) with a viable count of 240 X 10^7 cfu. The inoculations were done in proportions of the press mud weight taken as 1:1; 1:2; 1:3; 1:4. (10ml; 20ml; 30ml; 40ml) The samples were drawn in ten days interval upto 30 days and analyzed for compost characteristics. Further the optimum inoculum was used in culture boxes and the compost formed was analyzed.

5.1.3: Study of effect of mixed bacterial consortium (BM) on composting of press mud:

Two identical culture boxes of size 15cm x 20 cm x 30cm were set up as presented in plate 5.1. Culture boxes containing a few brickbats which were introduced to facilitate water-holding capacity and 25 earthworm species of *Eudrilus eugenae,* were inoculated with 40 ml of 24 hrs old bacterial consortium and with a consortium of cellulose degrading bacteria.

The experimental and control culture boxes were maintained at the laboratory at 70 % moisture. The visual observations were carried out every day for the spread of vermi-

casts on the beds. Filter mud obtained from the sanjeevani sugar factory was fed on the beds to give a thickness of 5 cms. (Plate 5.2) The beds were further observed for visual bioconversion and the samples were drawn at regular intervals for physicochemical, geotechnical characters and microbiological counts.

A control of each culture box with 5 cms filter mud without earthworms and microbial consortium was also maintained and analyzed.

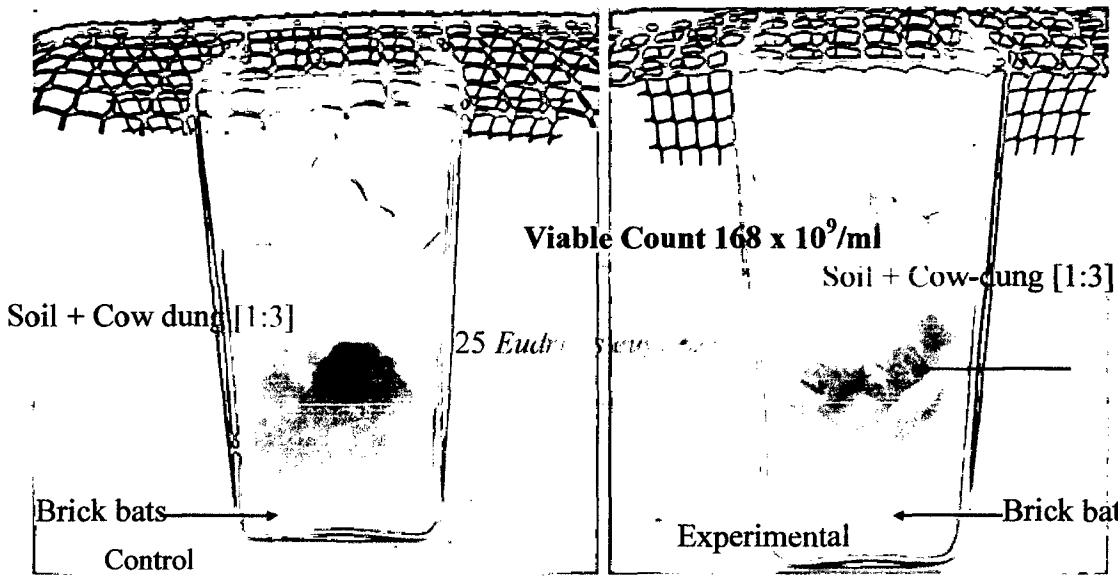
5.1.4 Analysis of compost

The total viable count was determined by plating on nutrient agar. Total solids organic carbon, volatile solids and ash content, were determined as per the procedures outlined in the Appendix B

Earthworms were measured by hand sorting from the samples drawn in the core cutter and complete culture box.

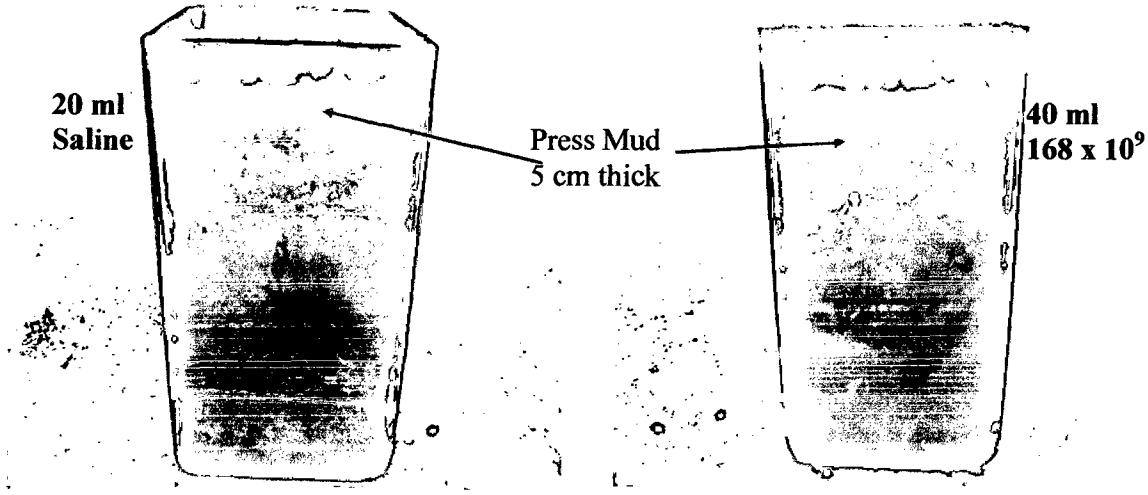
5.1.5 Study of soil aggregation using bacterial consortium (BM).

Petri plates containing 100 g of industry soil passed through 420 and 850 sieves were inoculated with 10 ml, 20ml, and 30 ml of bacterial consortium with a total viable count of 168 x 10^9/ml (Plate 5.13, 5.14). The plates were incubated for 30 days at room temperature, the soil in each Petri plate was sieved by using IS sieves, and the weight retained on 425 μ and 850μ sieves was recorded. Control plates without inoculum were also analyzed for soil aggregation.



Industry Soil passed through 850 micron IS Sieve

Plate 5.1: Study the effect of bacterial consortium on biodegradation of press mud



Control **Press mud inoculated with Bactrial consortium (BM)**

Plate 5.2: Organic loading of Press mud

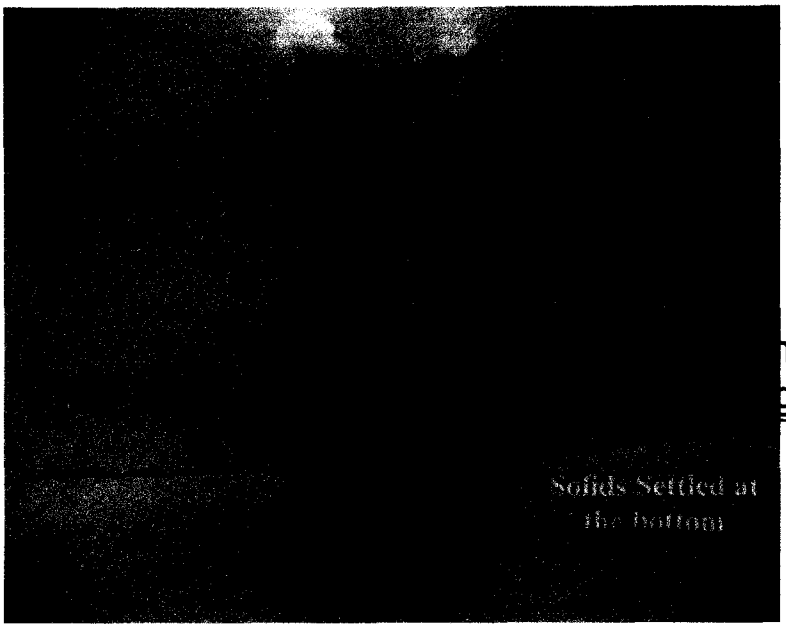
5.2 Results and discussion:

Consortium of microorganisms performing the biological tasks under controlled conditions has been found to be of immense use in industrial applications (Alex Lye, 1994, Sharma Alka, 2000.) Keeping in view the application of consortium in the industrial organic solid waste management the bacterial consortium were prepared and used for the present experiments.

5.2.1 Biodegradation of filter mud with cellulose degrading organisms

A) Preliminary studies

The Petri plates inoculated with cellulose degrading organisms having viable count 270 x 10^6, gave compost after 20 days almost similar to that of press mud with earthworm in the field trials. Further the dilutions of press mud and this compost (plate 5.3) showed that the press mud floated in the test tube whereas the compost settled at the bottom of the test tube. This demonstrated the change in the particle size of the filter mud and its specific gravity. Such a change in specific gravity becomes an essential factor in handling large volumes of industrial wastes in a given time.



n Cellulose
ganisms

Changes in Specific Gravity

Plate 5.3: 20 Day dilutions of filter mud compost with Cellulose degrading Bacteria (BC)

The analysis of the compost on subjecting to oven drying and ashing in muffle furnace showed the decrease of organic carbon 34.14% within 20 days, confirming the role of microorganisms in bioconversion, mainly the consortium of cellulose degrading bacteria. This gave a new direction in which the industrial solid waste management could be looked upon using microbial consortium to achieve desired level of bioconversion in a specified time period.

The proportions of press mud and the consortium (plate 5.4 and 5.5) showed variation in the changes occurring in the chemical parameters The total solids showed decrease for all the proportions especially for 1:1.1:3 (fig.5.1) with a steady decrease shown by the 1: 2 proportion The proportion 1:2 and 1:3 showed (5.4 to 5.5) a decrease in organic carbon of 13.7%, 41.64%in 10, 20 days respectively The ash values are of interest because each proportion has shown decrease up to 10 days after which the increase is seen from 10 to 20 days (fig.5.4).

The density bottle experiment (plate 5.6, fig 5.5) revealed that the proportion 1: 2 increased the specific gravity as compared with the other proportions. The order of increase in specific gravity was 1:2>1:3 and 1:4. The specific gravity is an important parameter to be considered for handling the large volumes of filter mud produced by the sugar industries. The cumulative data on compost showed that the 1:2 proportions gave consistent gradual changes with significant ash content after 20 days therefore this proportion was used for culture box studies.

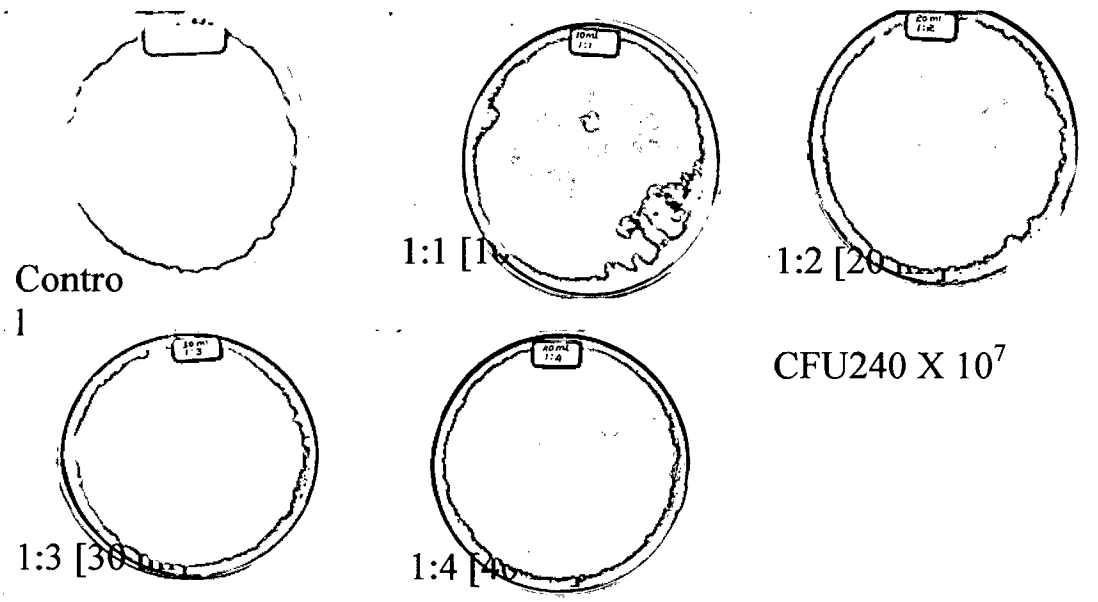


Plate 5.4 Effect of different proportion of inoculum

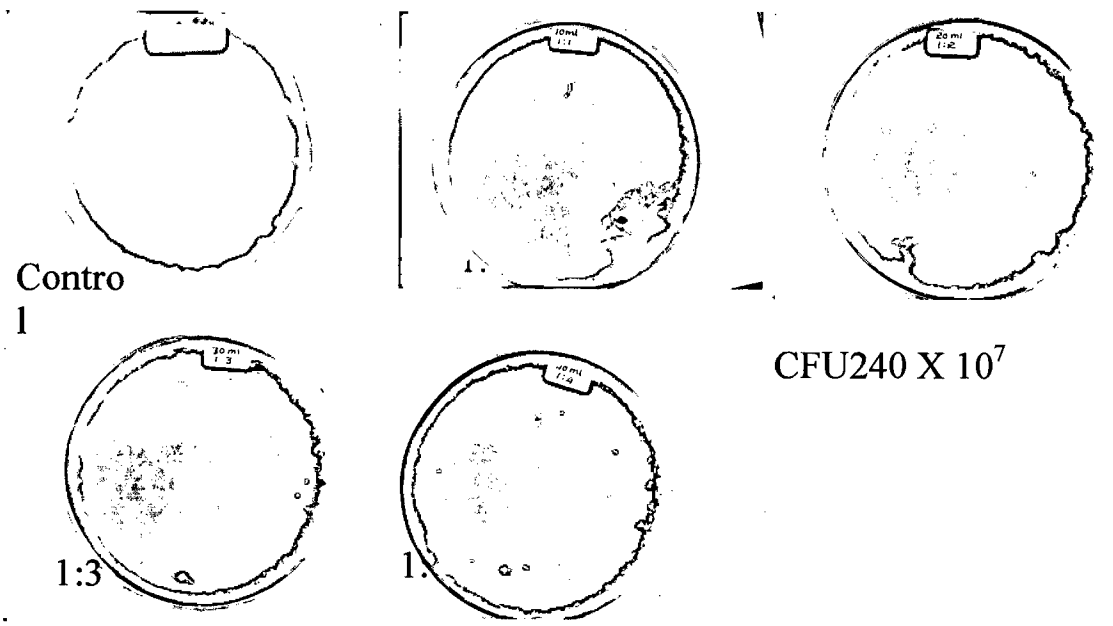


Plate 5.5: Effect of optimum proportion of inoculums 20-day pictures

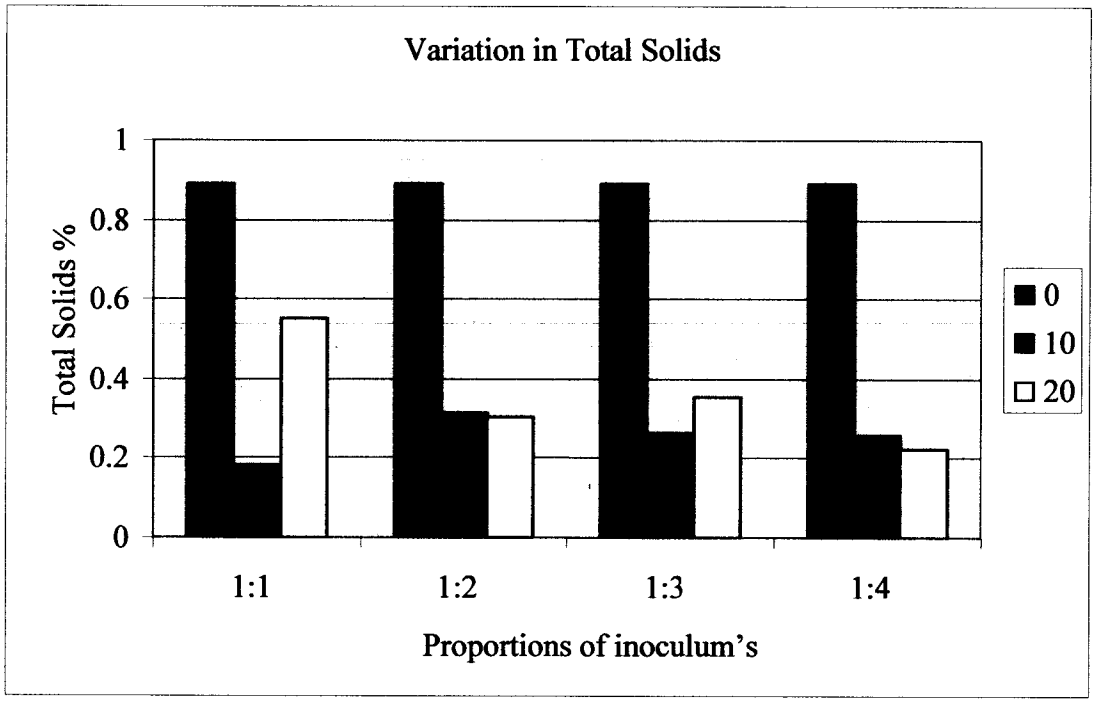


Fig. 5.1: Changes in total solids with different proportion of inoculum

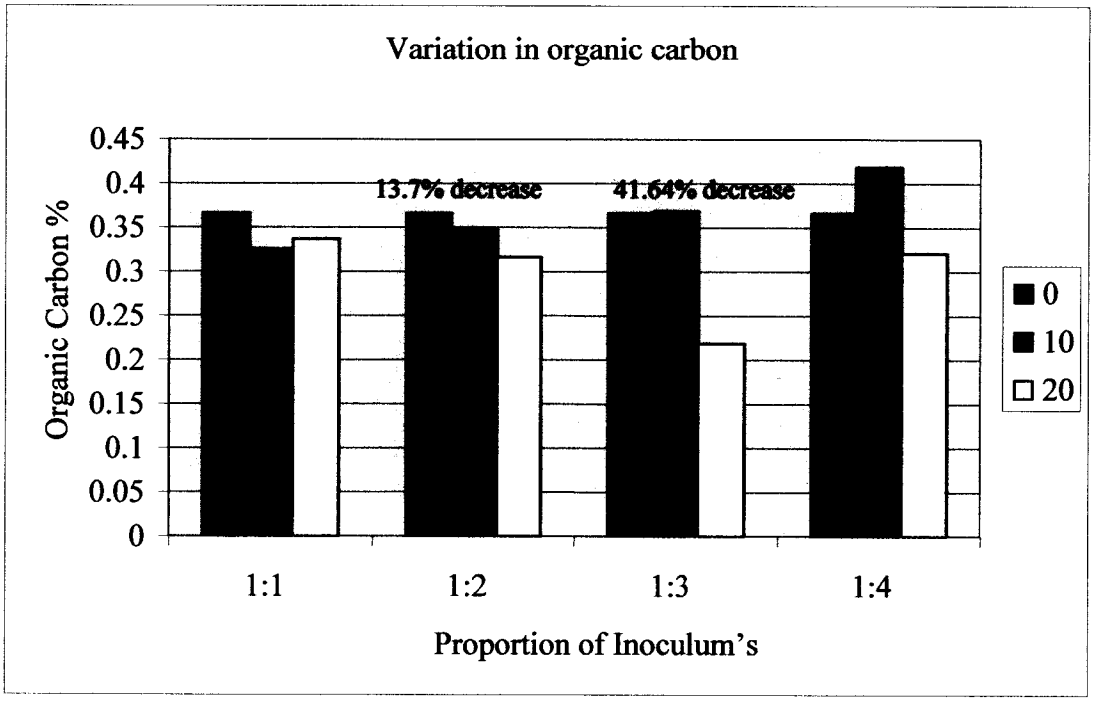


Fig. 5.2: Changes in organic carbon with different proportion of inoculum

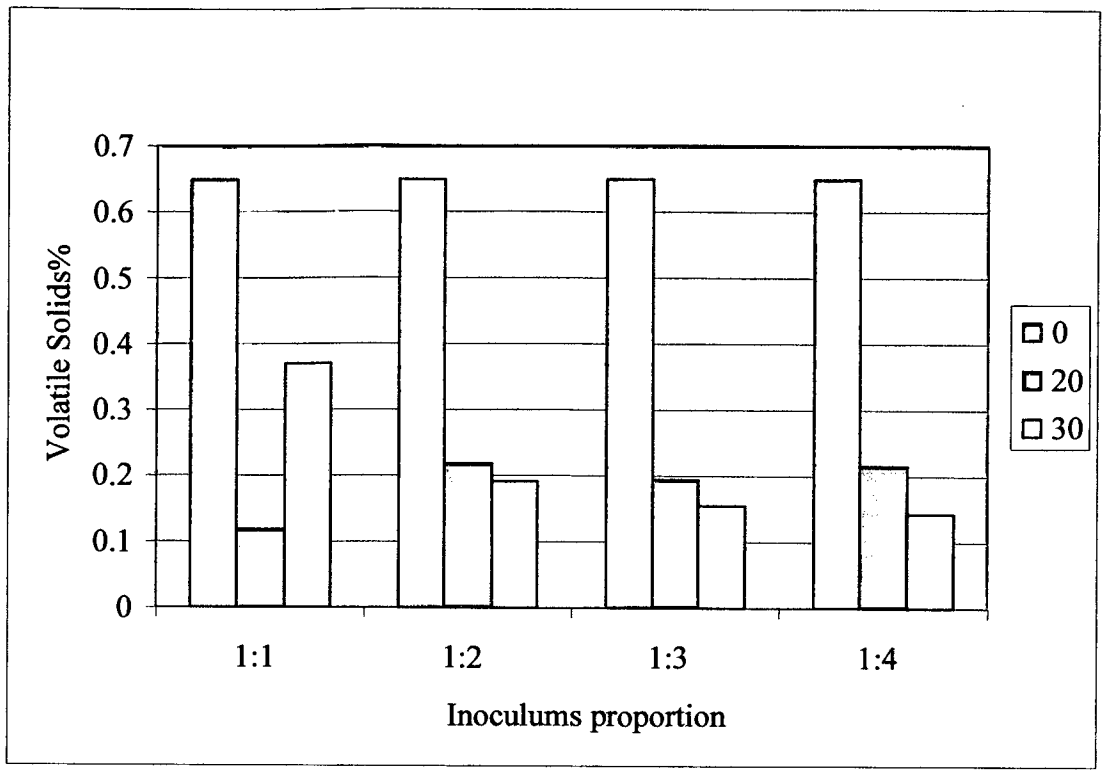


Fig. 5.3: Changes in volatile solids with different proportion of inoculum

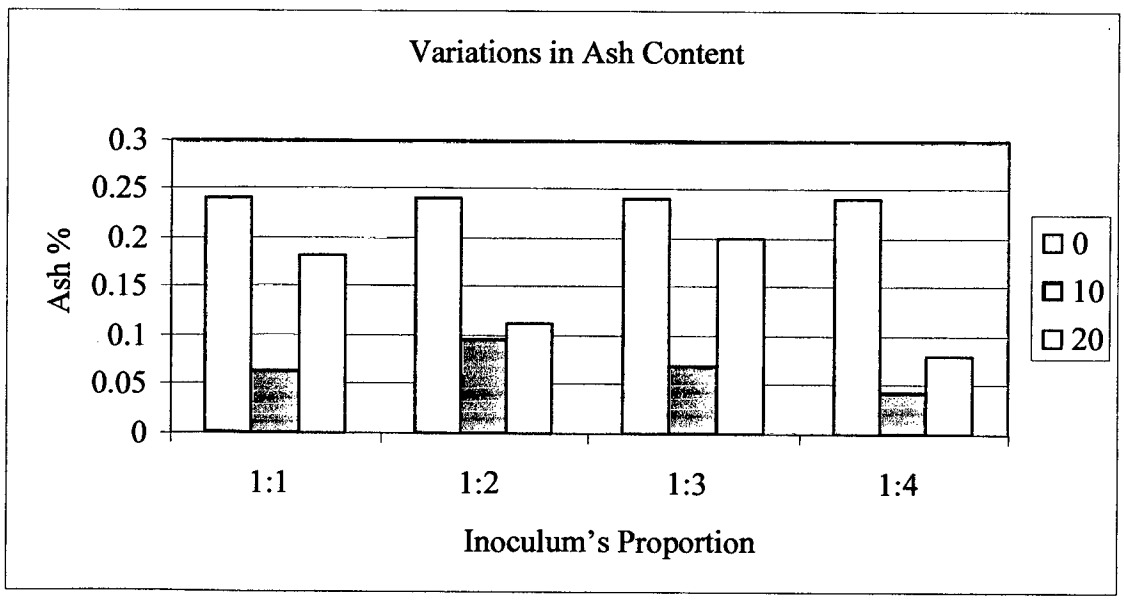


Fig. 5.4: Changes in ash content with different proportion of inoculum

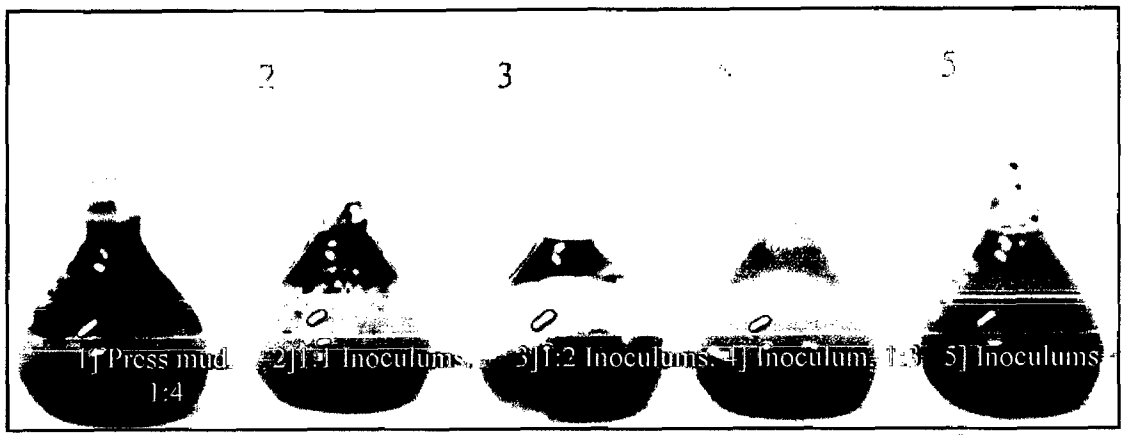


Plate 5.6: Press mud in density bottles (For Specific gravity)

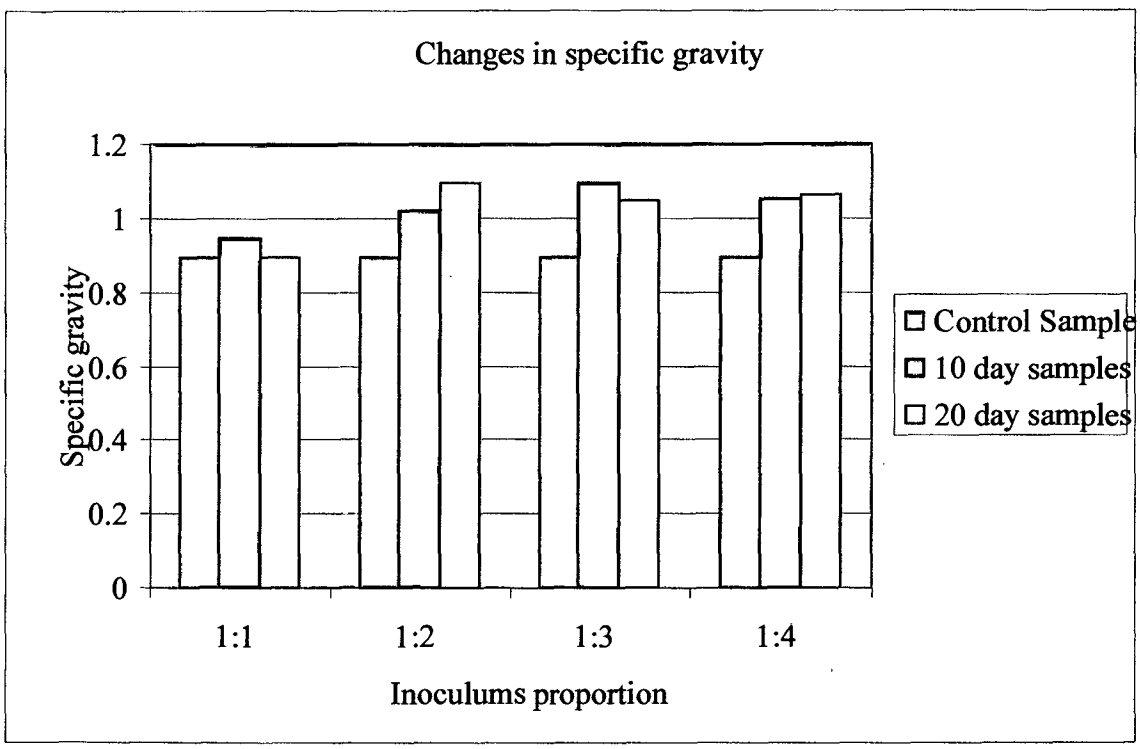


Fig. 5.5: changes in specific gravity of compost samples from

B) Bio degradation of press mud with optimized proportion (1:2) using BC

The changes in geotechnical properties of press mud subjected to cellulose degrading bacterial consortiua are presented in Table 5.3. The specific gravity of the filter mud was found to be (Table 5.2) far less as compared to the compost obtained from cellulose degrading organisms Which also had higher water content (Table 5.2). This is based on one time inoculation after that no additional water was added to the experiment at any stage of experiment. This facilitated in controlling the compost formation without regularly maintaining the moisture as was required in vermi-compost system.

The voids ratio and porosity (Table 5.2) was much higher as compared to the filter mud values showing the amorphous compost without earthworms

The densities have shown increasing trend in bulk and saturated densities by 0.22 and 0.27, where as the dry density remains almost the same with a marginal variation of decrease of 0.016.

The air content-recorded (Table 5.2) in case of filter mud compost treated with cellulose degrading organisms is 61 %, which was found to be higher than what is recorded, with control (48%)and experimental culture (38%) boxes with *Eudrilus eugenae* . This suggests that the treatment with bacteria alone provides better geotechnical properties as compared with that of earthworm experiments. The same is true in case of degree of saturation.

The air voids recorded(Table 5.2) in case of filter mud treated with cellulose degrading organisms as 48% is comparable with the air voids obtained from control and experimental culture boxes which showed similar results.

The total solids, organic carbon, volatile solids, ash content etc are presented in the fig. 5.6.The total solid of the sample due to the addition of inoculum showed the decreasing trend in 10, 20 day and 30-day samples. Whereas, comparison with the experiment with earthworms the trend is increasing in total solids. The same is true with volatile solids.

The organic carbon (Fig. 5.6) showed a decreasing trend up to 20 days there after for 30 days it showed a higher value. This indicates the experiment is contributing to additional organic carbon due to inoculum. Therefore, it was concluded that the bioconversion reached its maximum up to 20 days and thereafter further increase in organic carbon suggests that the compost could be harvested after 20 days. The volatile solids have shown decreasing trend, where as ash showed increasing trend for 20 days and decrease for 30 days findings.

The total solids, organic carbon, volatile solids, ash content etc are presented in the Fig. 5.6. The total solid of the sample due to the inoculums showed the decreased in 10, 20 day and 30-day samples. Whereas, comparison with the experiment with earthworms the trend is increasing in total solids. The same is true with volatile solids.

The particle size distribution of filter mud alone and its compost with cellulose degrading microorganisms are presented in Fig. 5.7. It is evident from the graph that the finer particles less than 425 μ have aggregated with the bioconversion where as the higher size aggregates > 425 μ has decreased in the particle size. The particle size is more of uniform distribution and is almost parallel to the filter mud distribution

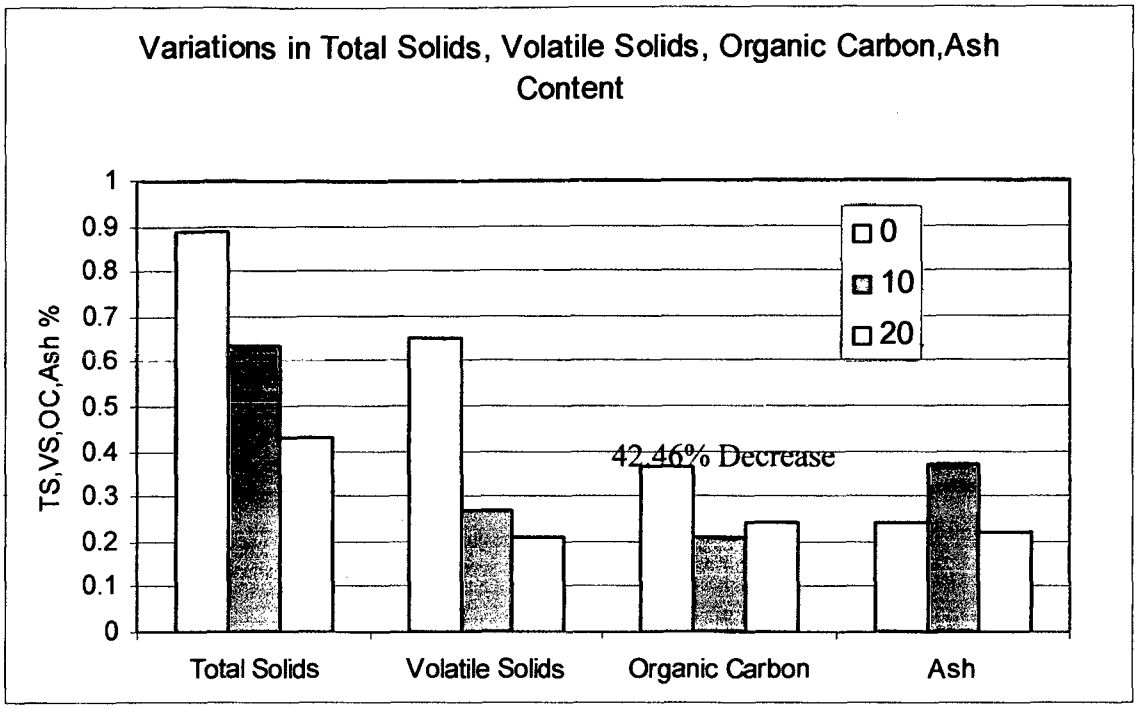


Fig. 5.6: changes total solids, volatile solids, organic carbon, and ash content with consortium of cellulose bacteria (BC)

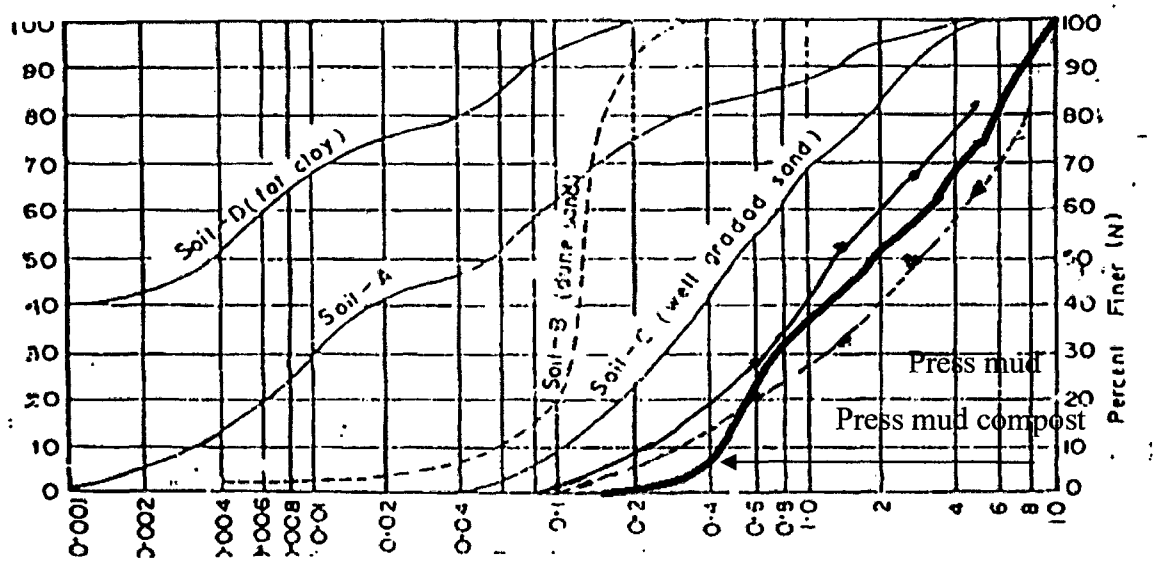


Fig. 5.7: Particle size distribution of filter mud compost with consortium cellulose degrading bacteria (BC)

5.2.2 Role of mixed bacterial (BM) consortium on vermicomposting system:

A) Biodegradation of press mud:

The studies carried out on role of bacteria on vermi-composting system and on press mud biodegradation showed significant observations. For this study the earthworm used was *Eudrilus eugenae* along with consortia for the vermiculure system. As the number of isolates showing enzyme activity was found to be maximum, it was evident that the *Eudrilus eugeniae* was most viable as compared to other two.

The consortia of vermicompost microorganisms showed viable count 168 x 10^9 /ml having 37 vermi-compost microorganisms isolated from the vermi-composts of press mud from field trials using *Eudrilus eugeniae, Eisenia fetida,* and *Megascolex megascolex*

The appearance of the casts on the top surface of the vermi-beds after 10 days showed (Plate 5.7) better earthworm activity in the test culture box with uniform distribution of vermi cast the observations indicated that the beds were ready to receive the organic load of selected substrate, in the present case the press mud. . The total viable count on nutrient agar was found to range from 10^7 to 10^8

Control vermi composting system

Experimental vermicomposting system with bacterial inoculum

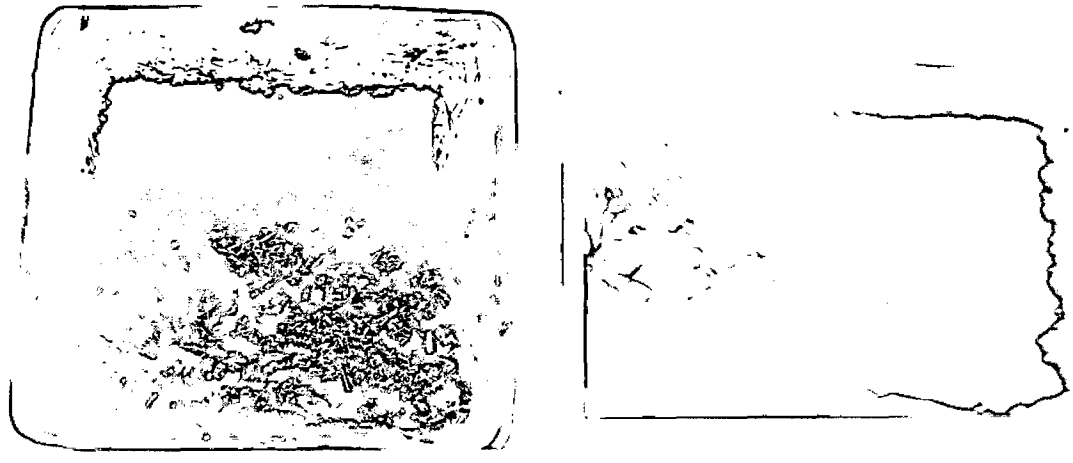
Plate 5.7: Top views of culture boxes after 10 days of introducing earthworms.

Edwards and lofty (1977), reported the large amounts of soil from deeper layers was brought to the surface and deposited as casts and the casts deposited ranged from 2 to 250 tonnes per hectare. This amounts to bringing up 1mm to 5cm thick, to the surface every year. At the same time major part of the casts are also deposited as either subsurface casts or within burrows, so that the total soil turnover is even greater. Barley (1961) reported that earthworms trans-located the subsoil to the top layers and thus unproductive subsoil undergoes biochemical changes during its passage through the alimentary canal of earthworms and turns productive for growing plants.

In the present experiment, the turn over in the experimental culture box was found more than the control experiment with more fine casts deposited uniformly all over the surface. This demonstrates that either adding vermi-casts to the soil not only increase the fertility but also microbial population, which in turn contributes to greater earthworm activity leading to formation of soil layers.

It was observed that after ten days partial biodegradation of press-mud occurs in control culture box whereas biodegradation is more and uniform in the test culture box. The top surface of experimental culture box showed more amount of soil brought as cast on the surface depicting increased earthworm activity (Fig 5.8). This also suggests that the press-mud and soil can be easily blended together to upgrade the value of the compost for addition in the fields.

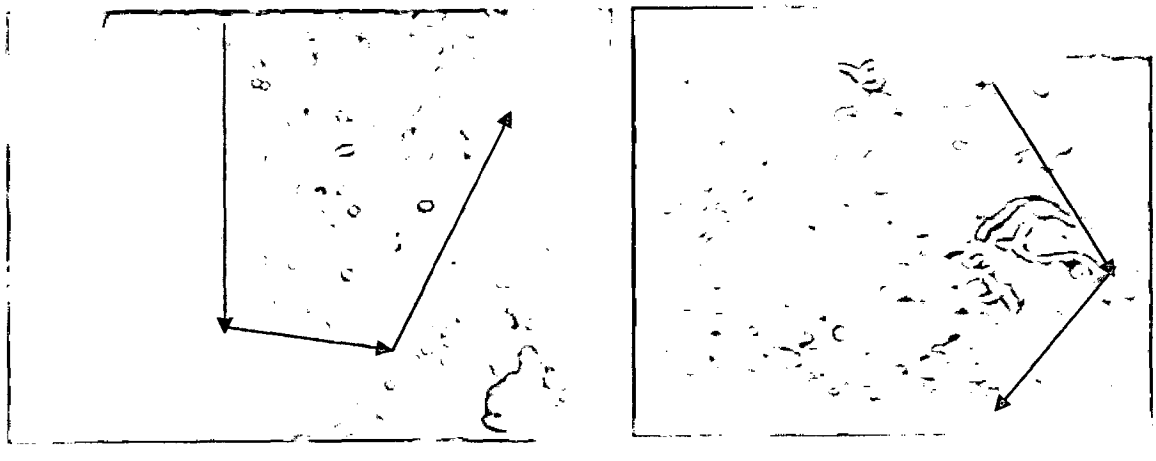
A-10 days



Control pot

Experimental pot

B-20days



Control pot

Experimental pot

Plate 5.8: Culture boxes inoculated with microorganisms

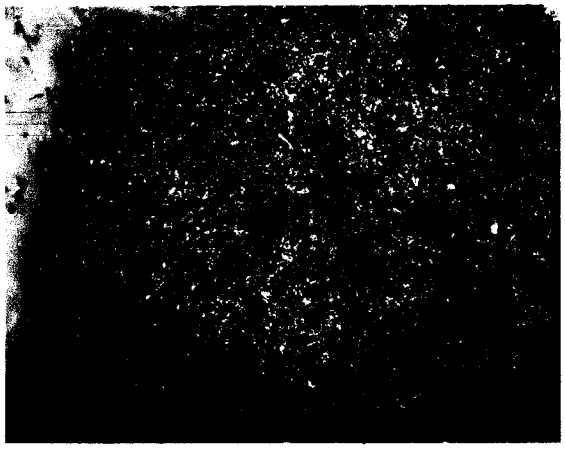
The visual show that the complete bioconversion of the filter mud has taken place in case of experimental culture box where as in control culture box a small portion was yet to under go bioconversion. (Plate 5.9)

Based on the observations it was evident that the experimental culture box had shown a bioconversion time of 25 days. However the control culture box required additional time for complete bioconversion to occur of 5 to 10 days.

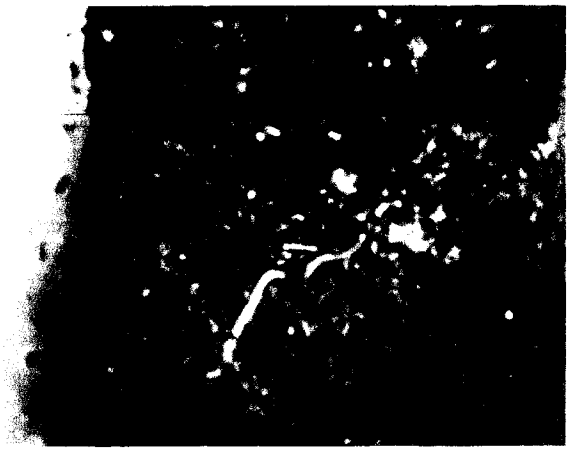
Singh (1997); Munnoli,(2002), have reported that bioconversion of industrial waste with *Pheritima elongate* using substrates press mud and potato peel as 35 and 40 days respectively. There are fewer reports on conversion time with microorganisms added to vermi-composting system. In our earlier chapter the press mud bioconversion time in field trials was found to be of 45; 40 and 35 days with *Eisenia fetida, Eudrilus eugeniae* and *Megascolex megascolex* respectively.

The size of the worm cast isolated from the experimental culture box with *Eudrilus eugenae* varied from 2 mm to 6 mm in length and 3 mm in diameter and the temperature recorded during experimentation varied from 28 ^{0}C to 36^{0}C. Also worm cast and cocoon production (5.10 and 5.11) was high in experimental culture box

Gates, (1961) reported that *Notoscolex* earthworm in Burma produced large tower shaped casts up to 20-25 cm weighing 1.6 Kg. where as Madge, (1969) reported that *H africanus* also produced similar casts from 2.5 cm to 8.0cm length and 1.0 to 2.0 cm in diameter.



Top View 25 day control culture box still unutilized substrate is visible



Top View of experimental culture box 25 days showing complete bioconversion

Plate 5.9: Control and experimental culture boxes top views after 25 days

Plate 5.10 (Fresh cocoons)

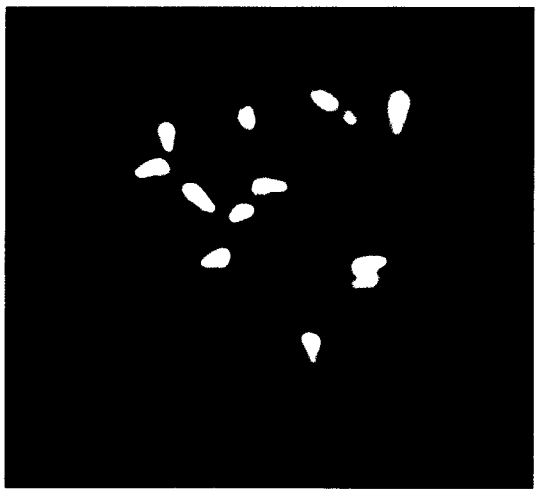


Plate 5.11 (Dry cocoons)

Plate 5.10 and 5.11 Cocoons of *Eudrilus eugeniae*

The efficiency of worms for effective waste management in the given environmental conditions is to their full potential and produce maximum cast in any period of the year. Gates (1961) reported that earthworms in India are active mainly in the four to six months of the rainy season between May and October. Therefore, castings by tropical earthworms are limited to wet season Madge (1969) and Gates (1969). Our field trials conducted on filter mud as substrate through out the year showed successful breeding of *Eisenia fetida, Eudrilus eugeniae, Megascolex megascolex.*

The observations of the dilutions of vermi-compost samples[1g vermi-compost in 10 ml normal saline] drawn from control and experimental culture box(Plate 5.12.) revealed that there are more suspended particles of composts and juveniles of *Eudrilus eugeniae* in the experimental culture box as compared with the control culture box.

There are reports that fresh moist cast is 26 to 41 percent more dispersible than un ingested moist soil and ageing reduces the dispensability of moist casts to 49 per cent than uningested soil (Shipitalo and Protz, 1988).

The total viable count (VC) of samples from control culture box showed increasing trend from 10 to 20 days and remain same up to 30 days ranging from 10^6 to 10^8. Where as experimental culture box also showed increase in VC from 10 to 30 days with the cast ranging from 10^6 to 10^{10}. The symbiotic relationship between the microorganisms and earthworms, which has resulted in many fold increases in the number of microorganisms in both control and experimental box (Fig.5.8). However the increase in total viable count of experimental sample was 100 fold more than the control sample.

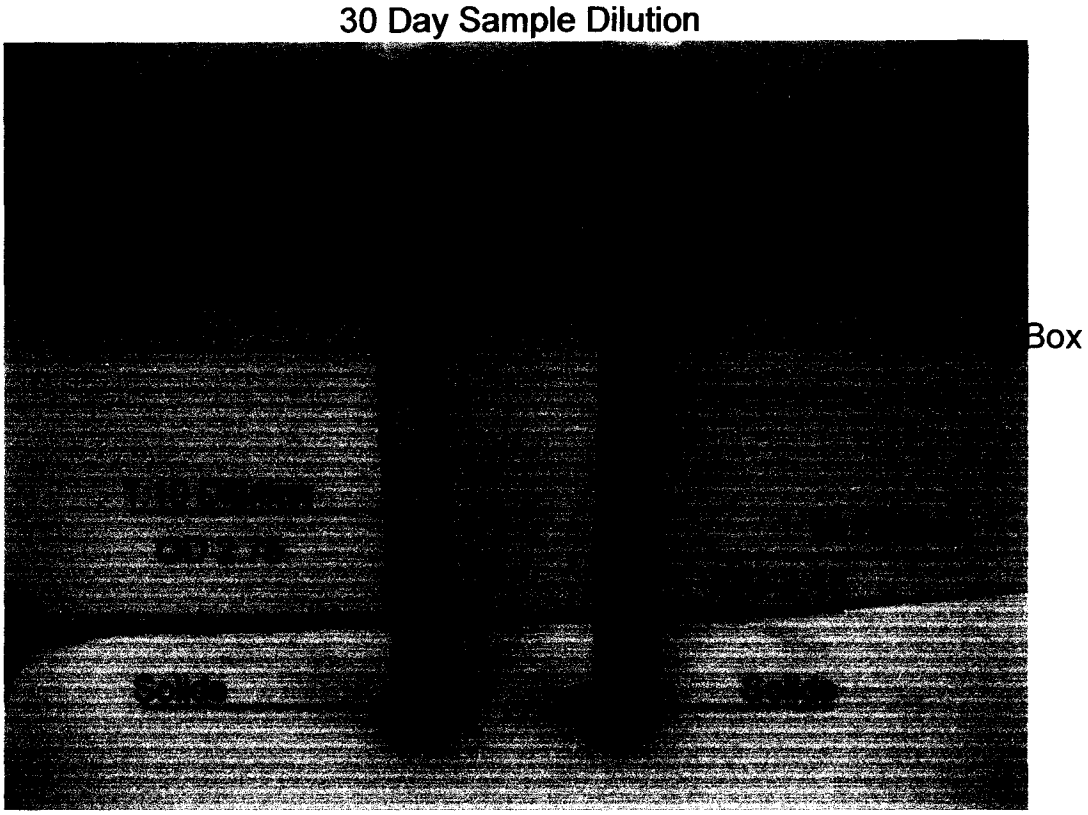


Plate: 5.12 Dilutions of vermi-compost samples from control and experimental culture box after vortexes.

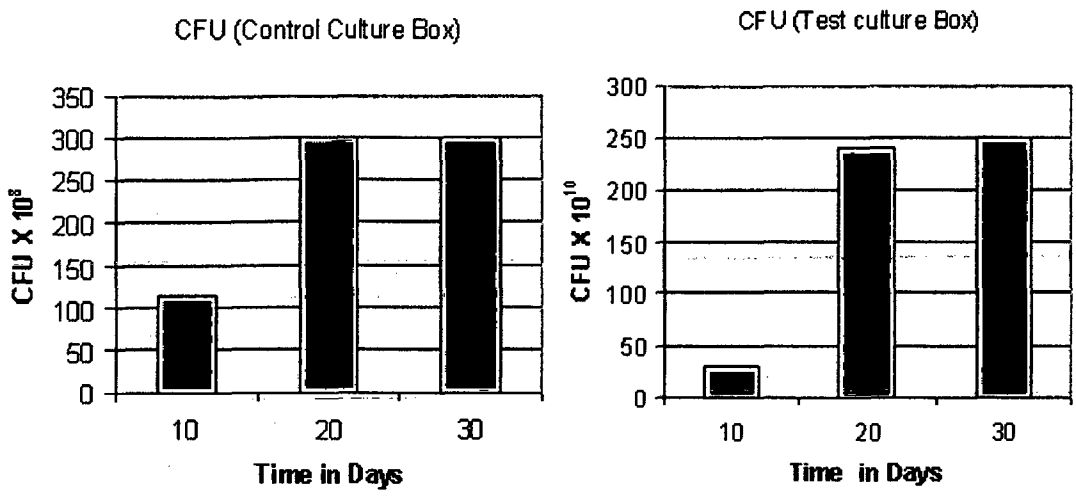


Fig. 5.8: Comparison of colony forming unit's Control and experimental culture box

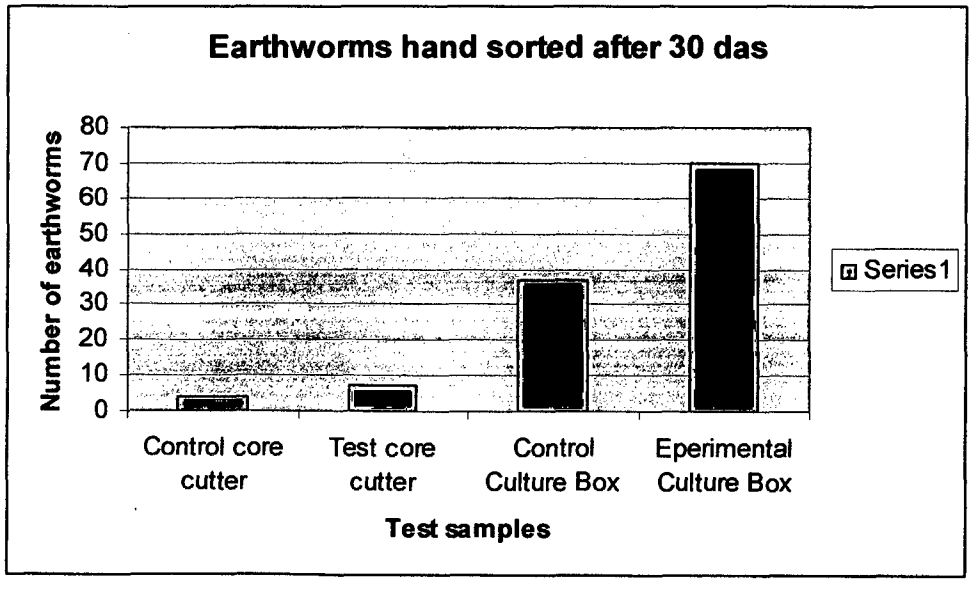


Fig. 5.9: Number of earthworms in core cutter, control, and test culture boxes

The samples drawn in the core cutter when removed showed increased earthworms in the experimental culture box. Perhaps the earthworms and the microorganisms, helping each other with nutrients to survive. The results presented in the Fig. 5.9 showed increase in number of earthworms in the experimental box.

Edwards and Lofty,(1977) reported an A increase of earthworms 1.71;3.72:2.75;2.1;0.77;14.5;3.56 times *L.terristris; L.castenus ; A.calignosa; A rosea;A nacturna ; O cyaneum* respectively in grass land experiment with and without cow dung supplementation. Where as *L terristris*; *A.calignosa; A chlorotica; a longa:* showed 46.96; 19.25; 13.93; 3.91 times increase in Aerable land.

The changes in total solids, organic carbon, volatile solids and ash content are presented in Fig. 5.10, to 5.13. The total solids have increased in both the culture boxes up to 20 days. The control culture box sample shows further increase in total solids depicting that the process of conversion of substrate to humus is still continuing. Where as the sample form experimental culture box shows increasing trend in total solids up to 20 days and suddenly falls before 25 days. These shows the microorganisms have utilized all the substrate available.

The samples drawn from the control and experimental culture boxes showed a decrease t in organic carbon (Fig.5.12) up to 20 days in both the culture boxes. Further, there was a decrease in organic carbon in control sample even up to 30 days and the process of bioconversion was continuing. Where as if you see the experimental sample graph from 20 to 30 days it has reached its minimum value around 25 days and indicating that the bioconversion time for press mud was 25 days. The organic carbon, volatile solids have

decreased where as ash content increased (Fig 5.13. and 5.14)up to 20 days and further increase in control sample and sudden decrease in case of experimental sample.

The decrease in pH values was seen in both the culture boxes from 7.2 to 7.0 and 7.2 to 6.7 for control and experimental samples respectively. The decrease was up to 20 days in case of control sample and further decrease up to 30 days is seen only in case of experimental sample. A wide range of decrease from 9 to 4.5 is being reported by Edwards (1995).and Singh (1997).Such decrease in pH values are useful in the amending the alkaline soils and bring the pH to nearly neutral value.

The geotechnical properties presented in the (Table 5.2) reveals that the characteristics of the experimental culture box sample are showing better results in all the selected parameters.

The specific gravity (Table 5.2) of the samples showed higher specific gravity as compared with the specific gravity of the filter mud itself. The comparison of the specific gravities between these two samples showed that the sample drawn from experiential culture box is (1.15) is more amorphous and lighter in weight than control sample(1.275) This is further conformed with weight of sample (Table 5.2) in core cutter .

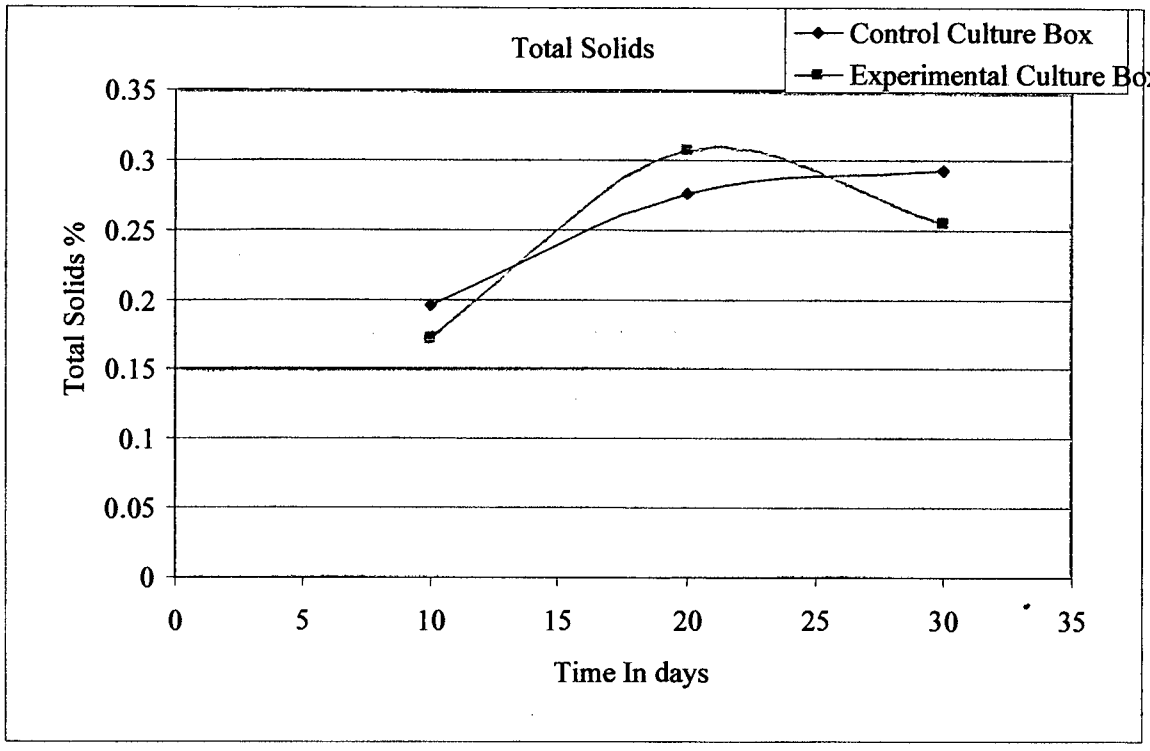


Fig. 5.10: Changes in total solids, with consortium of cellulose bacteria (BM)

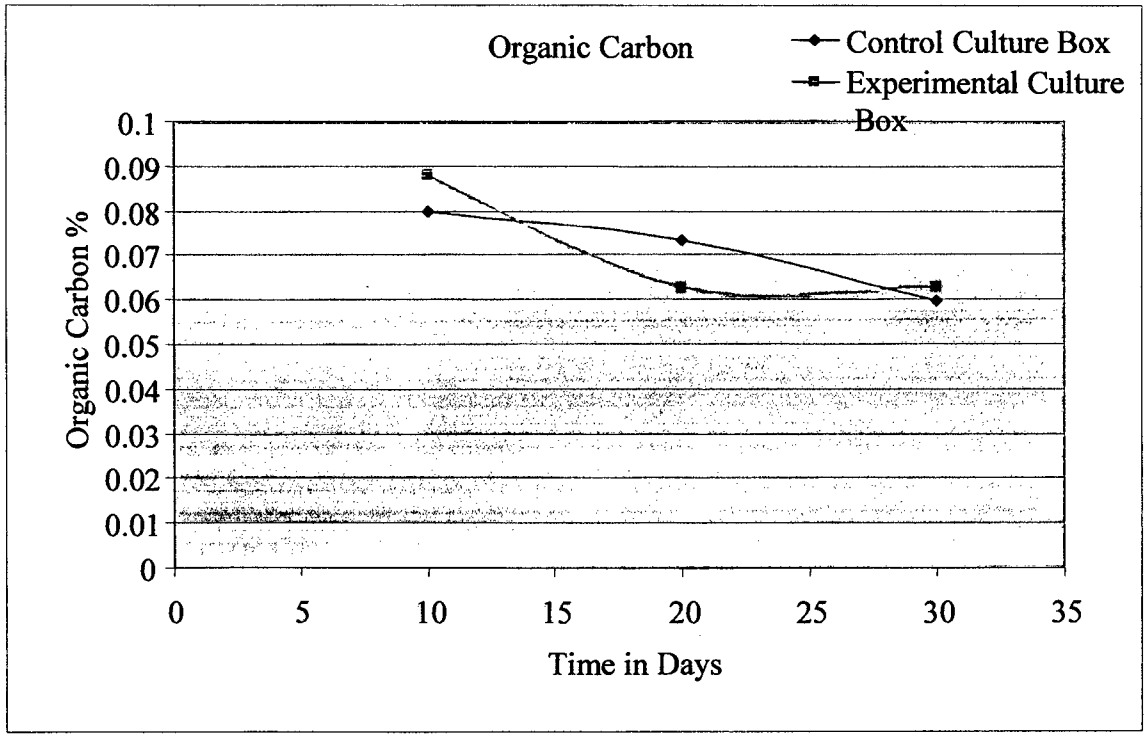


Fig. 5.11: Changes in organic carbon with consortium of cellulose bacteria (BM)

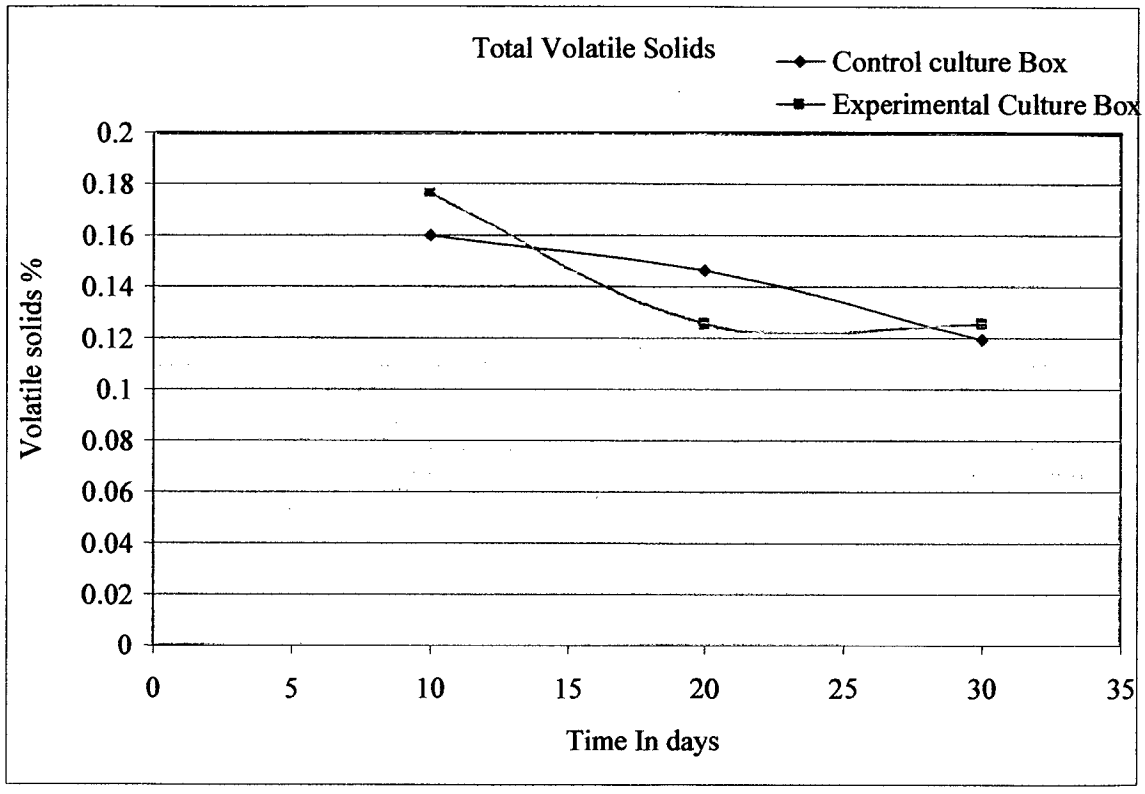


Fig. 5.12: Changes in volatile solids with consortium of cellulose bacteria (BM)

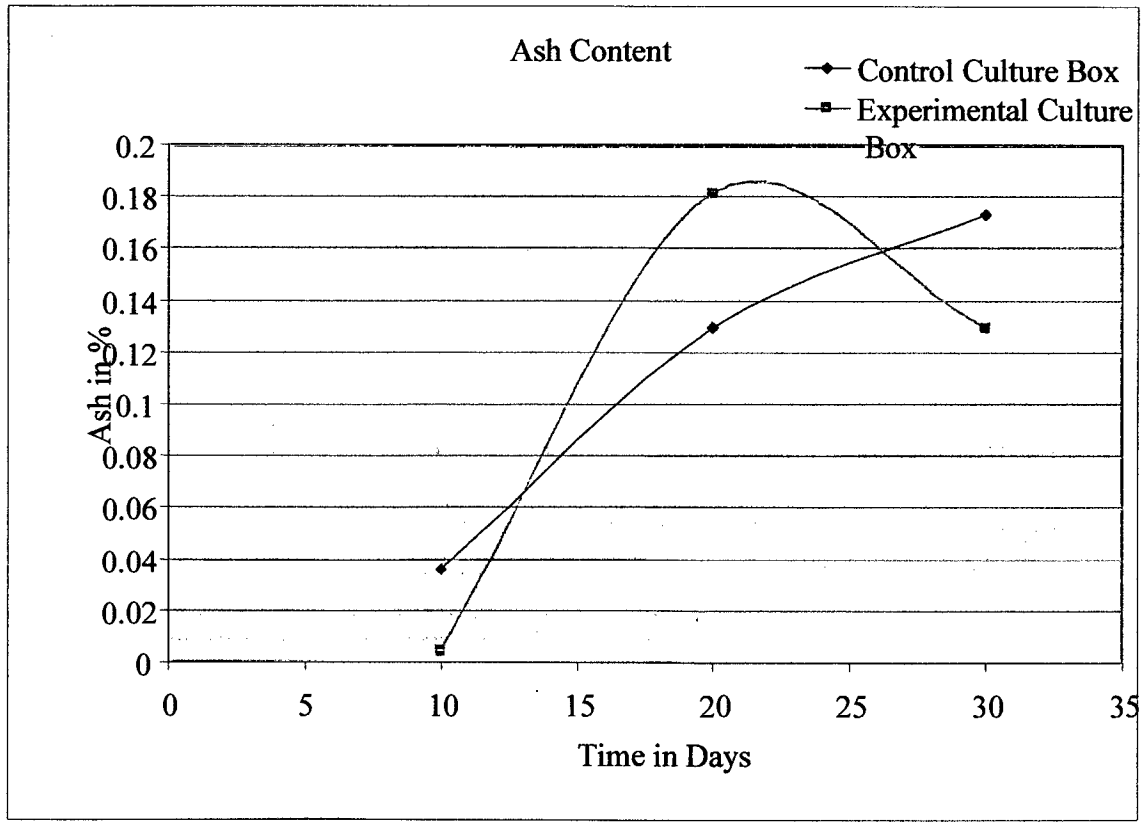


Fig. 5.13: Changes in ash content with consortium of cellulose bacteria (BM)

The water content, voids ratio and porosity (Table 5.2) are showing negative difference indicating the experimental vermi-compost has more water content, voids ratio and porosity as compared to the control culture box reports by Shaw & Pawluk (1986); Stewart *et al.* (1988); Zhang & Schrader (1993). Accordingly, the degraded soils with earthworms alone, or in combination with plants, can develop decreased bulk density and enlarged porosity and structural stability. The positive differences in all the densities show the superiority of vermi-compost from experimental culture box as compared with the control culture box. The experimental culture box vermi-compost had shown 39.03 % increase in degree of saturation as compared to the control culture box samples. The air content is the highest in control box as compared to experimental culture box; this is because of the degree of saturation, which is more in experimental culture box. Table 5.2 represents higher percentage air voids in experimental culture box as compared to control culture box.

The particle size distribution and sieve analysis of sample drawn from control and experimental culture boxes presented in Fig. 5.14. The cumulative percent finer of the two samples appear to be same in the results (Table 5.1) .while plot drawn with these readings is showing a clear interpretation that, the sample with inoculum has undergone a change in particle size from bigger to smaller depicting the role of biodegradation by microorganisms.

A marginal aggregation of particles has also taken place from 400μ to 2mm.Bothe samples control and experimental have shown a lesser particle size distribution (Table 5.2) as compared to particle size of filter mud alone.

There fore these two graphs figure 5.14 indicate the combine role of earthworms and microorganisms. The plot of experimental sample indicates the difference which is due to the additional inoculums of bacteria.

Table5.1: Sieve analysis of oven dried sample (Cumulative percent finer)

IS Sieve Size	Particle Size	Cumulative % Finer (N) Control sample(479 g)	Cumulative % Finer (N) Experimental sample(573 g)
100 mm	100 mm	100%	100%
63 mm.	63 mm	100%	100%
20 mm	20 mm	100%	100%
10 mm	10 mm	100%	100%
4.75 mm	4.75 mm	97.20%	97.03%
2.36 mm	2.36 mm	94.49%	93.02%
1.18 mm	1.18 mm	86.97%	87.26%
600µ	600µ	70.27%	71.20%
300µ	300µ	39.79%	38.91%
150µ	150µ	13.69%	11.68%
90µ	90µ	4.72%	26.00%
70µ	70µ	0.00%	0.00%

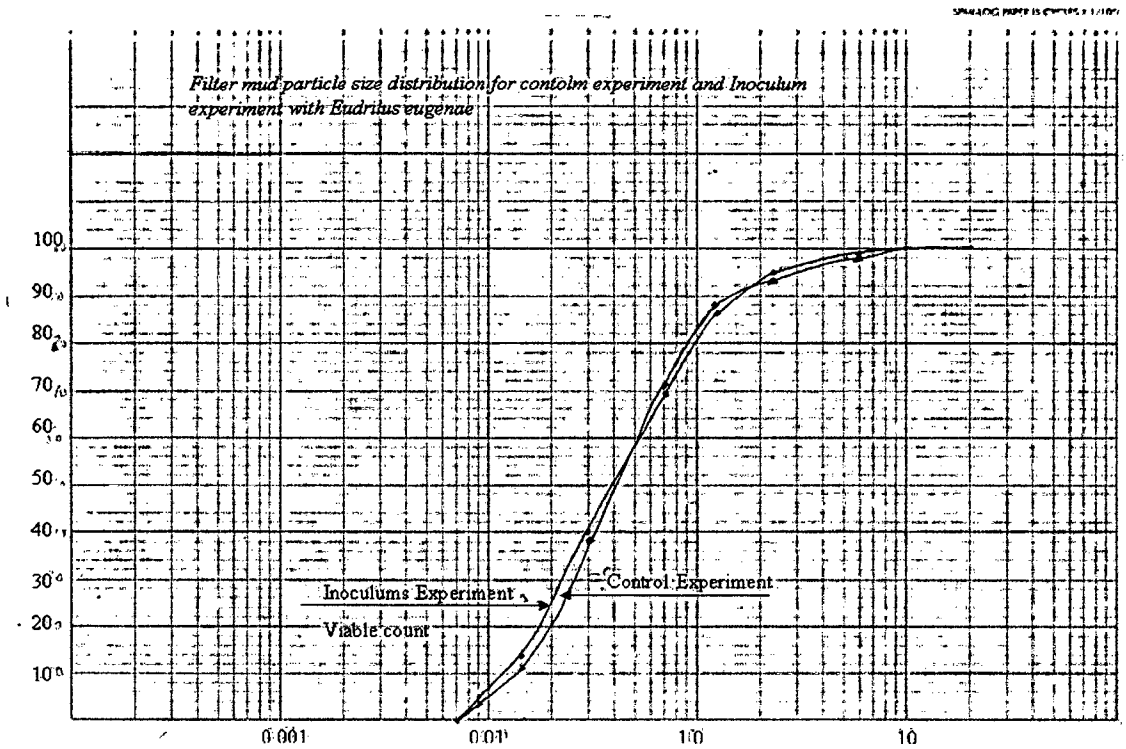


Fig. 5.14: Particle sine distribution curve for control and experimental sample

Table 5.2: Comparison of geotechnical properties of compost in control and test with cellulose degrading organisms

	Control and test experiment				Experiment with cellulose degrading organisms	
Parameters	Filter mud	Control compost	Test compost	variation	Filter mud compost *	Variation
Specific Gravity	0.5	1.28	1.15	10.156	1.06	-0.56
Water Content	24.3	1.385	1.44	-3.971	131.5	107.2
Voids ratio	1	1.98	2.29	-15.66	3.53	-2.53
Porosity	0.5	0.66	0.7	-6.06	0.78	-0.28
Bulk Density	0.32	1.02	0.853	16.373	0.54	-0.22
Dry Density	0.25	0.43	0.35	18.6	0.23	0.016
Saturated density	0.75	1.09	1.046	4.0366	1.02	-0.27
Degree of saturation	0.14	0.52	0.723	-39.04	0.393	-0.253
Air Content	0.86	0.48	0.38	20.833	0.61	0.25
% air voids	93.9	0.07	0.2	-185.7	0.48	0.459

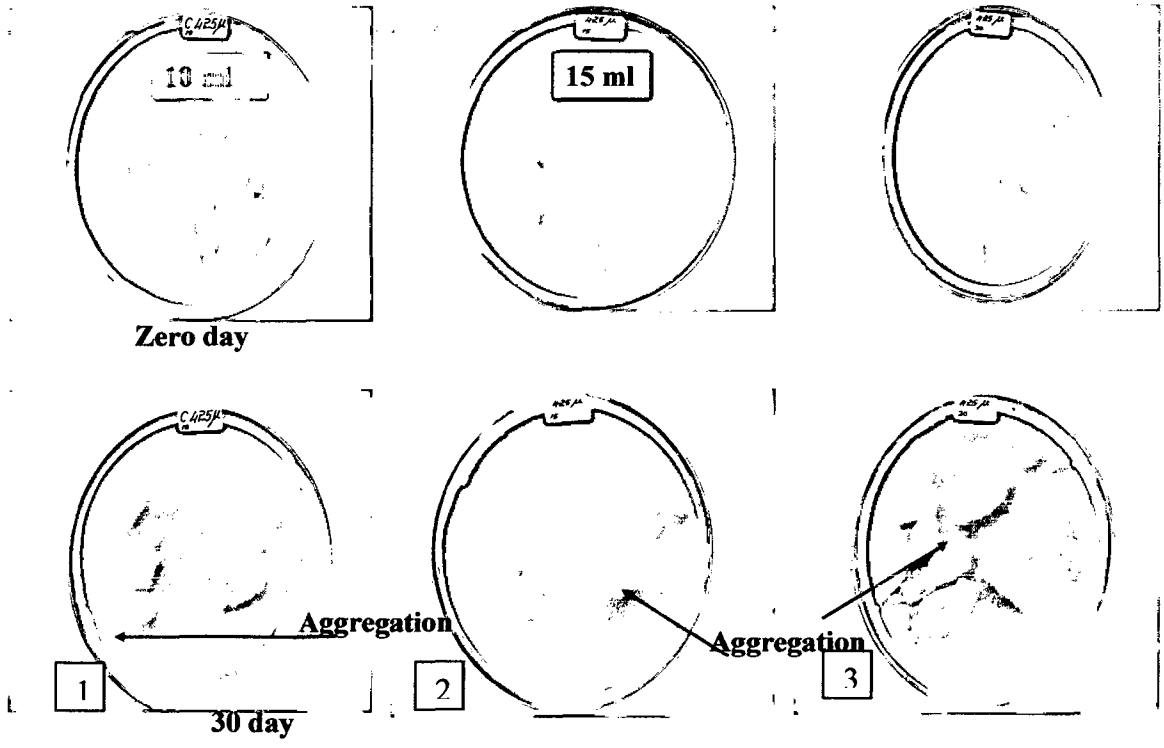
* Filter mud compost obtained from experiment with cellulose degrading organisms

B) Studies on Soil Aggregation using BM

The results of the soil aggregations by bacterial inoculums alone on soil passed through 425 μ and 850 showed aggregation in Plates (Plate 1> Plate 2> Plate) 3 after 30 days indicating for a given particle size of the soil there is a increase in the aggregation by increasing the amount of inoculums of the same population (Plate 5.13 ,5.14).

The weight retained (Table 5.3) indicates that for a given weight of soil sample there is an increase in the aggregation of soil by bacterial inoculum having viable count of 168 X 10^9/ml/g. The aggregation in both 425 and 850 μ showed increased pattern as the inoculum proportion increased. The aggregation for soil sample out of the 100g taken in each plate 49.13 %, 45.72%, 51.67% aggregated soil passing through 425 μ. The remaining soil got aggregated and automatically falls in the category from 425 μ 850 μ. The cumulative aggregation on 425-μ sieve increased.

The comparison of soil aggregation passing through 425 μ and 850 μ are presented in Table 5.3. & 5.4. This depicts that even though both showed an increasing trend the aggregation of particles of size 425 μ was more predominant than the 850-μ size particles. The comparison of 425 μ sieves of the two experiments indicates that the one which conducted exclusively with 425 μ size particles having greater aggregation with 15 ml and 20 ml inoculums. The fact that revealed from this experiment that the microorganisms aggregate finer fraction of soil particles more as compared to the larger size particles. One of the reasons could be the specific surface of a circular particle as it is easier to microorganisms to bring biochemical changes in finer particles as compared to the larges size particles.



Plates 5.13: Soil aggregation in 425 µ soils with different proportion of inoculums of bacteria consortium (BM) [168 x 10^9/ml]

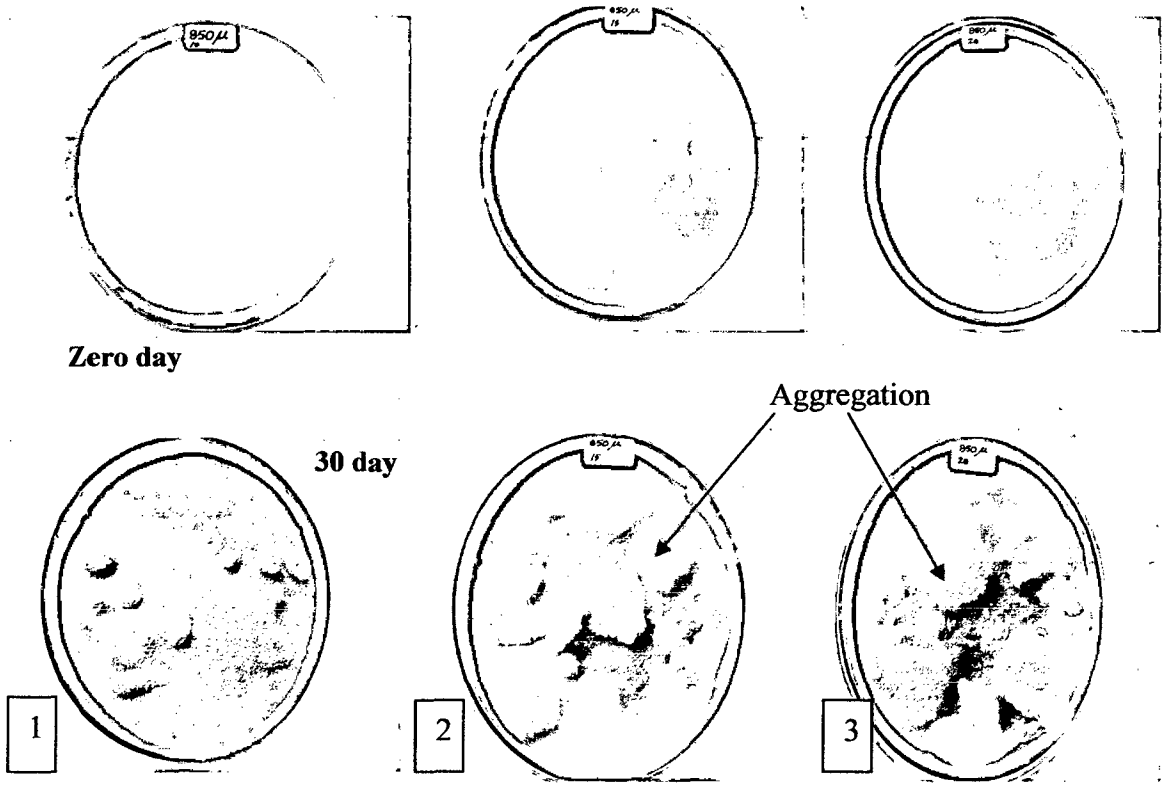


Plate 5.14: Soil aggregation in 850 μ soils with different proportion of inoculums of bacteria consortium (BM) [168 x 10^9/ml]

Table 5.3: Weight retained for 425-μ soil

	10 ml Inoculums	15 ml inoculums	20 ml inoculums
IS sieve size	425 μ	425μ	425μ
Soil retained	0	0	0
30 days soil retained	26.02 %	48.12 %	68.97 %

Table 5.4: Weight retained for 850-μ soil

IS sieve	Weight retained (%) 10 ml inoculums	Weight retained (%) 15 ml inoculums	Weight retained (%) 20 ml inoculums
850 μ	8.1	31.1	44.52
425μ	49.13	45.72	51.67
Pan	42.77	23.18	3.81

The effect of earthworm activity influences the physical structure of the soil the proportion of the water stable aggregates and formation of burrows. There is evidence that presence of coarse sand in the worm cast than the surrounding soil. (Shrikhande and pathak, 1951 Zhang and Schrader, 1993; Chan and Hena 1995)

One of the important effects of the earthworm activity is its influence on the crumb structure of fertile mull soils.In several studies, worm casts were found more water stable aggregates than non cast soil (Teotia et al., 1950; Nijhawan and Kanwar, 1952; Hamilton and Dindal, 1989a; Chan and Heena, 1995)

Zhang and Scharder, 1993 compared the effect of earthworms (*Lumbricus terristris, Alotobophora longa and Apporrectodea calignosa*) on stabilization of soil aggregates from casts and burrow wall with those of the natural soil. The results revealed that the total content of polysaccharides increased by 35 to 87 per cent for casts and 33 to 46 per cent for the burrow wall material which had the strongest effect on the inter-particle bonding of the reformed aggregates in terms of tensile strength and water holding.

Bhandari *et al.,* (1967) reported that the soil particles in the worm casts were stabilized by polysaccharide gums produced by bacteria present in the intestine of earthworms, there by in general, entry of water I to soil was enhanced and reduced soil erosions Earthworms also helped in increasing the rate of infiltration due to construction of cemented macro pores.

5.17 Significant points of study

- Just after 20 days the press mud resembles the appearance of vermi-compost.
- There is a 34.14 decrease in organic carbon indicating the role of cellulose degrading organisms.
- The effect of 1:2 proportions of inoculum showed better performance. The specific gravity of the 1:2 and 1:3 proportions had increased as compared to other two with press mud
- The visuals on biodegradation of filter mud in the experimental culture box with inoculums of additional bacteria showed higher rate of earthworm activity in terms of bringing the soil to top surface, distribution of vermi-cast, earthworm activity, and bioconversion.
- The samples showed highest number of earthworms in core cutter after 30 days and vermin reactors with inoculum.
- Dilutions of samples showed highest juveniles, population of microbes, solids settled at bottom in case of experimental culture box with inoculums.
- The organic carbon and ash content have decreased. There is a decrease in pH in both the culture boxes
- The geotechnical properties have shown the superiority in all the parameters for the compost of experimental culture box.

- There is a higher specific gravity shown by the compost from experimental culture box. Higher water content, porosity, voids ratio, Increasing densities, Degree of saturation, Air content. Decreased air voids.

- The finer particles less than 425 μ have aggregated with the bioconversion where as the higher size aggregates > 425 μ has decreased in the particle size. The particle size is more of uniform distribution and is almost parallel to the filter mud distribution.

- The 100g taken in each plate 49.13 %, 45.72%, 51.67% aggregated soil passing through 425 μ. The remaining soil automatically got aggregated and falls in the category from 425 μ 850 μ. The cumulative aggregation on 425-μ sieve shows the increasing trend.

The study on press mud with inoculums of vermi compost bacteria has clearly demonstrated the role of microorganisms in bringing down the organic carbon and the time of bioconversion to 20 days. Further the vermicompost of press mud with inoculum of BM in presence of earthworm *Eudrilus eugeniae* requires about 25 days for full bioconversion. It is of interest to note here that the experiment with earthworms with additional inoculums of bacteria could be easily converted to a suitable industrial vermi-composting unit.

Therefore, it is envisaged that the work in this direction should continue and presently a process package using earthworms and inoculums in the form of vermi-compost suspensions is expected to solve the solid waste disposal problem in sugar industry.

The process package designed keeping in mind the local environmental conditions and bringing sustainability is presented separately.

Design of process package

For Sanjeevani co-operative Sakhar

Kharkhana Dayanand Nagar goa.

Process Package for Sanjeevani Co-operative Sakhar Kharkhana

Dayanand Nagar, Goa

Introduction:

Sugar industry occupies an important place in the Indian economy. It is the second largest agro based industry in India. Sugar is one of the commercially valuable products of India for a very long time. There is a definite link between sugar industry and the rural development and farm sector. A direct employment of the tune of four lakh and about 40 lakh people connected with industry indirectly. It sustains about 400 lakh sugar cane growers which represent more than 7% of rural population. Therefore, the present study on design of process package is of importance in bringing the environmental sustainability and profits by way of waste recycling. This leads to the sustainable growth of the rural development.

1) Sanjeevani sugar factory

It was felt on the similar lines of growth of rural development in Maharashtra and Uttar Pradesh, Karnataka; Goa will also cultivate and process sugarcane for rapid rural development. The factory started its first operation in the year 1972, with a capacity 1700 tones / day. The factory struggled to achieve the optimum capacity utilization right from day one due to reasons of i] Inadequate production of cane in Goa.ii] No proper incentives to farmers to undertake cane cultivation. This forced overwhelming dependence on sugarcane to be procured from Karnataka. The factory requires about 1 to

8 lakh tones per annum in order to run profitably. This target cannot be achieved due to local cane production of 60,000 tones /a from an area of 1170 hectares.

The fact that the sugar recovery in Goa is 8.5% coupled with low yields per hectare 52 tones/hec has made the situation worse. Goa's climate is disadvantages to achieve higher recovery whereas the recovery rate in neighboring states is 10.5%. As a result the factory has to pay higher price for gate cane

The factory has a higher social acceptance, employ's about 250 people during off-season, and 550 people during crushing season. About 1500 farmers and their families depend on it.

2 Present Practices of waste disposal of filter mud.

The industry is disposing the solid wastes like Baggasse and press mud openly in the fields which are creating a disposal problem. (Present disposal are placed in Plate 1 to 4). The problems caused by disposing press mud are not only related to the volume to be handled. It has potential of source of pollution in the form of bad odors and toxic effects due to growth of fungus. The large number of insects such as house flies. The industry generates about 1200 t/day of press mud, which is presently disposed in open fields along with furnace ash. This has created bad odors in the premises and in the vicinity, along with aesthetic disturbance of land. Annexure I

Even though the press mud finds its uses in various forms, the use of press mud as an animal feed and fertilizer is yet to be given authenticity by competent authority because of presence of agricultural wax and sulfur coming from sulphitation process.

Recently farmers are also reluctant to lift the press mud because of the cost of transport. Steam generation from press mud cake by burning along with bagasse in a definite

proportion for generating power supply is slowly gaining momentum. Press mud cake for bio-gas from press mud also is a better option for the rural set up. Use of press mud as a cementing building material is studied by Central Building research Institute by mixing lime.

The sugar industry under consideration is situated neat Tisk Goa on NH 4 A. This has a crushing capacity of 1200 tons per day. The factory is a seasonal one and works for a period of 6 to 8 months depending on the locally available cane and sugar cane received from the neighboring states. The process of package has been designed keeping in view the benefits the industry is gong to derive. The advantages the technology is offering are multifold 1] it is a win win situation for the industry 2] the industry earns profit by way of selling the value added product 3] benefits of cleaner house keeping increases the overall productivity.4] fulfills the pollution board regulations. 5] involvement of stake holders like sugar cane supplying farmers and encouraging them to use the organic bio-fertilizers saves the partially cost of chemical fertilizers. The industry can have a MOU with mine owners to supply assured supply of organic fertilizers for the development of mining wastelands.

There are reports stating the increase in yield of sugar cane wherein vermi-compost of cow dung along with agricultural residues is applied to the tune of 80 tones per acre where as the neighbors harvested around 50-55 tones per acre [Laxmanan, 2006]

Out of all the options available to use the solid waste [press mud] as a resource, the conversion of it as a bio-fertilizer in a given time within the premises of the industry itself

will be of great advantage because the benefit's derived will go directly to the industry itself. Therefore, in the process of design an attempt was made to bring down the bioconversion time to a minimum at the same timeto supply quality biofertilizer to farmers.

Our study on role of microbes in the vermi-culture as system has brought down the bioconversion time to 25 days against 40 days in a normal vermi-culture system.

The application of vermi-culture and supplying additional microorganisms to the vermi beds was suggested to bring down the bioconversion time to around 25 days. The bio-fertilizer obtained by recycling press mud is sold back to farmers proportionately to the extent they are supplying the cane will not only solve the problem of marketing but also brings additional revenue to the industry.

3) Waste Generation:

i) The total waste generated in 2003-04 days

= 230 x crushing capacity /day x Rate of waste generated /day

= 230 x 1200 t x 3.5/100

= 9660 tonnes.

ii) Average waste generated per day = 42 tones.

As the quantity of waste generated per day is large, the greater area of vermi bed will be required. Therefore 10% of total waste generated per day is taken for process design.

The said industry and the other industries that have more area available can utilize the total waste generated for vermi processing. The process package presented here can be extended even on open field with availability of water.

Storage: the waste cannot be stored for a long period as it starts decomposing with foul odor and fungal growth. Therefore a suggested time of storage before it is taken to vermin beds is 4 days.

4) Waste characteristic:

1. Solid waste to be treated per day = 4.2 tonnes
2. Particle characteristics = Fine powdered
3. Bulk density = 500 -600 Kg/ m3
4. Moisture = 70 %
5. pH = 6.4
6. Temperature = 38 -45 ^{0}C
7. Soil Type = Well graded
8. observation on excavation pit = Native earthworms found at the factory site

5) Process Design

i) Area of bins:

The waste will be applied to the beds in 5cm thick as a top feeding after storage of 4 days. The waste will be spread in the bins after a gap of 20 days thus the total number of vermin bins required is 21 including one bin for storage.

Area of bin required = [4.2 x 1000] / [610 x 0.05] = 137.37 Sq m.

ii) Design of bins:

Area required per day = 137.7 Sqm.

Adopting a size of = 20 m x 7 m

Area provided = 140 Sqm.

Total number of bins = 21

iii) Other requirements

1) Vermicompost @ 10 Kg/m^2 = 20 x 7 x 21 x 10 Kg/m3

= 29,400

2) Cow dung 5 cm thick = 20 x 7 x 0.05x 21

= 150m3

3) Bricks 5 cm thick = 147~150m3

4) Irrigation = Sprinkler system

5) Source of water supply and sprinkler system along with pump and control value.

6) Roof: Of the treatment, process is to continue in rainy season, a temporary bamboo shed and thatch roof is recommended.

6) Preparation of vermiculture beds:

Vermibeds for fields trails testing of press mud conversion were prepared as mentioned below.

i) Deep borrower species:

The beds were prepared on a tank of 1m x 1m x 0.6m budget. Initially 2.5cm thick brickbats were spread everywhere above. This cow dung about seven days old (easily biodegradable

substrate) having pH nearly neutral was spread in the tank. Inculumm at the rate of 10- 15 kg/sqm. were spread over it. This was covered with cow dung of 5 cm thick. Finally the beds were covered with paddy and jute bag on top. The tank was covered by a wire mash and jute on the top to minimum evaporation; regular sprinkling of water was done to maintain moisture level of 60-70%. The earthworm hatched out is nearly 20-25 days. The bed further used for experimental purpose.

ii) Surface feeder species:

A layer of bedding material (grass) was laid to a thickness of about 5 cm at the bottom of beds (1 x 1 x 0.06m dimension). Above this a cow dung layer of 5 cm thick (7 days old) was evenly spread. There after the worms were introduced on to the beds. The worms developed within 2 to 3 weeks and the beds were used for further experimental purposes. The bags were covered with paddy and jute bags to minimize evaporation.

7) Harvesting Vermicompost:

The vermin compost of press mud is harvested after 45 days the last layer is laid. Incase of harvesting intermediately applying press mud should be stopped and collect it after 45 days.

If the vermi-compost suspension of 1:10 is supplied continuously through a separate pipe line system as shown in figure the compost will be harvested in 25 days only after the first cycle with normal 45 days.

8 Cost of the project: The details of the items involved are being presented in the Table 5.6 and the summary of materials presented in Table 5.7

Table 5.5: Cost of the project

Sl .No	Description of the Item	Unit	Rate/ unit	Qty.	Amount Rs.
1	Excavation in foundation	m3	120	190.0	22800.00
2	PCC 1:4 :8	m3	1400	63.93	89544.00
3	Laterite masonry in cm 1:6	m3	1550	316.8	4,91,040.00
4	12 mm plaster in cm 1:5	m2	80	1270	1,01,600.00
5	vermicast	tonnes	5000	30	1,50,000.00
6	Cow dung	m3	200	150	30,000.00
7	Pump and sprinkler	LS	--	1	1,50,000.00
8	Overhead tank with pipe line	LS	---	1	30,000.00
			TOTAL		= 1064984.00

= Rs 10.64 lakh

9 Summary of the materials/works/ Manpower

Table 5.6: Summary of materials/other requirements

1.Material	
[i] Vermi casting	30 tonnes
[ii] Surface feeder inoculums	10 Kg
[iii] Cow dung	150m3
2. Total Area	4257 m2
3.Earth work in foundation	190 m3
4.P.C.C [1:4:8]	63.93 m3
5. Laterite Masonry in cm 1:6	316 m3
6. 12 mm thick cement plaster	1270 m2
7. Source of water along with sprinkler	1 No
8. Source of electricity	1 No
9. Man power	1 skilled
10. Tools and equipments	1 No
11. Pump set	1 No

Running Cost (Monthly)	**(Rs)**
1. Salary of skilled labor	= 6000.00
1. Salary of two unskilled labor	= 6000.00
2. Electricity and water supply	= 2500.00
3. Miscellaneous including maintenance	= 16500.**00**

10) Payback Quality of the project.

The project can generate revenue of Rs 25, 00, 000/- per year by marketing vermi-compost of press mud at 5000/t. The industry will achieve the break even in the first ear itself.

11) Lay out of embeds:

The complete lay out of vermin-beds along with water supply line and sprinkler system is presented in Fig.5.28

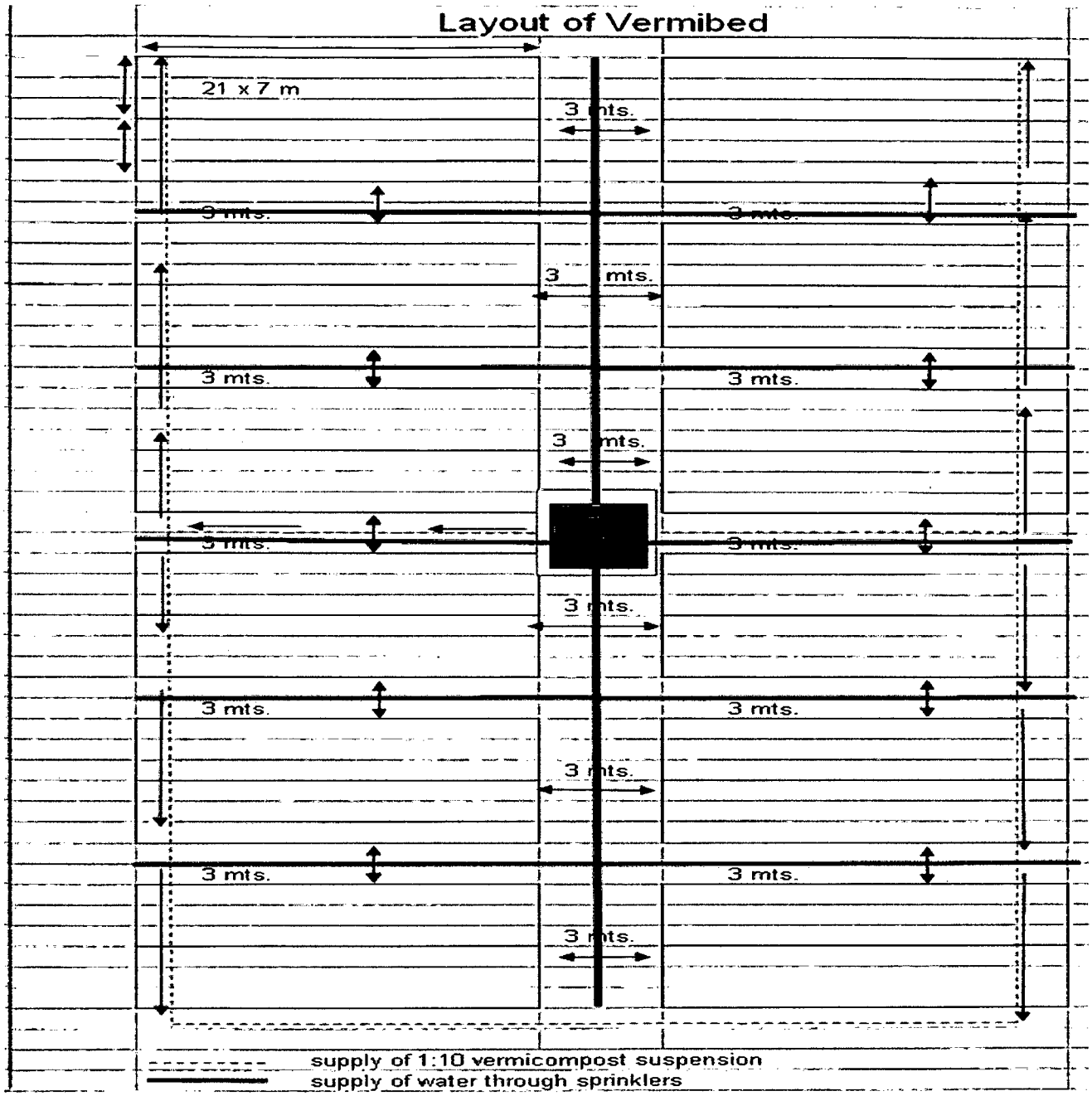


Fig.5.15 Lay out of vermi beds along with water supply line

In addition, sprinkler system.

12 Significant points of the design

- The industry can opt for either of the species.
- The species *Eudrilus eugeniae* will give superior quality compost. The composting time can be reduced by supplying vermi compost suspension in each bed.
- The species *Megascolex megascolex* besides yielding effective waste management also can give better management of soil both from pollution point and fertility point of view because of its superiority in water holding capacity, infiltration, aggregation and geotechnical properties.
- Deviced a market mechanism for sale of vermi-compost with its registered farmers who supply sugar cane.
- Vermi compost to develop mining waste lands.
- Openness to industrial waste management brings in enabling ecosystem culture in the industry.
- A sugar industry with its own Effluent treatment plant, solid waste management system, proper house keeping and green belt will be looked upon as an environmental friendly enterprise.

CHAPTER VI

Application of vermin-compost for soil aggregation and growth of garden plants on saline soils

6.0 Introduction:

The soil salinity has become a major problem due to irrigation practices, water logging and excess application of chemical fertilizers etc., Salinity results from the accumulation of free salts to an extent that causes degradation of vegetation and soils (Krishnamurthy, 1978). When salinity has affected a landscape, warning signs appear. These include sick or dying trees, declining vegetation, the appearance of salt-tolerant weed-like plants such as sea barley grass and spiny rush, salty bare patches where all of the vegetation has died, and saline pools in creek beds. As salinity affects any remaining native vegetation and the wildlife that depends on it for survival, the loss of biodiversity escalates (Reddy and Pasha, (1993). Salinity also reduces the productivity of crops and the sustainability of agriculture and population of earthworms.

It is therefore important that salinity of these soils be reduced by technology means, one of the technologies could be to use irrigation methodologies and the other could be the application of bio-fertilizers. However, application of water and bio-fertilizer will always be together in a planned way to reduce considerably the salinity. Therefore, a study on application of vermi-compost derived from the industrial waste (press mud) being tried on saline soils for the salinity parameter and growth of garden plants.

6.1 Materials and methods:

6.1.1 Soil aggregation using vermi-compost.

The vermi compost of press mud with *Eudrilus eugenae* and bacterial inoculums was used for the experiment (Chapter V).

1g of this vermin compost is placed in the Petri dish and by taking a sterilized straight wire the vermi-compost was spread around the plate as shown in photograph.

20 g of industry soil passing through 90 micron was spread above the vermi-compost. The plates observed at every 2 hr interval. The plates 6.1 and 6.2 taken after 10 hrs, in vertical and inverted positions presented in section

TheProcedure for sieve analysis carried out is outlined in appendix B.

6.1.2 Selection of saline soil:

The saline soil was obtained from a village Jayashingpur near Miraj, Maharashtra and the same was maintained in the laboratory.

6.1.3 Soil Characterization:

The saline soil obtained was characterized for the visual parameters like color, texture. The salinity was measured in terms of electrical conductivity by using salinity meter with 1:2 suspensions. Nitrogen, Phosphorus, was and Potassium determined as per procedure given in Appendix B.

6.1.4 Pot Experiments on saline soils using garden plants *Dieffenbachia* and *Aglonima.*

The experimental pots (h 21 cms, d 16.5 cm) were filled with ½ Kg. saline soils + ½ Kg. press mud vermi-compost and ¼ Kg. sand(2:2;1) ratio. The mixture laid in the pot above 2 cm thick river pebbles and coarse sand. The controls were filled with 1 Kg. of saline soil and ¼ Kg. of sand (4:1) ratio.

Indoor plant *Dieffenbachia* and *Aglonima* having complete green color with one shoot and height 24 cm were planted in control and experimental pot respectively.

The pots were kept and growth of plants was being monitored (Plate 6.3, 6.4)

Vermi-compost of press mud obtained by using *Eudrilus eugenae* from the field trials was used in the experimentation.

6.2 Results and discussion

6.2.1 Soil aggregation

Soil flora and fauna cause aggregation due to mechanical binding by mycelia fungi and actinomycetes and cementation products of decomposition and synthesis by bacteria (amino-plyuronides and polysaccharides). The extent of aggregation depends upon the nature of organisms, the amount of growth produced and the nature of substrate (Martin and Waxman, 1940). It is observed that only after three days of earthworm's action, test soil beds had over 50 % larger aggregates, clumps of silt, clay and sand, particleswhich form in earthwoem guts. These aggregates are essential components of productive soil which remains aerated and resists erosion (Kale, 1994: Noble et al., 1970)

In this study the Petri plate with 20 g of soil and 1 g of vermi-compost of filter mud using *Eudrilus eugenae* showed soil binding capacity. The vertical and inverted positions show cohesive nature of forces brought in by inoculating 1 g of vermi-compost (Plate 6.3, 6.4). This property of formation of aggregation of soil also reduces the soil erosion in the field. (Bhandari et al., 1967).The important property of vermi-compost was to retasin the water holding capacity and release of water in drought conditions could maintaining the health of the soil for longer periods. The bacteria in 1g of vermi-compost with cfu 100 x 10^9 /ml/g could hold 20 g of soil in position, As water from vermi-compost gets released when dried conditions prevail in the environment, The sporulationg *bacilli* will survive in adverse conditions, when the moisture levels in the soil are regained in the rains, the bacteria will become active and continue their metabolic activities. (Edwards and Lofty, 1977)

This experiment also demonstrates the tensile strength added to the soil by vermi-compost, which is held freely when the Petri plate is inverted (Plate 5)... The results of the sieve analysis presented in Table 6.1, Fig. 6.1 shows the diagrammatic presentation of aggregation of soil which is found to be74.10% retained on 90 μ sieve. The reason could be attributed to the smaller particle size have more surface area available for the bacteria to act upon. And the binding by polysaccharides is easier.

In the earlier chapter it is evident that the vermi-culture system as a whole provides a complete answer to soil processes which has also been proved that by using *Eudrilus eugenae*, *Eisenia fetida*, and *Megascolex megascolex*. Our comparative study revealed

that the deep burrower showed highest aggregation of soil as compared to the surface feeder species. Also there is a experimental evidence that *Pheritima elongate* when used with potato peel waste in open vermi beds leads to aggregation of soil. (Munnoli, 2000; 2002).Similar aggregation property of vermicomposts was reported by Guild,(1951) Kale et al., (1981) Bhawalkar, (1992) Singh, (1998). The aggregation of soil becomes an important factor as far as the percolation of water and water holding capacity of soil is concerned. The vermi-cast is rich in the micro and macronutrients therefore the aggregation of soil with vermicast also helps in storage of nutrients, which will be available for plants. The stability of aggregates depends upon cementation of domains by organic and inorganic cements or microbial films (Ghildyal and Gupta, 2002).

The more realistic answer to soil aggregation can be given by theory of soil structure genesis. The aggregation by humus absorbed on soil particles was reported by (Ghildyal and Gupta, 2002). Baver (1935) says aggregation was more in soils containing, 25% clay, where as (Pearlkamp, 1950) increase in granulation occurs after addition of organic matter and which decreases with time. Edwards and Bremner (1967) linked the role of organic matter and polyvanal metals and found that, the basic structural units in soil of high base is fine sand and silt size micro aggregates consisting largely of C-P-OM (Clay-Polyvynal Metal- Organic Matter) complex. The earthworm casts were formed by cementation of soil particles(Bhatkin and Plsky,1950) and also by calcium humate in intestine of earthworm are found to be quite stable.

It is noted that soil colloids clay particles, organic and inorganic compounds have high electro kinetic potential and repel each other in colloidal suspensions but flocculate when potential is lowered significantly with stable aggregation formation(Hopp & Hopkins,1946 :Dawsan, 1997). However, this further requires cementation or binding together of primary and flocculated particles so that they are held firmly and do not dispense in water (Ghildyal and Gupta, 2002).

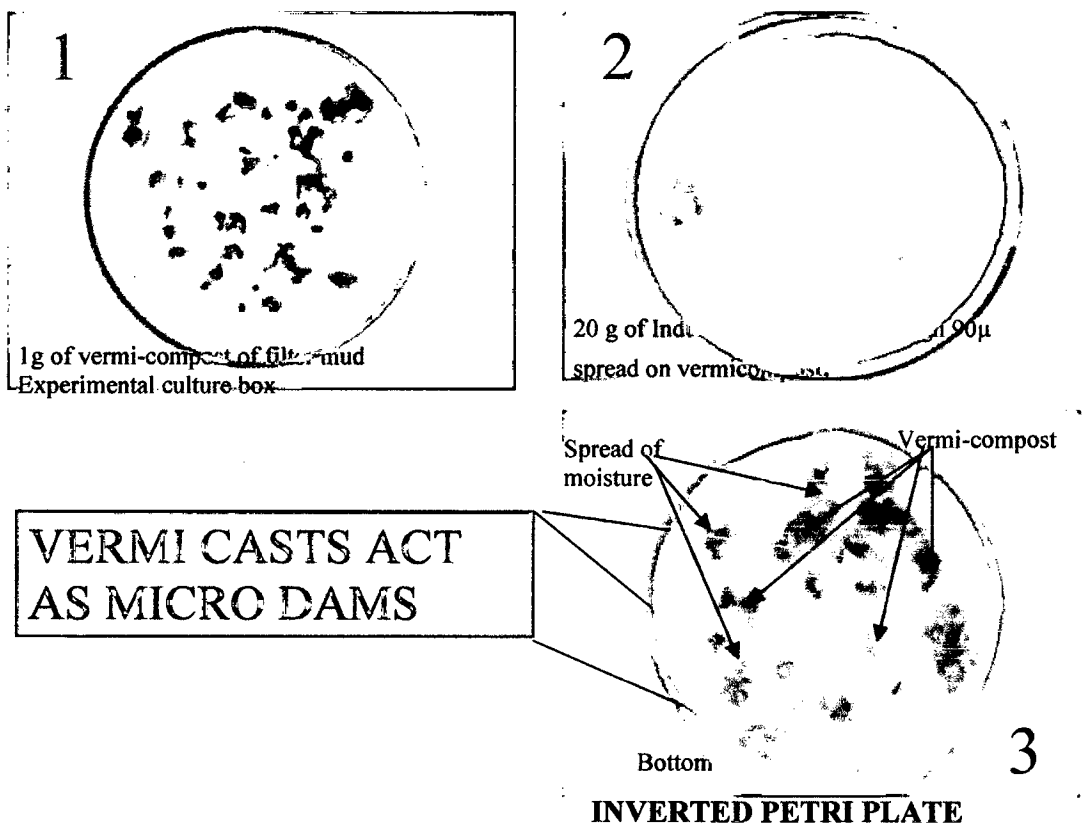


Plate 6.1: Vermi-compost as micro dams

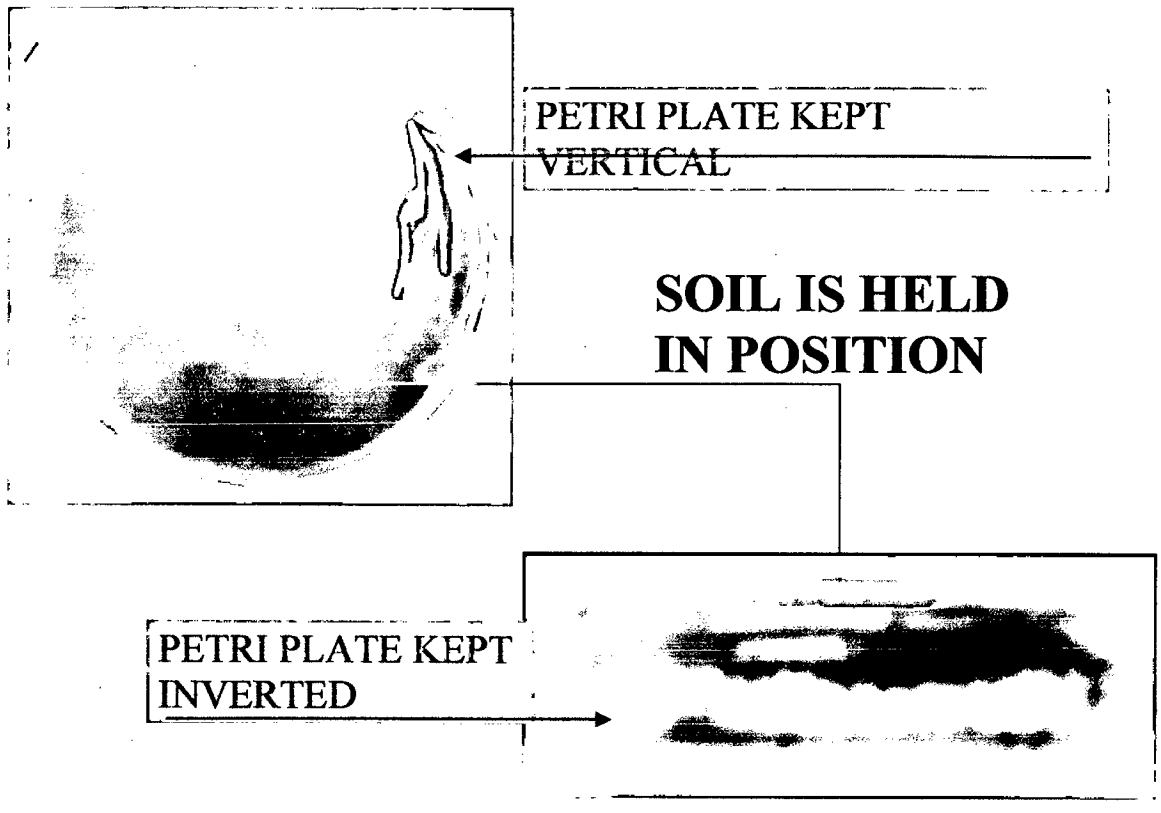


Plate 6.2: Soil held vertical and inverted in position in Petri plates.

The soil passed through 90μ

Table: 6.1: Weight retained on sieve 90μ

Particle size	Weight retained	% Retained	Cumulative %retained	Cumulative% finer(N)
90μ	0	0.00	0.00	100%
90μ	14.82	74.10	74.10	25.9

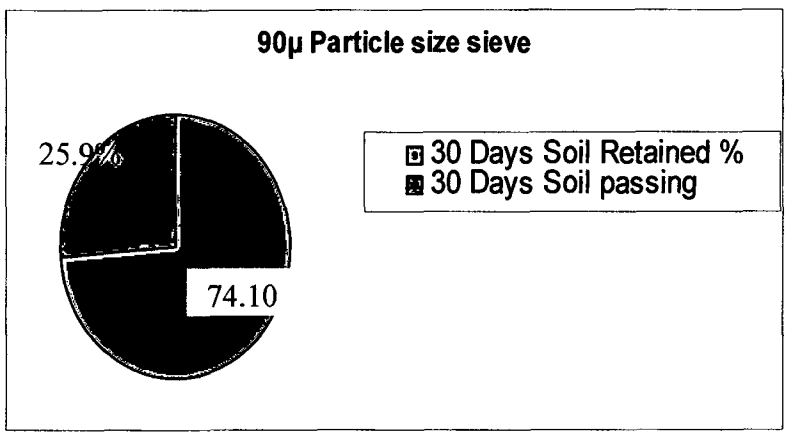


Fig. 6.1: Pie diagram of sieve analysis

6.2.2 Study of growth of garden plants on saline soil

Soil characteristics:

The soil characteristics with the selected parameters are placed in Table 6.2. The decrease in salinity are placed in Table 6.3, Fig 6.2 and Table 6.5 Fig.6.5 where as the decrease in pH are presented in Table 6.4 and 6.6,the parameters of plant growth height and number of leaves are placed in Fig 6.3, 6.4 and Fig. 6.6,6.7 for *Dieffenbachia* and *Aglonima* respectively. The variations in nutrient values with respect to N, K& P placed in Fig 6.8, 6.9 for control and experimental pots with *Dieffenbachia* and *Aglonima* respectively.

The soil obtained from the Jayashingpur village showed blackish color with medium texture with a pH range of 7.8 to 8.0. The electrical conductivity measured was 2.5 indicating the soil is saline and growth resistant for salt resistant plants.

Table 6.2: Characteristics of saline soil

Parameter studied	observation	Remark
Color	Blackish	As the electrical conductivity is > 2.0
Texture	Medium	The soil is saline and growth resistant
pH	7.8-8.0	for salt resistant plants.
Ec mmhos/cm	2.5	
N Kg/ha	1.24	
P Kg/ha	77.48	
K Kg/ha	184.8	

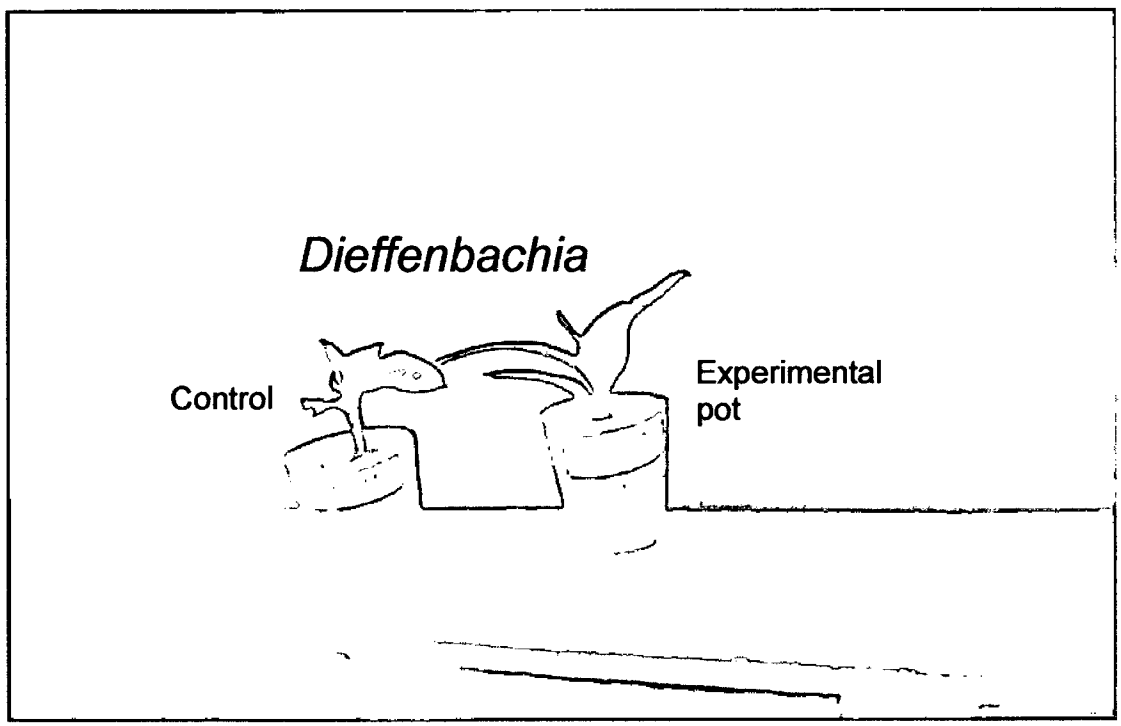


Plate 6.3: Control and experimental pots with *Dieffenbachia*

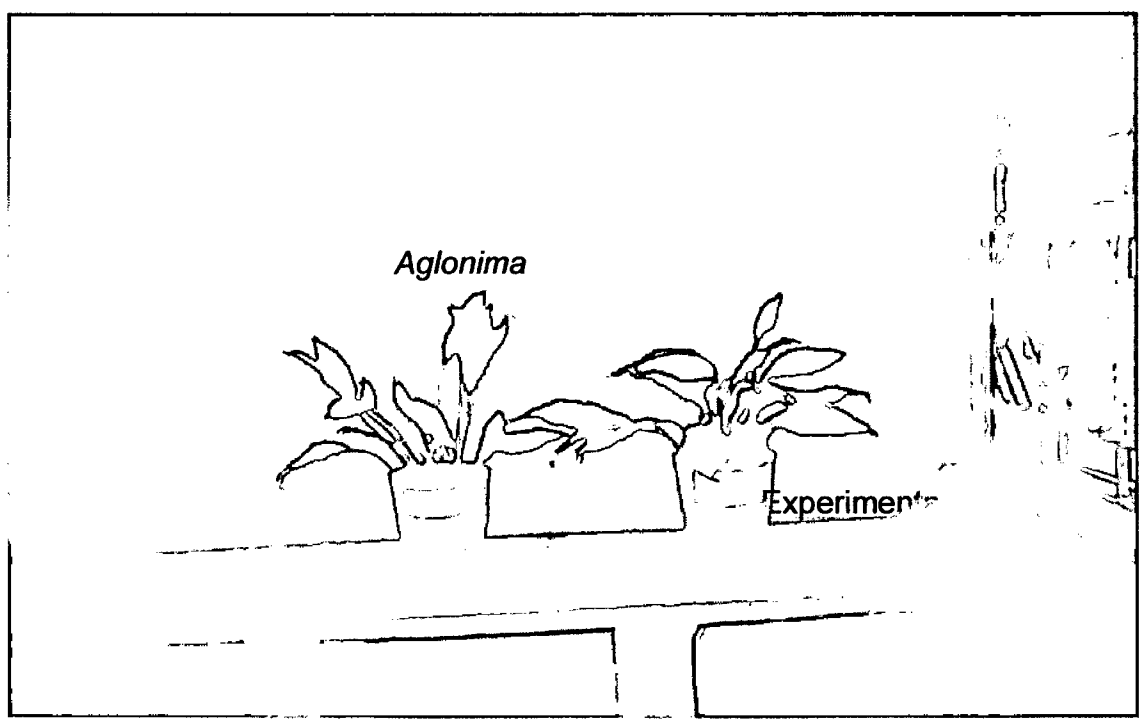


Table 6.3: Pot culture experiment with *Dieffenbachia* and *Aglonima* in saline soli

Observation in days	*Dieffenbachia*		*Aglonima*	
	Soil EC mmhos/cm	Soil +VC EC mmhos/cm	Soil EC mmhos/cm	Soil +VC EC mmhos/cm
0	2.70	2.70	2.70	2.70
30	2.40	2.00	2.14	2.01
60	1.95	1.41	1.62	1.14
90	1.01	0.55	1.05	0.69
120	0.41	0.30	0.49	0.35
150	0.26	0.20	0.30	0.22

VC =Vermi compost

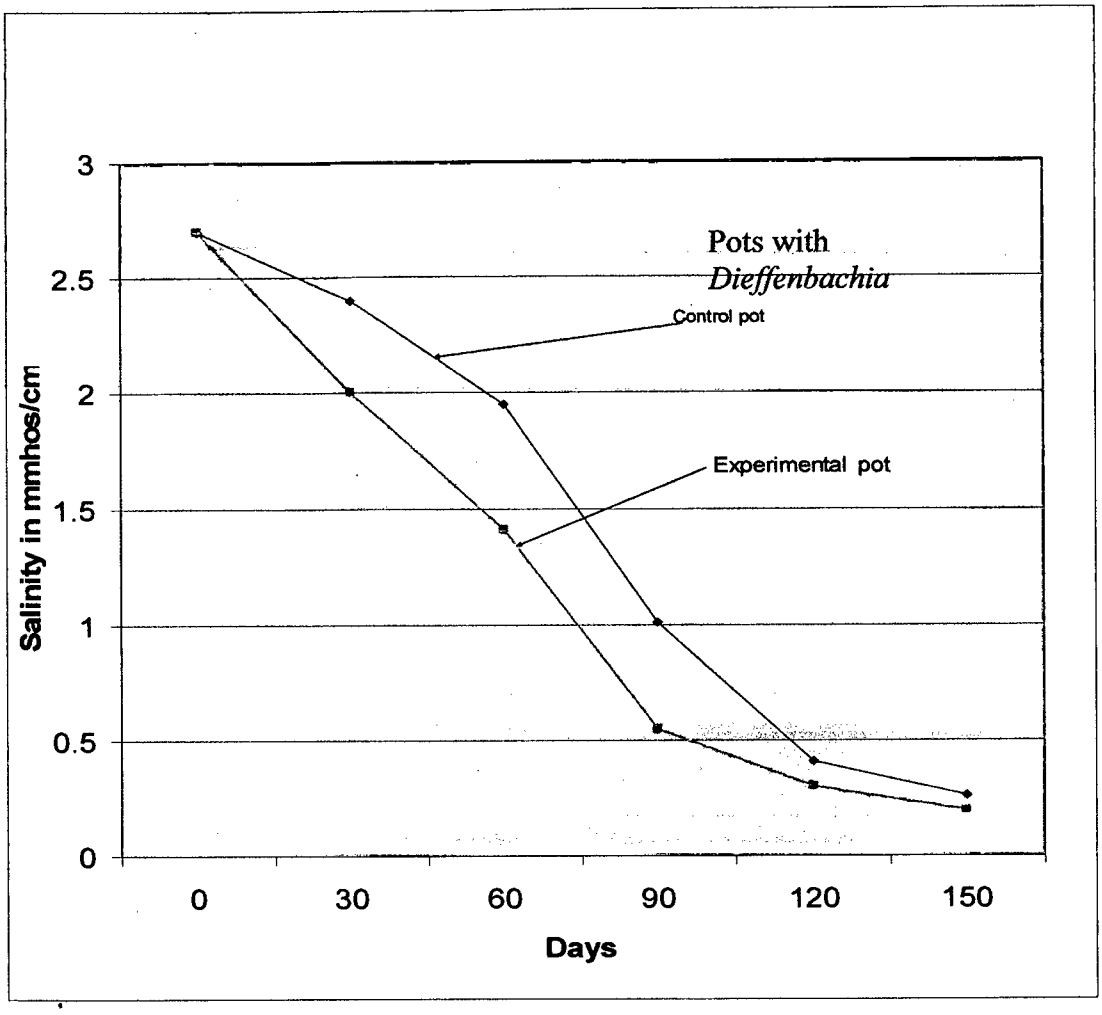


Fig. 6.2: Variations in salinity in experiment and control pots

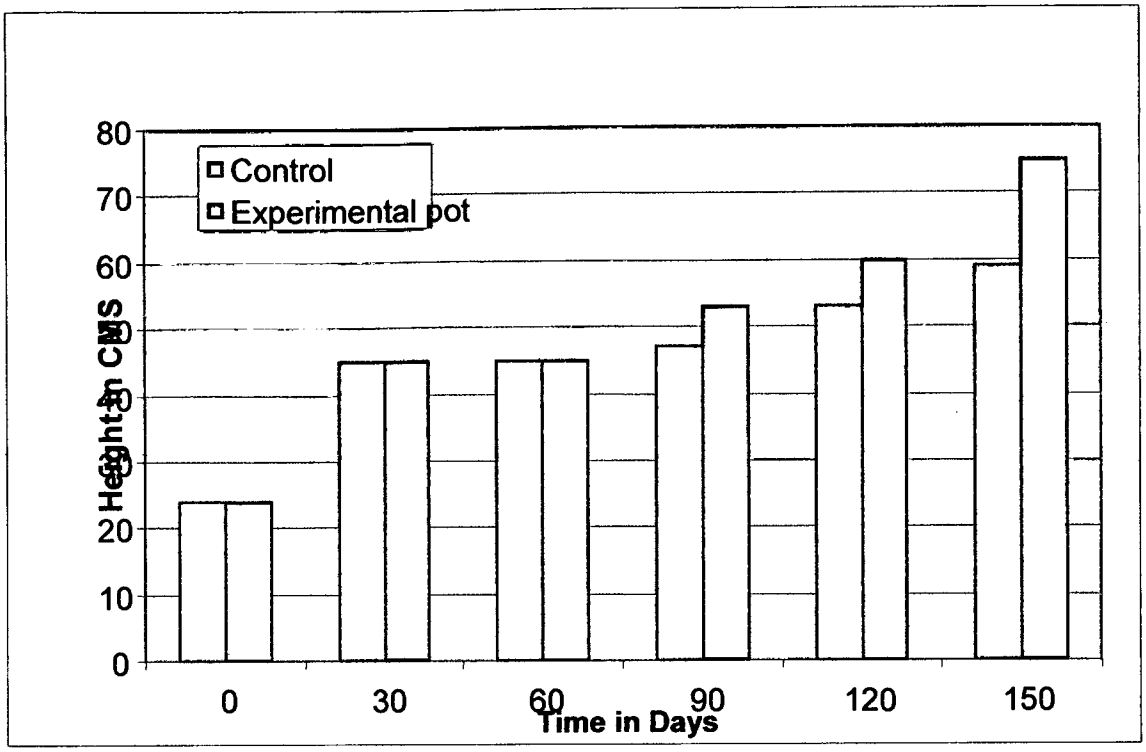


Fig. 6.3: Changes in height (*Dieffenbachia*)

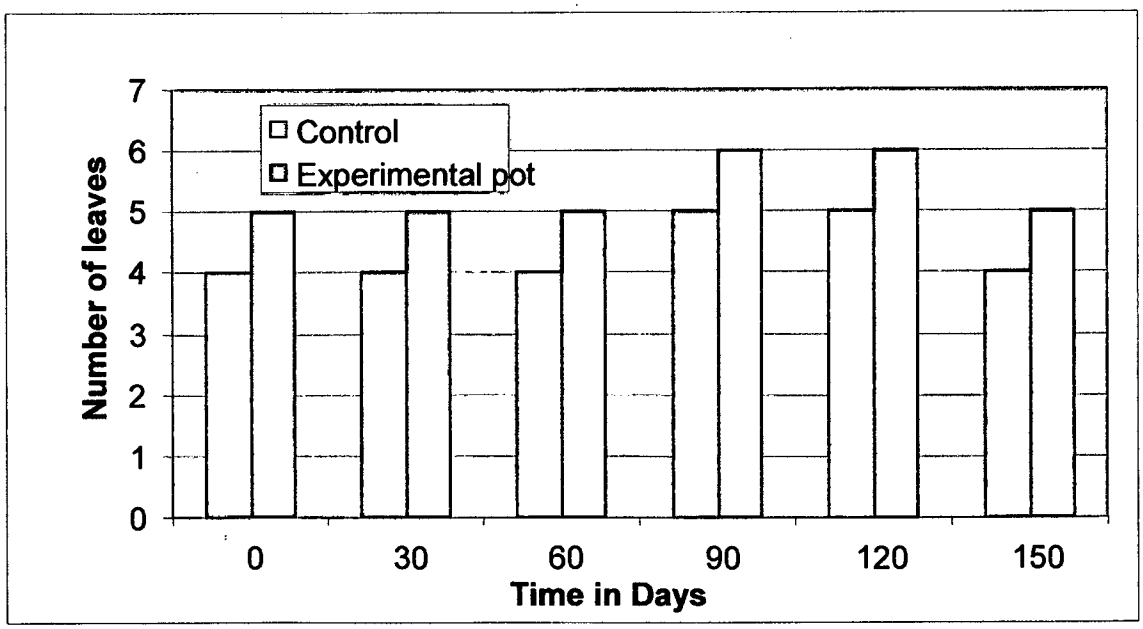


Fig.6.4: Changes in number of leaves (*Dieffenbachia*)

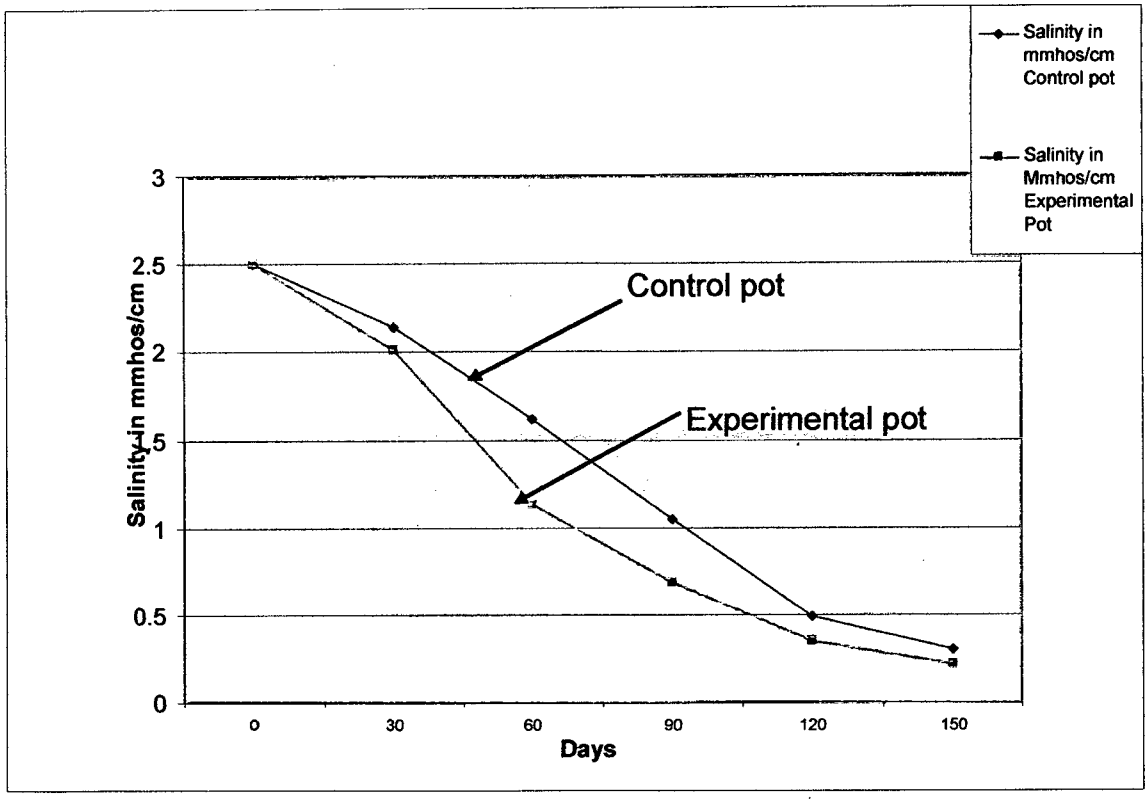


Fig. 6.5: Variations in salinity for *Aglonima*

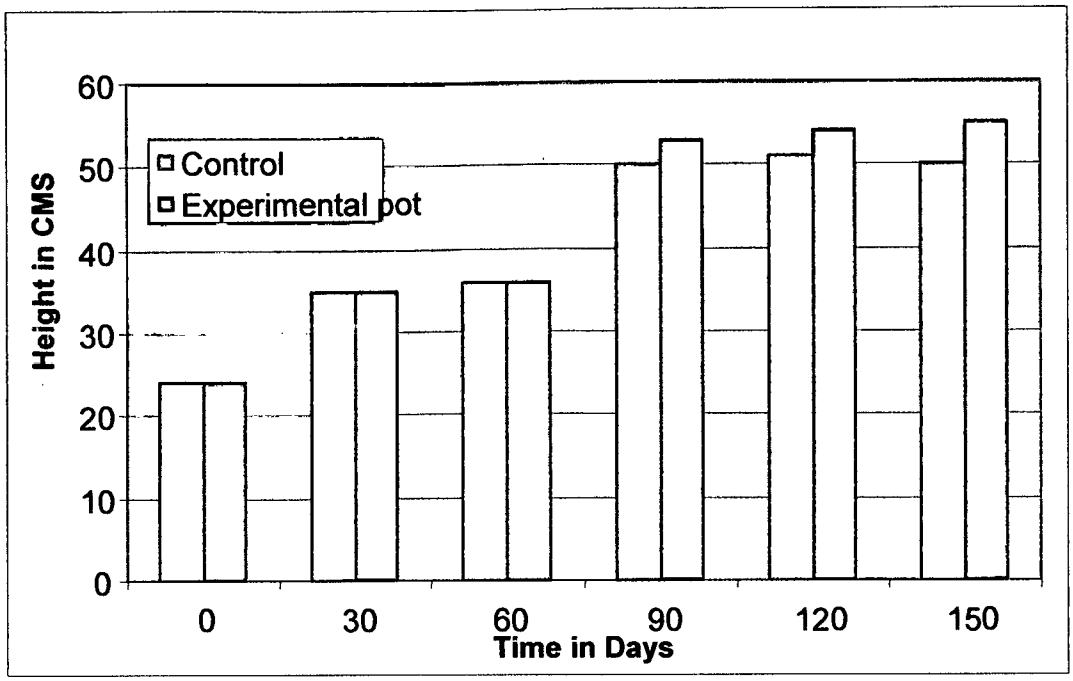


Fig. 6.6 Changes in height (*Aglonima*)

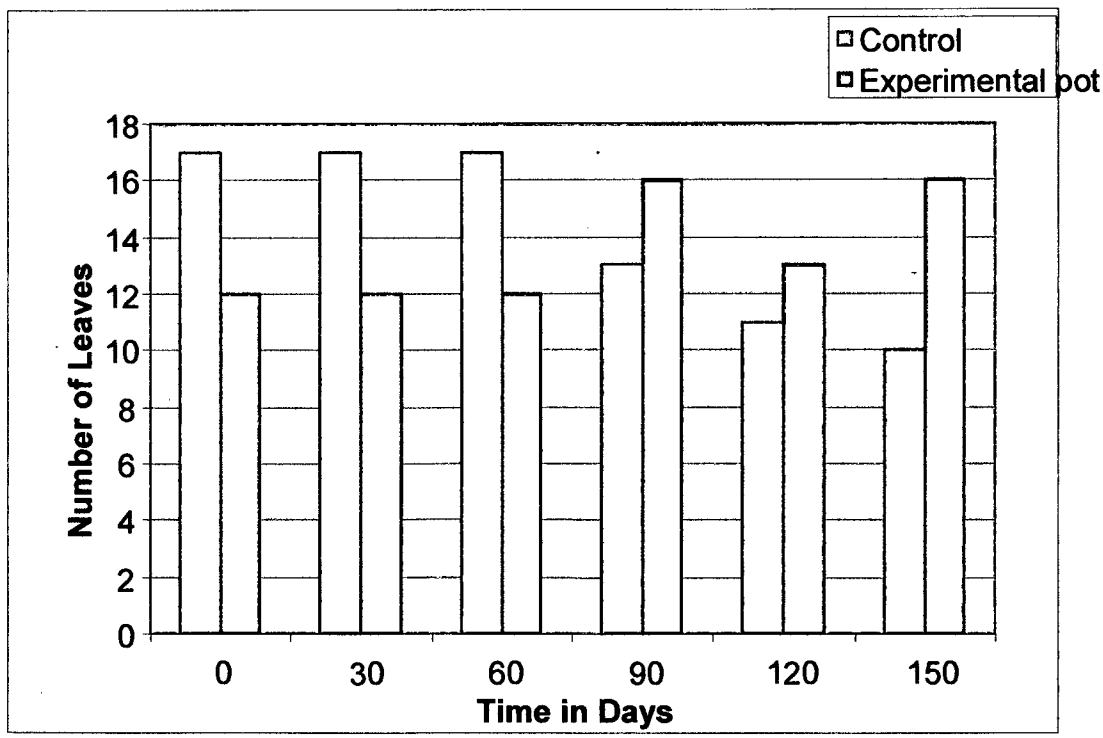


Fig. 6.7 Changes number of leaves(*Aglonima*)

Table 6.4: Variations in Soil pH values for control and experimental pots

Time in days	*Dieffenbachia* pH		*Aglonima* pH	
	Control pot	Expt .pot	Control pot	Expt. pot
0	8.0	7.8	8.0	7.8
30	7.9	7.7	7.9	7.5
60	7.7	7.2	7.8	7.3
90	7.5	7.0	7.4	7.1
120	7.2	6.6	7.2	6.9
150	7.0	6.4	7.1	6.6

Table 6.5: Analysis of variance applied to salinity observations.

Table ANOVA	(P< 0.05)			
Square of variance	df	sum of squares	Mean sum of squares	F ratio
Between Individual observations	4-1=3	0.33	0.11	
within individual observations	23-3=20	19.826	0.9913	9.01
Total	24-1=23			

Table 6.6: Comparison of soil characters with *Diffenbachia* and *Aglonima*

Parameter studied	*Dieffenbachia*		*Aglonima*	
	Control Pot Soil + Sand[1:1/4]	Exit .Pot Soil+sand+VC	Control Pot Soil + Sand[1:1/4]	Expt. Pot Soil+sand+VC
pH	8.0	7.9	8.0	7.9
Ec mmhos/cm	0.25	0.20	0.33	0.26
N kg/ha	0.8	2.1	0.93	2.1
P Kg/ha	33.9	169.7	38.74	121
K Kg/ha	308	464.8	352.8	873.6

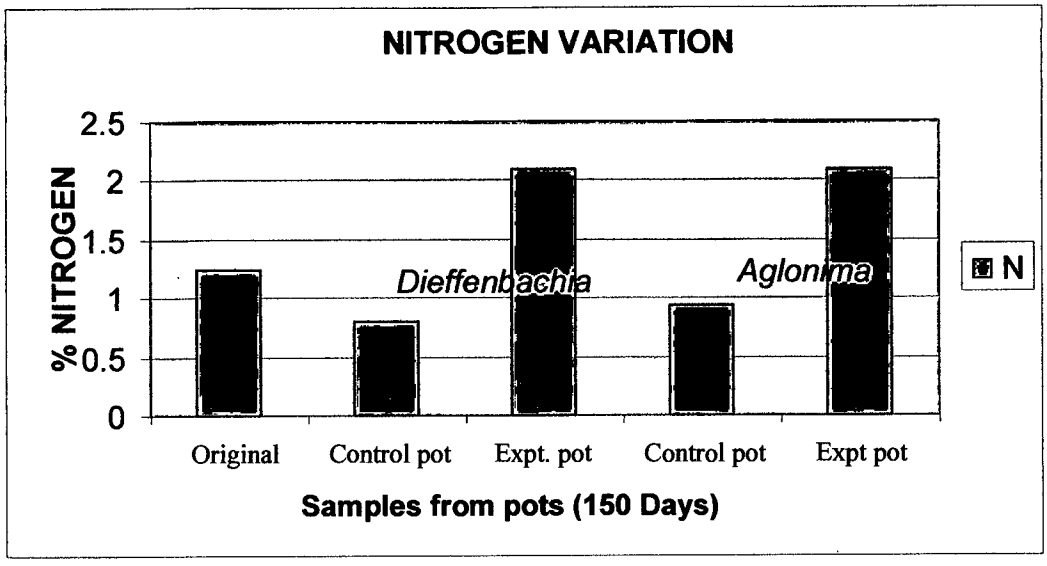
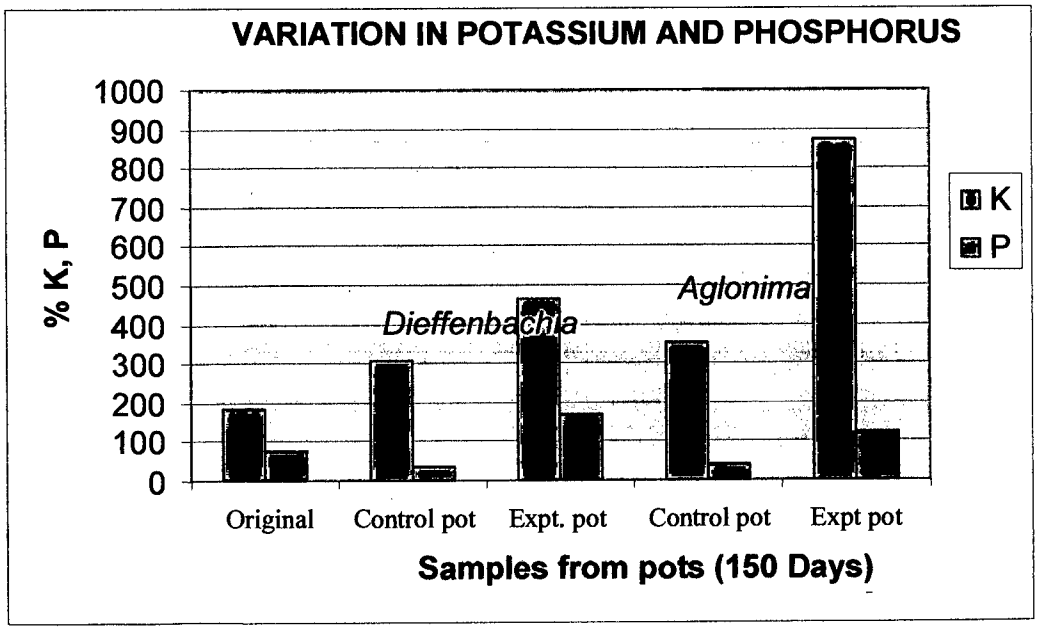


Fig. 6.8: Percentage of Nitrogen in pot soil samples



. Fig. 6.9: Percentage of Potassium and Phosphorus in pot soil samples

i) Growth of garden plants:

The change in height of *Diffenbachia* and *Aglonima* observed for 150 days in control and experimental pots(Fig.6.3 and 6.6) showed that, the increase was comparable up to 60 days thereafter the increase in height was significant in the experimental pots for *Difffenbachia.* Whereas, the *Aglonima* plant growth was slower and showed similar trend with marginal increase in height in experimental pots. The increase in height was 3.5, 3.0 and 2.75, 2.5 times in experimental and control pot as compared with the original height for *Diffenbachia* and *Aglonima* respectively.

The observations recorded for the number of leaves up to 150 days (Fig.6.4and 6.7) showed that for *Diffenbachia* the number remained the same up to sixty days, after this there was increase in one leave in both pots up to 150 days, there was a weeding of one leave after 150 days, where as the results obtained on *Aglonima* plant for number of leaves showed no change up to 90 days. The experimental pots gained 4 leaves against 4 losses in control pot. The 20^{th} day record showed weeding of two and three leaves for control and experimental pots. Further, there was a weeding of one leave in control pot against a gain of two leaves in experimental pots after 150 days.

There are several reports on application of vermicompost for the increased growth and yield of banana is reported by Barve,1992, Gubbuck *et al*,1993 Ushakumari,1997, Athani *et al.*,1999 where as on grape by Barve,1992, Venkatesh,1995 and Custard apple Patnaik,(1992).The application of vermin compost on the growth of sugar cane was reported by (Katharissan,1991:Virendra kumar,1991;Varamali,1993;Laxman,2006).

ii) pH: The pH values for pot soils drawn at regular intervals ispresented in Table 6.4. The vermiculture system has revealed that there is always a decline in pH values of soils in the vermi beds as well as vermi compost applied beds. Our results in the earlier chapters showed a decline in pH values and was supported with experimental results by Kale et al.,1986; Bhawalkar, 1992;Raut,1997;Singh,1998;Munnoli,2002).

iii) Salinity:

The results of the ANOVA analysis following the procedure given by Indrajit Singh,(1990) and Mahajan,(1997) are presented in Table 6.5, which showed that there ws not much variation in between the individual observations of the salinity, where as there was a greater variance within the individuals. (For $P<0.05$, F ratio 9.01) was highly significant.

It was clear from the salinity decrease that even though the individual did not show the variations, the decrease within itself of great significance to show the application of vermi-compost in bringing down the salinity which is of a great importance in solving the problems of salinity of agricultural lands and wasteland development especially the salt affected soils.

The decrease in salinity in vermi compost used pots is 12.27, 11.36 times with *Dieffenbachia* and *Aglonima* plant species respectively, whereas the pots without plant species i.e. controls have shown a decrease of 10.38, 8.33 times respectively as compared with original salinity value. Indicating the fields with saline soil can be well recovered

with both irrigation methods or by applying vermi composting prepared out of press mud.

According to Kale and Krishnamurthy (1978) and Grant (1995) reported that soil moisture, pH, salinity and organic matter were the principle factors controlling the distribution and bionomic of earthworm population around Bangalore. Significant influence on earthworm population, seasonal pattern of soil salinity was reported Reddy and Pasha (1993).

Our work clearly demonstrates the decrease in pH values, salinity by using vermi-compost of press mud. There is a conformity report from Bhawalkar and Bhawalkar, (1992) successfully improved saline soils by inoculating earthworms with organic manures, and heavy mulching. They also reported good yield of tondli a vegetable crop in saline soil and sugar cane grown on saline soils with saline ground water irrigation. A reduction of salinity from 1.15 to1.0 and 1.15 to 0.9 mmhos/cm, in case of press mud treated with cow dung 25% and 50% by weight Giraddi and Kitturmath, (2002).

Nutrients:

The sample soils drawn after 150 days were analyzed for nutrients and the results presented in Table 6.7 and Fig. 6.8, 6.9. The results showed more left over nutrients in both the experimental pots as compared to control pots with *Dieffenbachia* and *Aglonima* respectively.Bhoovireddy and Kanamadi (2003) and Athani et al., (2000) had reported that effect of vermi compost on nutrient status of leaf and ield in banana Cv Rajapuri.The application of vermi-composts has brought the significant growth of garden plants at the same time solving the salinity problems.

Therefore, to conclude the study on application of vermi-compost of industrial wastes has got a greater potential to be undertaken as a priority be all the sugar industries and food processing industries and harvest the benefits derived from vermi-compost either by selling or by utilizing it for its own consumption.

6.3 Significant points of study

Out of hundred grams of soil 74.10g of soil passing through 90μ aggregated. 1 g of vermi-compost of filter mud prepared by using *Eudrilus eugenae* holds 20 g of 90μ soil

The decrease in salinity in vermi compost used Pots is 12.27, 11.36 times with *Dieffenbachia* and *Aglonima* plant species respectively, whereas the pots without plant species i.e. controls have shown a decrease of 10.38, 8.33 times respectively as compared with original salinity value.

There is availabilty of nutrients for the growth of plants after 150 days.

The property of vermi-compost to hold water and in turn reduce salinity of soils will be of great help in recovery of salinity-affected lands.

CHAPTER VII

Summary and Future Scope

SUMMARY

Investigations were carried out on biodegradation of filter mud as an agro industrial waste using *Eisenia fetida, Eudrilus eugeniae, Megascolex megascolex,* both at laboratory and field trials. The influence of cow dung as additive on the degradation process was also studied. The compost samples subjected to analysis of geotechnical properties. The isolation and identification of microorganisms isolated from vermi-composts and skin of earthworm species were also checked for the enzyme activity. The experiments were also conducted with and without additional inoculums of bacteria isolated from vermi-composts in two identical vermi-compost systems couture boxes to assess the role of microorganisms. The consortium of cellulose degrading microorganisms inoculated on filter mud showed a decrease of organic carbon of 34.14%. Further the geotechnical properties of filter mud compost with inoculation of cellulose degrading organisms determined. The demonstration of aggregation of soil by bacteria carried out with 100g of soil inoculated with vermi-compost microorganisms.

The filter mud with specific gravity 0.5 to 0.7, showed an organic carbon ranging from 17-35 % and microbiological counts of fungi, yeast, bacteria (cfu) 3.6×10^8, 9.0×10^6, 2.4×10^9 respectively. The same is tried for vermin processing. Initial experimentation of species selection with different proportions of soil cow dung showed 1:3 proportion gave better results in terms of juveniles and number of earthworms. The industry soil used passed through 850μ in the above experiment designated as (SW) Well graded sand is suitable for vermi-processing without any amendments in it.

The microbiological counts of fungi, yeasts, and bacteria 11 x 10^5, 6 x10^4, and 1.28 x 10^7 respectively. The order of increase in number of earthworms is *Eudrilus eugeniiae* > *Megascolex megascolex* > *Eisenia fetida.* The particle size distribution of filter mud is higher than that of industry soil.

The substrate preference (Cow dung-soil), grinding capabilities of *Eudrilus eugeniae*> *Eisenia fetida*> *Megascolex megascolex.* The order of aggregation of vermi-compost of 1:3 soil cow-dung is *Megascolex megascolex*>*Eisenia Fetida*>*Eudrilus eugenae.* The aggregation is more in the soil with 90μsieve

Using this optimized proportion as the base the laboratory and filed trials were taken in culture boxes and field units respectively. The results showed the order of biodegradation is *Megascolex megascolex* > *Eudrilus eugeniae* > *Eisenia fetida.*

The optimum temperature up to 38 °C, moisture 70-80% and pH range 5-9 will be suitable for setting up vermi-processing units. The higher decrease in organic carbon is obtained in case of vermi-compost of press mud with *Megascolex megascolex*>*Eudrilus eugenae*> *Eisenia fetida.* The higher aggregation of soil finer than 850 micron is 54.85%, 50.26, 45.23 in case of *Megascolex megascolex* >*Eisenia fetida* > *Eudrilus eugeniae.* The order of aggregation in case of composts is also the same. Grinding Capabilities *Eudrilus eugeniae*>*Eisenia fetida*> *Megascolex megascolex.*

The press mud has shown larger size particle size distribution > industry soil. Larger size particle distribution is shown by *Megascolex megascolex.* The *Megascolex megascolex* vermi-compost has shown its superiority in all the geotechnical properties as compared to vermi-composts of Eudrilus *eugeniae* and *Eisenia fetida*, the higher nutrient values of N, P, and K is shown by *Megascolex megascolex.*

There is a remarkable increase in the bacterial count of the vermi composts of the press mud as compared to the industry soil sample and zero day samples. The many fold increase is 16, 6.4; 42.7, 17.1; 41.6, 16.6 times in case of vermi-compost with *Eisenia fetida, Eudrilus eugeniae,* and *Megascolex megascolex*, as compared to industry soil and zero day samples respectively.

The many fold increase due to supplementation of cow dung, varied from 1.68,2,23, 2.45, 2.05 times in case of *Eisenia fetida*, Co culture of *Eisenia* and *Eudrilus eugeniae*, *Eudrilus eugeniae*, *Megascolex megascolex* respectively.

Following the biochemical tests the comparison of organisms with the Bergy's Manuel for determinative bacteriology revealed the occurrence of organisms belonging to *Bacillus* group. The order of preference of earthworm species based on the enzyme activity of skin organisms is *Eisenia fetida> Megascolex megascolex > Eudrilus eugeniae.*

Based on the number of isolates showing the enzyme activity it is evident that the *Eudrilus eugenae* stand as superior as compared to other two and the order of preference is given as *Eudrilus eugeniae>Megascolex megascolex> Eisenia fetida.*

Based on the individual enzyme activities recorded by each organisms the species of earthworms rank showing enzyme activity as 68.11%,66.66%,52% for *Eudrilus eugeniae, Eisenia fetida> Megascolex megascolex.*

The biodegradation of filter mud in the experimental culture box with inoculums of additional bacteria showed the higher rate of earthworm activity in terms of bringing soil to top surface, distribution of vermicast, earthworm activity, and bioconversion.

The samples showed highest number of earthworms in core cutter and after 30 days and the dilutions of samples showed highest juveniles, microbial population, solids settled at bottom in case of experimental culture box with inoculums.

The organic carbon and ash content have shown decreasing trend. There is a decreasing trend of pH in both the culture boxes. The geotechnical properties have shown the superiority in all the parameters for the compost of experimental culture box.

The enzyme activities have shown a increased amylase activity up to 20 days and the values have shown decrease in trend incase of protease and cellulase activity indicating complete utilization The organic carbon and ash content have shown decreasing trend.

Just after 20 days, the filter mud resembles the appearance of vermi compost.

There is a 34.14 decrease in organic carbon indicating the role of cellulose degrading organisms. There is a higher specific gravity shown by the compost from experimental culture box. Higher water content, porosity, voids ratio, Increasing densities, Degree of saturation, Air content. Decreased air voids.

The finer particles less than 425 μ have aggregated with the bioconversion where as the higher size aggregates > 425 μ has decreased in the particle size. The particle size is more of uniform distribution and is almost parallel to the filter mud distribution.

74.10g of soil passing through 90μ aggregated. 1 g of vermi-compost of filter mud prepared by using Co culture of *Eisenia fetida* and *Eudrilus eugenae* holds 20 g of 90μ soil.

The 100g taken in each plate 49.13 %, 45.72%, 51.67% aggregated soil passing through 425 μ. The remaining soil automatically got aggregated and falls in the category from 425 μ 850 μ. The cumulative aggregation on 425-μ sieve shows the increasing trend as in figure 68.

The proportion 1:2 and 1:3 has shown a decrease in organic carbon of 13, 7%, 41.64%in 10, 20 days respectively. The experimentation with varied proportions, showing a decrease in total solids from 0 to 10 days and then increasing from 10 to 20 days.

The density bottle experiment has reveled that the proportion 1: 2 has shown increase in specific gravity as compared with the other proportions. The order of increase in specific gravity is 1:2>1:3 and 1:4.

The decrease in salinity in Vermi compost used Pots is 12.27, 11.36 times with *Dieffenbachia* and *Aglonima* plant species respectively, whereas the pots without plant species i.e. controls have shown a decrease of 10.38, 8.33 times respectively as compared with original salinity value.

Further Scope

- From the results of the present study, it can be conclusively stated that the microbes are playing a greater role in the field of industrial organic solid waste management. The synergetic role of earthworms in maintaining the pH, moisture, aeration makes it more interesting to study the different indigenous species of earthworms in treatment of filter mud and other industrial solid wastes.

- The filter mud composting process standardized for its bioconversion time and concerning quality in terms of nutrient values by using earthworms and only bacteria to make the product available to the end users.
- The study on the microbes isolated from skin and vermi-composts with distinct characteristic with enzyme activities has revealed that the same could be used for treatment of other industrial wastes depending on the major constituent of the waste.
- There fore the research in waste management using microbes should aim towards studies on enzyme activities such as xylanase, cellulose, lipase, amylase, protease etc. for the potential uses in organic waste treatment and wastewater treatments.
- As the industrial waste management field is new there is a larger scope for new experiment design and setting pin pointing experiments to get a realistic answer to the process of biodegradation using bacteria, fungi, yeasts, atinomycetes etc or in combination of these in a definite proportion.
- The measures have to be taken to maximize the efficiency of the waste management system and make the industry to realize the value addition in terms of environmental management and at the same time earn profits.
- Gathering data from various food processing industries itself will be a great task and make these industries realize about the resource utilization. A team effort and in house R& D facilities within the industries will bring greater sustainability in the industrial development.

CHAPTER VIII

APPENDICES

APPENDIX A: Sugar industry manufacturing, waste generation, disposal and vermi-composting practices.

APPENDIX A1: Present disposal of solid and liquid wastes.

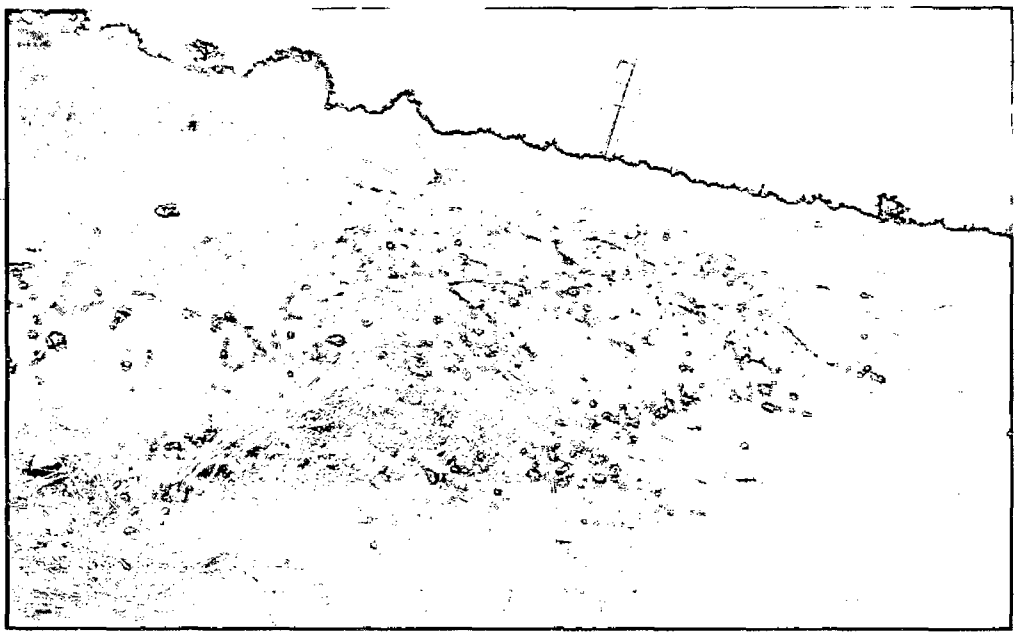


Plate A1: Open dumping of furnace ash.

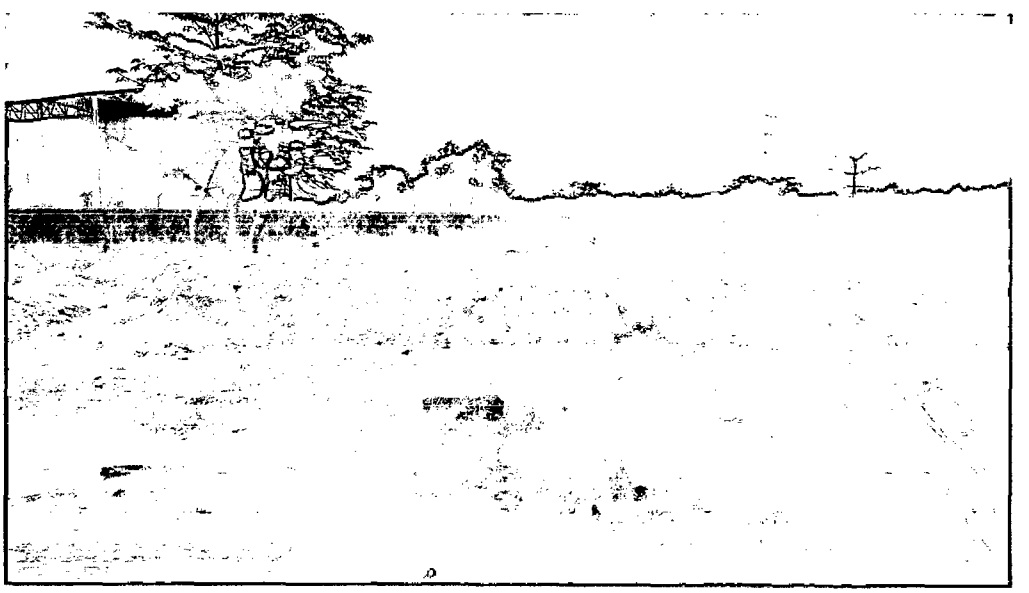


Plate A2: Open dumping of furnace ash.

Plate A3: Open dumping of filter mud.

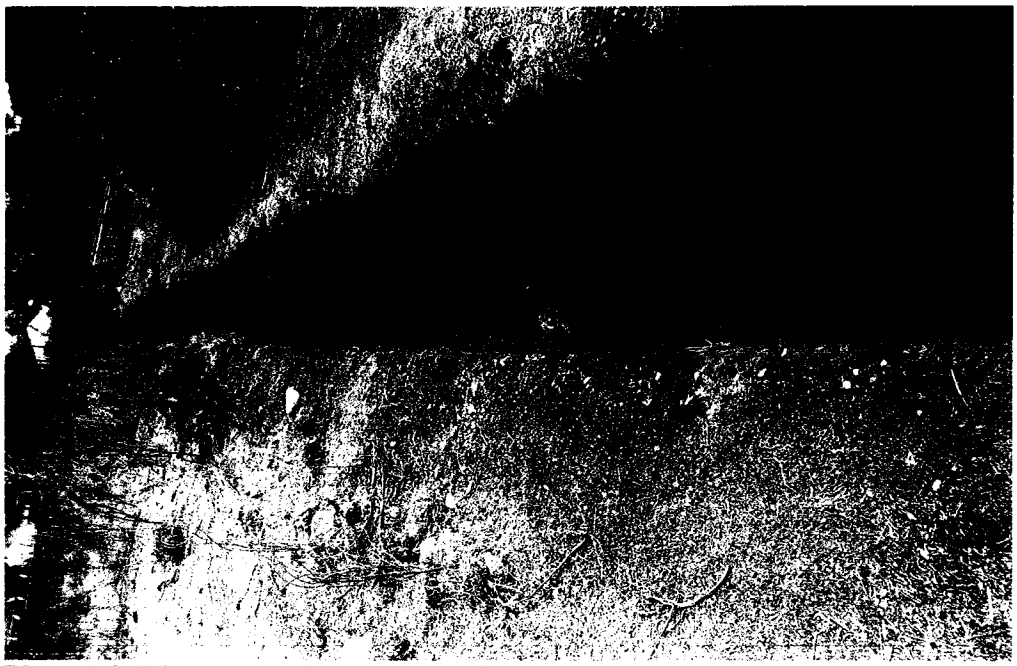


Plate A4: Discharge of combined effluents.

APPENDIX A2: Vermi-processing in industrial units

Table A1: List of on going vermi processing of industrial wastes:

Sl.No	Type of industry	Waste/generation rate/state	Earthworm inoculums	References/Remarks
1	Tanning	Salt,haur,likme, fleshmyrobdust,buffing dust and trimmed waste.	*Perionyx exavatus*	Shahul Hameed et.al, (2000)
2	Pepsi Foods Ltd.Channo.,Punjab	Potato Peels	*Pheritima elongata*	Arora ,1998
3	Doaba Co operative Sugar Punjab	Press mud	*Pheritima elongata*	Singh , 1997
4	Indian Aluminiam Company Belgaum	Canteen solid waste	*NA	White ,1996
5	Citric India Ltd	Citric acid effluent	*NA	White,1996
6	DWM bauxite mines	Waste land developement	*NA	Jamble,1996
7	FDC,Ltd., Roha	Soya Residue 3tons/day		Singh, 1997
8	Marmgoa Potrt Trust	Canteen waste	*Eisenia foetida*	
9	Hindustan Motors Ltd., Indore	NA		Singh,1997
10	Hindustan Lever Ltd., Zahura Ltd	Tomato skin seed	*Pheritima elongata*	Singh,1997
11	MICO Bangalore	*NA	*NA	Nayak and Rath,1996
12	HMT Bangalore	*NA	*NA	Nayak and Rath,1996
13	Military Dairy Farm Bangalore	*NA	*NA	Nayak and Rath,1996
14	Orient vegetexp Ltd., Nasik	Onion residue	*NA	White,1996
15	Sakthi Sugar Ltd.,	Bio methanisation of distillery spent wash	*NA	Singh ,1996
16	Sesa Goa Ltd.,	Canteen waste/ Land reclamation	*Eisenia fetida*	Singh,1997
17	Venkateshwara Hatcheries Pune	Poultry residue4 tons/day	*NA	White,1996
18	VST Industries Hyderabad	*NA	*NA	Singh,1997
19	Thermax Ltd., Pune	Canteen Waste		Singh,1997
20	Spice Board a	*NA	*NA	Nayak and

	Central Govt. Org.,			Rath,1996
21	Oceanic Distillaries Mumbai	Vermi filter for utilization of distillery spent wash	*NA	White,1996
22	JagatJit Industries ,Punjab	Distillery Sludge	*Pheritima elongata*	Singh,1997
23	Electronic Division Nanjangud	Processing Canteen Waste	*NA	Jamble and Mannivannan,1996
24	M P Glychem Industries Ltd., Indore	Soya Residue	*NA	Singh,1996
25	Paper mills Shimoga Karnataka	Punjab, Ropar	*NA	Sudhir Ghatnekar,1999
26	Soyabean Oil E#xtractin Plants Dewas M.P	Soya Residue	*NA	Sudhir Ghatnekar,1999
27	Naptha Based Organo Chemicals Thane Mumbai		*NA	Sudhir Ghatnekar,1999
28	Dairy and Chese Manufacturing	Dairy waste	*NA	Sudhir Ghatnekar,1999
29	Food and vegetable processing industries	Nasik Maharashtra, Valsad Gujarat	*NA	Sudhir Ghatnekar,1999
30	Slaughter Huse	Slaughter waste	*NA	Sudhir Ghatnekar,1999
31	Coir processing waste	Tenkasi Tamilnadu	*NA	Sudhir Ghatnekar,1999
32	Dye ndustry ,Chiplun	Maharashtra	*NA	Sudhir Ghatnekar,1999
33	Oil Refinery Trivendrum	Kerals	*NA	Sudhir Ghatnekar,1999
34	Paper /board mill sludge	Board mill sludge Finland	**Lumbricus terristris*	Kevian,(2005)

*NA: Details not available

Appendix A3: Organic waste producing industries

Table A2: List of Important Organic Waste Producing Industries with United Nations Classification Codes.

Sr. No.	Type of industry	UN classification codes
1.	Slaughter houses	3111a
2.	Packing houses	3111b
3.	Poultry processing	3111c
4.	Dairy products	3112
5.	Canning of fruits and vegetables	3113
6.	Canning of fish	3114
7.	Olive oil extraction	3115a
8.	Vegetable oil refining	3115b
9.	Grain mills	3116
10	Cane sugar factories	3118a
11.	Beet sugar manufacturing	3118b
12.	Starch and sugar manufacturing	3121a
13.	Yeast manufacturing	3121b

WHO Report, 1992

Appendix A4: Waste minimization opportunities

Table A3: Waste minimization opportunities in various Industrial sectors

Industrial Sector	Clean Production	Recycling through EOP Treatment	Waste Utilization
Iron And Steel	B	B	A
Metal Planting	A	B	C
Non-ferrous Metals	B	C	A
Pulp and Paper	A	A	A
Textile	A	B	A
Tannery	B	B	B
Fertilizer	B	B	C
Distiller	B	B	A
Food Processing	B	B	B
Thermal Power	C	B	A

A: High B: Moderate C: Low

Source: Khanna, (1996) ; EOP; End of pipe

Appendix A5: Flow diagram for process of sugar manufacturing

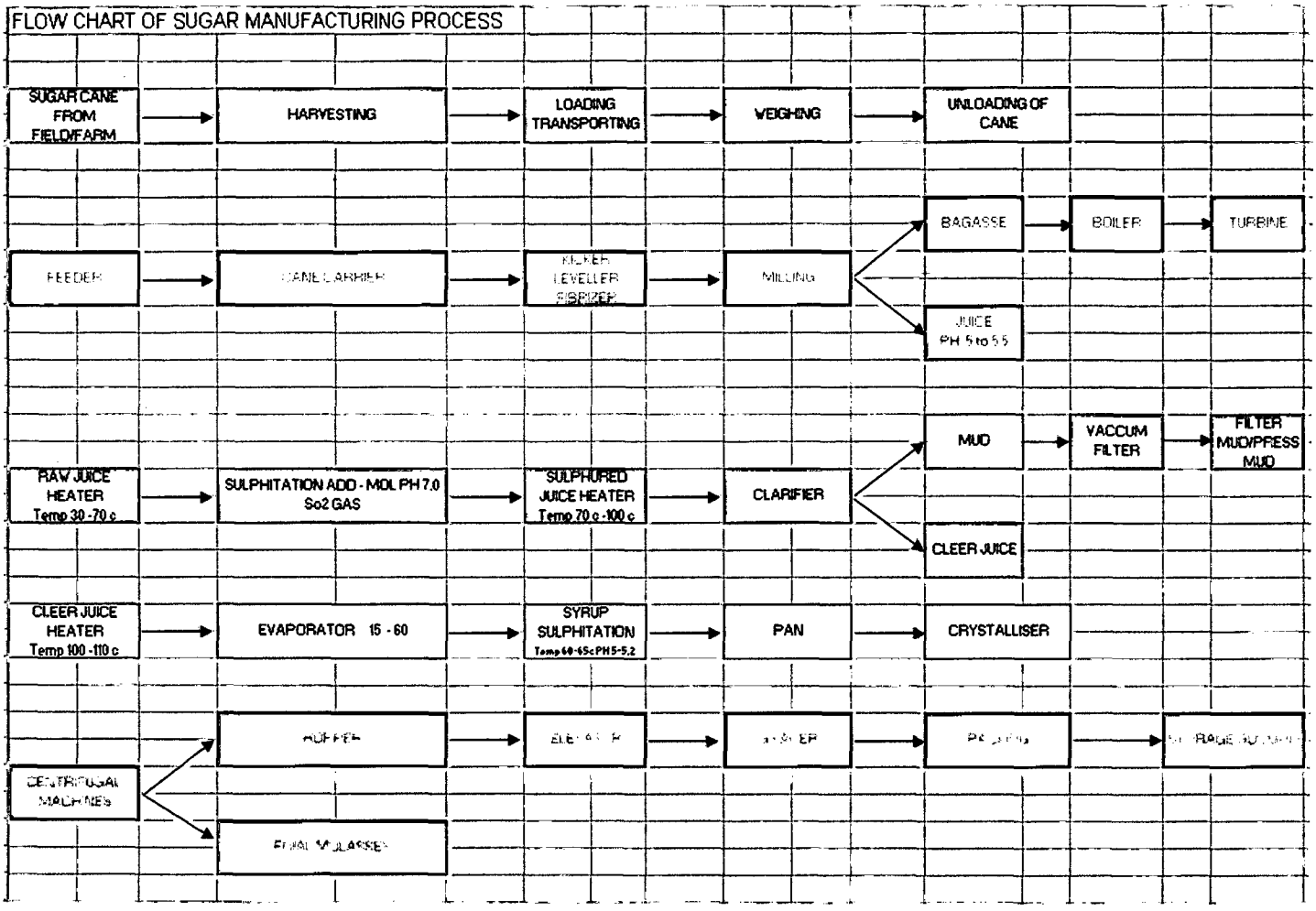
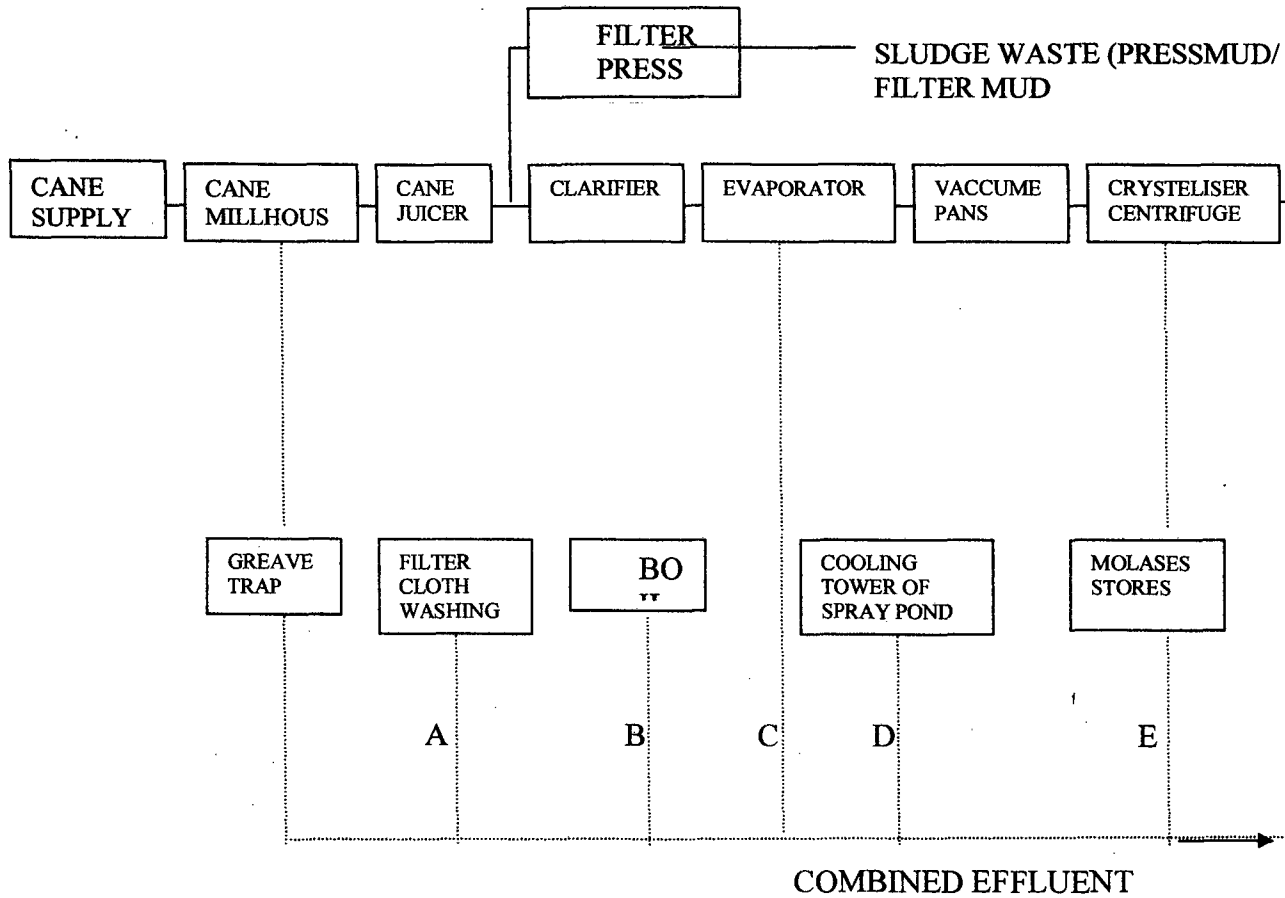


Figure A1: Flow Diagram for Sugar Manufacturing

Appendix A6: Sanjeevani sugar factory Dayanand Nagr Goa:

The sugar industry under consideration is the only one in Goa situated near Tisk Goa on NH 4 A. This has a crushing capacity of 1200 tons per day. Generates about 45 ty^{-1}. The disposes the solid wastes openly on land creating environmental problems of odor, huge dump waste, aesthetics' flow diagram of solid waste generation and combined effluents presented in figure

Figure A2: Solid waste generation and combined effluents



A – Washing of filter cloth, B – Boiler House leakage etc, C – Excess condensed water.

D – Pond Overflow, E – Spill over and handling losses

Source : Munnoli,2002*

APPENDIX B: Physicochemical characteristics and Geotechnical properties

B1: Determination of moisture Content

The moisture contents of the soil and compost samples were determined by the oven drying method. A known weight W1of the sample was taken. The sample is kept in the oven for 24 hrs at 100 ^{0}C and weighs W2. The difference in weight gives the water evaporated (w).

Water Content = [W1 – W2] / W2 x 100

B2: Determination of Lose bulk density

The waste procured s n taken in a container of known volume. The container filled with waste and the empty containers were weighed.

Weight of the container = X gms

Weight container with waste = Y gms

Volume of the container = V cc

Loose bulk density = [Y - X] / V

B3: Determination of Total solids

Clean silica crucible was heated at 100 ^{0}C for one hour in a hot air oven; it was cooled in a desiccator's and weighed. Then weigh about 10 Gms of sample in the silica crucible. It was dried in a hot air hot air oven at 1000C to a constant weight for overnight, cooled in desiccators and weighed again

Weight of empty silica crucible = A gms

Weight of crucible + sample = B gms

Weight of crucible + sample after drying at 100 ^{0}C = C gms

Moisture (%) = [C- B] / [B-A] X 100

Total Solids = [C-A] / [B-A] X 100

The dried samples were retained for further estimations

B4: Determination of organic carbon

Empty and clean silica crucible is heated at 550 ^{0}C for 3 hours in a muffle furnace, and then cooled in a desiccators and weigh. Accurately weigh about 2-4g of sample in a silica crucible. The sample was dried in an oven as described above cooled in desiccators and weighed. The crucible is transferred in to a muffle furnace and the sample is ignited at 550 + 50 0C for 3 hours. There after the same is cooled and weighed.

Weight of empty silica crucible = A gms

Weight of crucible + sample = B gms

Weight of crucible + sample after drying at 103 to 105 0C up to constant weight = C gms

Weight of crucible and ash after ignition = D gms

$$\text{Total volatile solids (\%)} = \frac{C-D}{B-A} \times 100 \qquad \text{and Ash Content} = \frac{D-A}{B-A}$$

Organic Carbon is calculated as:

$$\text{Organic Carbon (\%)} = 50\,\% \frac{\text{Volatile solids}}{\text{Total solids}}$$

B5: Determination of pH

About 5g of sample is placed in 100 ml distilled water and shaken vigorously. The sample is allowed to settle down for 1 hour. These solutions were used for determination of pH using a digital pH meter.

B6: Water content determination

W1 = empty wt of container

W2 = empty wt of container + Vermi-compost

W3 = empty wt of container + Vermi compost + after oven drying for 24 hrs @ 100 ^{0}C

$$\text{Water content (w)} = \frac{W_3 - W_2}{W_3 - W1} \times 100 \quad \text{Expressed as \%}$$

B7: Determination of nitrogen in press mud sample

Principle:

Nitrogen content of plant is converted into $(NH_4)_2\ SO_4$ by digesting with conc. $H_2\ SO_4$. The acid digest is distilled for NH_3 by using 40% NaOH. The distilled NH_3 is trapped in boric acid is mixed indicator solution. The amount of ammonia trapped is estimated by titrating against standard acid.

Reagents

1. Conc. H_2SO_4
2. Digestion mixture – Na_2SO_4 or K_2SO_4 : $CuSO_4$: selenium powder in 50:10:1ratio.
3. 40%NaOH
4. 2% or 4% Boric acid
5. 0.01N H_2SO_4
6. Mixed indicator (Bromeresol green (0.1g) + Methyl red (0.07g) in 100 ml of 95% ethanol.

Transfer 15 ml of mixed indicator to 1 liter of boric acid. Adjust the boric acid + mixed indicator solution to the bluish purple mid-color at pH 4.5 by using dil HCL or NaOH (0.1N) to get sharp endpoint. This indicator is pink at pH 4.2, bluish purple

mid-color at pH 4.5 and bluish green as the pH rises to 4.9 or above when ammonia is trapped.

Procedure:

1. Transfer 0.5 g powdered dried plant sample to Kjeldhal flask.
2. Add 10ml of conc. H_2SO_4 and 0.2 to 0.3g digestion \ catalytic mixture (Na_2SO_4 or K_2SO_4 helps in increasing boiling point, Cu_2SO_4 and se- powder act as catalysts in quick conversion of organic- N to inorganic N).
3. Digest on low flame initially for 10-15 minutes until frothing stops, pre-digestion is necessary for oil seed crops or seeds.
4. Then digest at high flame (temp.) for 1-1 1\ 2 hours till the contents of Kjeldhal flask become clear
5. Cool the flask and transfer the contents of flask quantitatively to 50ml volumetric flask and make up the volume by adding distilled water. This dilution may be necessary when micro Kjeldhal method is followed (The capacity of distillation flask is about 20ml. therefore, the volume of digested material + 40%NaOH should be around 20ml. Moreover when sample handled is less, the loss of NH_3 is avoided.
6. Pipette out 10ml of acid digest and transfer to micro Kjeldhal distillation assembly.
7. Add sufficient quantity (about 10-15ml) of 40% NaOH to make the contents distinctly alkaline.
8. Before adding NaOH, boric acid –mixed indicator solution should be kept ready at the receiving end (Better to use higher concentration (4%) and lower volume about 20ml boric acid).However, neither the volume nor the strength of boric acid is important.
9. Carry out the distillation even after the color changes from bluish purple to bluish green. Continue for some more time to trap all the NH_3 released (Don't take out immediately after the color change).

   Litmus test: Test with red litmus after giving sufficient time after color changes (5-10min). No change in the color of red litmus indicates complete distillation.

10. After the distillation is over, estimate the quantity of NH_3 distilled (trapped in boric acid) by titrating against 0.01 N- H_2SO_4 till color changes to purple.

Reactions

During distillation

$H_3BO_3 + NH_3 \rightarrow NH_4H_2BO_3 \rightarrow NH_4^+ + H_2BO_3^-$

Boric acid (Ammonium borate)

(Color changes to bluish green)

During titration

$H_2BO_3^- + H \rightarrow H_3BO_3$

OR

$NH_4H_2BO_3 + H$ (from std. H_2SO_4) $\rightarrow H_3BO_3 + NH_4^+$

Calculations

$$\% N = \frac{\text{Titre value} \times N\ H_2SO_4 \times 0.014 \times \text{dil. Factor (if any)} \times 100}{\text{Wt. of pl. sample (g)}}$$

B8: Determination of Phosphorus in plant Sample

Principle: Orthophosphate (phosphorus) present in the plant digest when reacts with vanadate and molybdate gives a yellow colored complex i.e. phosphovanadomolybdate in acid solution. The yellow color is due to the substitution of oxyvanadium and oxymolybdenum radicals for oxygen of PO_4 to give a heteropoly compound. The intensity of yellow color is measured calorimetrically at 400- 490 nm using spectrophotometer.

Reagents:

1. Vanadomolybdate reagent (Ammonium molybdate – ammonium vanadate in nitric acid).

   Preparation: Solution A: Dissolve 1.25g ammonium metavanadate in 300ml of boiling water. Cool and add 250ml conc. HNO3 and again cool to room temperature.

Solution B: Dissolve 25g ammonium molybdate in 400ml of distilled water pour solution A to Solution B mix well and make up the volume to 1 litre with distilled water.

2. Phosphorus standard solution

Preparation: Prepare 100ppm of P standard stock solution by dissolving 0.2195g of pure KH_2PO_4 in 500ml of distilled water.

Working standards of P : Transfer 0,1,2.5,5,7.5 and 10ml of 100ppm P- stock solution into separate 50 ml volumetric flasks to get 0,2,5,10,15,and 20 ppm of P-working standards.

Procedure:

1. Transfer 5 ml aliquot of plant digest (Triacid or diacid digested plant samples) into 50 ml volumetric flask.
2. Add 10 ml of vanadomolybdate reagent to samples and to each standards and mix thoroughly and make up the volume to 50ml with distilled water.
3. After 30 minutes of color development read the intensity of yellow color on a spectrophotometer at 470nm (i.e. between 400 – 490nm)
4. Draw the calibration curve (standard graph) of P standard by plotting the P-absorbance against P- concentration.
5. Find out the P- content in plant digest sample by referring to the standard curve.

Calculations

$$\% \text{ in plant sample} = \text{Graph ppm} * \frac{\text{Vol. of dilution made}}{10^6} \times \frac{\text{Vol of PL. digest} * 100}{\text{Aliquot} \quad \text{Wt. of PL. sample}}$$

B9: Determination of potassium:

Principle: In flame photometry, also known as flame emission or flame atomic emission, the sample in solution is sprayed in to flame to vaporize, atomize, and exite the sample. The exited atoms of the element of interest emit light at certain discrete wavelengths, which are characteristic of that element. Light of the wavelength of interest is separated

from remainder of emitted radiations and its intensity is measured. The intensity measurement can be related directly to the concentration of the element of interest usually by comparing with the intensities, of standard or series of standards.

Reagents:

1. Potassium standard 100 ppm of K: Dissolve 0.191 g of KCl in some volume of distilled water and then make up the volume to one liter.

Materials required Flame photometer, pipette, volumetric flask, etc.

Procedure:

Preparation of standard curve:

Take 0, 1,2,3,4 and 5ml of 100-ppm K solution in a separate 50 ml volumetric flasks, make up the volume to 50 ml with distilled water and mix well. After adjusting needle of flame photometer to zero by feeding blank, adjust the needle to 100 by feeding maximum concentrated K solution. Then feed the standards to record the flame photometer readings. Plot flame photometer readings verses concentrations of standards and draw the standard curve.

Sample:

Feed the digested sample solution to the flame photometer and record the reading (if dilution is required one should do the dilution before feeding to the instrument). Compare the unknown sample reading with the standard curve to determine the percentage k in the sample

Calculations:

$$\%\,K = \frac{\text{Graph ppm} \times \text{Volume of digested sample}}{10^6 \times \text{Weight of sample}}$$

For diluted solutions:

$$\%\,K = \frac{\text{Graph ppm} \times \text{Volume of digested sample} \times \text{Volume made up}}{10^6 \times \text{Weight of sample} \times \text{Aliquot taken for dilution}}$$

B10: Determination of geotechnical properties.

A steel cylindrical core cutter of size 10 cm height and 2.8 cm internal diameter immersed in the compost for taking known weight of vermi-compost from the culture box to evaluate geotechnical properties.

B10.1 Determination of Specific gravit by density bottle

M1 = empty wt. of density bottle

M2 = empty wt + Vermi compost

M3 = empty wt + Vermi compost + Water

M4 = empty wt + Water

$$G = \frac{\{M2 - M_1\}}{(M2 - M_1) - (M3 - M_4)}$$

B10.2: Determination of bulk density of vermicompost:

A core cutter of known volume is taken; the core cutter is immersed in a vermi bed slowly until it is completely filled with the vermi-compost. The photograph showing different core cutters for the experimentation is placed in photograph 2.1.

V = Volume of core cutter

W1 = empty weight of core cutter

W2 = empty weight of core cutter + vermi compost

W = Weight of vermi compost in the core cutter = W1 – W2

Bulk density = γ = W/ V

B10.3 Determination of dry density:

The dry density a_d is determined by the relation

$\Gamma d = \gamma / (1+w)$

Where γ = Bulk density

W = water content

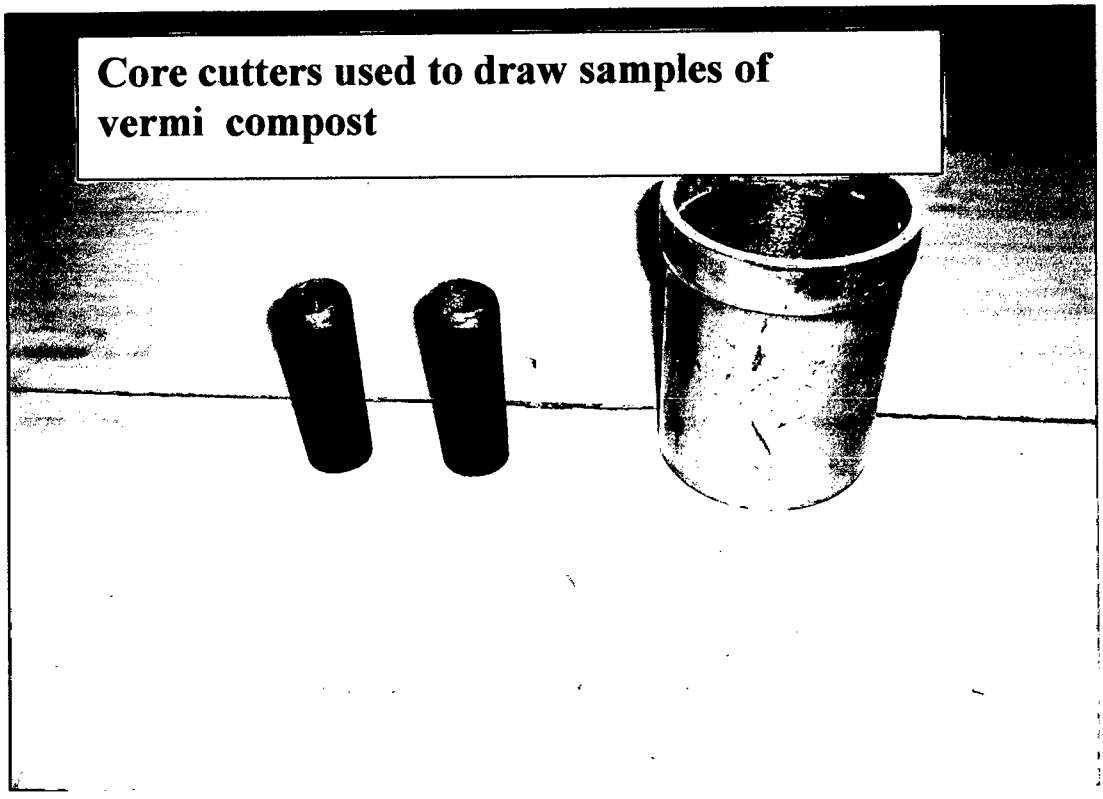


Plate B1: Core cutters

B10.4 Voids ratio 'e': The voids ratio e is calculated from the fallowing relation if the Specific gravity G is known.

e = $\{G * \gamma_w / \gamma d\}$ - 1

Where e = voids ratio

G = Specific gravity

Γw = Density of water

γ_d = Dry density

B10.5 Porosity n is obtained by the relation

n = 1 - γ_d / [G * γ_w] Saturate **density:**

B10.6 saturated density is obtained by the relation

$$\gamma_{sat} = \frac{[G + e] * \gamma_w}{[1 + e]}$$

B10.7 Degree of saturation Sr is obtained from the relation

S_r = [W * G] / e

B10.8: Air content a_c = 1 - S_r

B10.9: Percentage air voids n_a

$$n_a = 1 - \frac{[\gamma d * (1 + W G)]}{G * \gamma w}$$

B11 Characterization of soils:

Analysis of soil was done in accordance with IS:2720- Indian Standard Methods of Test for soils.

B11.1 Liquid Limit :

Liquid limit test was conducted by the cone penetration method as per IS:2720 (Part V) - 1985.

B11.2 Plastic Limit:

This test was also carried out as per the procedure outlined in IS 272(Part V) -1985.

B11.3 Particle Size Distribution:

Particle size distribution of the soil was determined in accordance with IS: 2720(part IV) - 1985 Using standard set of IS Sieves, Samples were machine sieved and percentage assign in various sieves was obtained.

Based on the test results, a graph representing particle size distribution prepared on a semi log graph paper.

B11.4 Identification of soil type:

Identification of soil type based on the samples obtained from the industry was carried out as per IS: 1498-1970. At first visual examination of soil was made to determine whether it is highly organic, coarse grained or fine grained. There after type of soil was ascertained by running sieve analysis and plotting grain size distribution curve.

B11.5 Determination of permeability:

The test was performed following the method in accordance with IS Code 2720(Part17) - 1986.Constant head method is performed on the soil samples and coefficient of permeability (K) is calculated as

$K = Q / Ait$

$$K_{27} = \frac{Kr\ Vr}{V_{27}}$$

Where

Kr = Permiabiity at temperature T 0C

Q = Quantity of water

A = Area of specimen

i = Hydraulic gradient

t = Time

V_T = Coefficient of viscosity at T 0C

V_{27} = Coefficient of viscosity at 27 0C

B11.6 Determination of pH

The test was carried out in accordance with IS Code (Part 2720) _1987 Using digital pH meter. 30g of soil was mixed with 100 ml of distilled water kept stirred continuously and recorded the pH meter reading after half an hour.

B11.7 **Water holding capacity**: The three-vermi-composts were subjected to a compaction test to evaluate the optimum water content and dry densities using a standard proctor compaction test. A representative soil sample of the industry 3 kg was taken and added press mud of 200g with 60-70% water content and given a compaction of 25 blows with a standard 2.5 kg hammer. The compaction was carried out in three layers and the excess soil trimmed off. The bulk density and water content are determined each case. The experiment repeated with second time 200g of press mud.

Appendix C: Composition of stains Biochemical Medias

1. Nutrient Agar

Peptone	10 g
Beef Extract	3g
Sodium Chloride	5 g
Distilled water	1000ml

Dissolve the ingredients; adjust the pH to 7.2 to 7.4 with 1N NaOH

Agar	20 g

Digest in boiling water bath for ½ hr Dispense in flasks and sterilize at 121^0C , 15lb for 20 min.
For ready made 28%/ liter.

2. Nutrient Broth

Same as Nutrient Agar but without agar

3. Sabaraud's Agar

Peptone	10 g
Sodium Chloride	Traces
Glucose 40%	100ml
D/W	900ml
pH	7.6
agar	29-30 g

Glucose should be sterilized separately and added to the melted sterilized medium before use (40g in 100 ml D/W)

4. Wicker hams Agar (Yeasts)

Peptone	5g
Malt Extract	3g
Yeast Extract	3g
Glucose	100ml
D/W	900ml
pH	5.5
Agar	30g

Glucose 10% sterilize separately

5. Normal saline:

0.85 g in 100 ml distilled water and sterilized

6. Skimmed Milk Agar test (protease)

Inoculate milk agar plates and incubated at R.T for 24-48 h. Then examine the plates for the presence or absence of a clear area around the organism. A clear area around the bacterial growth was indicative of positive proteolysis. Un-inoculated agar plate served as the control.

KH_2PO_4	0.1g
$MgSO_4$	0.02g
Agar	20g
Milk	20ml
D/W	80ml
pH	7.0

7. Carboxyl methyl Cellulose agar test (CMC)

Inoculate CMC agar plates and incubated at R.T for 24-48 h. Then examine the plates for the presence or absence of a clear area around the organism. Then flooded the plates with 0.1 % Congo red for 15 min and then rinsed with 1Nacl for15 min and poured off excess of stain. A clear area around the bacterial growth was indicative of positive cellulose activity. Un-inoculated agar plate served as

Peptone	5g
Yeast Extract	0.5 g
KH_2PO4	1g
MgSO4	002g
Agar	2g
CMC	10g
D/w	90ml
pH	7.0

Cango red:	1g
D/w	100ml

8. Starch Agar (Amylase test)

Inoculated starch agar plates incubated the plates at R.T. for 24 to 48 h. Then flooded the plates with Grams iodine forl min and poured off excess of stain. Clear zone surrounding the organism was a positive test. Un-inoculated agar plate served as the control.

Peptone	0.5g
Beef extract	0.3g
Agar	2g
Starch	10ml
D/w	90ml
pH	7.0

Grams iodine solution:	Iodine	-1g
	Potassium Iodide	-2g
	Distilled water	-300ml

9. Chitinase activity

Crude chitin of 10g is taken in a concentrated hydrochloric acid kept at 4^0C overnight. Tis preparation is filtered through a glass wool and centrifuged at 7000 rpm and washed with distilled water.

Inoculated chitin agar pates with fresh cultures incubated at R.T for 24-48 h. Fallowing the incubation the clear zones shown taken as positive.

Peptone	5g
Beef extract	3.0 g
Chitin	10 g
pH	7.2
Agar	20g
Distilled water	1000ml

10. Lipase test

Inoculated coconut agar by means of spot inoculation and incubated for 24-48 h at R.T. Fallowing the incubation, observed the plates for presence or absence of growth and a clear zone around the organisms. Un inoculated tubes serves as control.

Peptone	10 g
Sodium chloride	5. 0
Coconut oil	2 ml (g)
Agar	10g
Distilled water	1000 ml
pH	7.4

Sterilization 121 ^{0}C, 15 minutes .

11. Citrate utilization test

Inoculated Simmon's citrate agar slants by means of stab and streak inoculation and incubated for 24-48 h at R.T. Fallowing the incubation, observed the slant for presence or absence of growth. Citrate utilizes were indicated by the presence of growth(bue color) on the slants. Un inoculated tubes serves as control.

Ammonium di-hydrogen phosphate	1g
Potassium di-hydrogen phosphate	1g
Sodium chloride	5 g
Magnesium Sulphate	0.2g
Sodium Citrate	5g
Distilled water	1000 ml
agar	10 g
Bromothymolblue(0.2%)	0.2g
pH	7 . 4

Sterilization 121 ^{0}C, 15 minutes .

12. Oxidase test

Test reagent: 1 % aqueous solution of tetra methyl – p- phenylenediamine hydrochloride. Streak the inoculums from slants on what-man filter paper soaked in test reagent. Purple in 5 – 10 seconds indicated positive test result.

13. Catalase test

Test reagent: hydrogen peroxide

Place 1 ml of hydrogen peroxide in a test tube and add 1 ml broth culture .

Effervescences, was observerd indicated positive test result.

14.H_2S production and motility test

Hydrogen sulphide production was checked in SIM agar tubes by stab inoculating the organism and then incubating at R.T. for 24-48 h. After incubation, it was observed for black insoluble ferrous sulphide for a positive indication of test. . Fallowing incubation Motile cultures showed diffused growth where as non motile cultures grew only along the line.Un inoculated tubes served as the control.

Peptone	5g
Beef extract	3g
Ferrous ammonium sulphate	0.2g
sodium thiosulphate	0.025g
Agar	10g
Distilled water	1000ml
pH	7.4

Dispense 5ml in tubes and sterilize 20 minutes at 121 ^{0}C by autoclaving.

15.Carbohydrate utilization test

Inoculated sugar broth containing a carbohydrate source with phenol red indicator. Incubate the tubes at R.T for 24-48 h. Then examine the tubes for change in color and presence or absence of gas bubble. Positive reaction is indicated by change in color (red-yellow) of the indicator and appearance of air bubble in the Durham tube.

Peptone	10g
Sodium chloride	5g
Carbohydrate solution 10%	0 . 5 ml
(Sterilized and added separately)	
Distilled water	900 ml
pH	7 . 3
Phenol red	50 ml

Tube the medium (5 ml), put inverted Durham's tube before sterilization and Autoclaved at 120 and 15 lbs for 20 minutes .

Phenol red preparation 1 g phenol red + 10 ml 0 . 1 n naoh + 20 ml distilled water , gentle heating + 10 ml ,0 . 1 HCl make 500 ml by distilled water .

16.Hugh and leifson's medium.

Inoculated young cultures in the medium dispensed into two tubes. After inoculation, the medium of one tube overlaid with sterile liquid paraffin. Growth and color change of indicator was noted in the two tubes. Strict aerobes grow only in aerobic conditions. Facultative anaerobes grow in both aerobic and anaerobic conditions. The anaerobic organisms grow only in anaerobic conditions. Un inoculated tubes served as the control.

Peptone	2 . 0 g
Sodium chloride	5 . 0 g
Dipotassium hydrogen phosphate	0 . 3 g
bromothymol blue (1 %)	0.01g in water 0 . 3 ml
Distilled water	1000 ml
pH	7 . 1
glucose	10 % (sterile separately)

sterilization 121 c , 15 lbs

0 . 5 ml of glucose solution was added in each tube. Sterilized paraffin was added to Study the fermentative pathway of carbohydrate utilization.

17. Indole production test

Indole production was checked in SIM agar tubes by stab inoculating the organism and then incubating at R.T. for 24-48 h. After incubation,10 drops of Kovac's reagent were added and it was observed for coloration. The red reagent layer was a positive indication of indole production. Un inoculated tubes served as the control.

Peptone	5g
Beef extract	3g
Ferrous ammonium sulphate	0.2g
sodium thiosulphate	0.025g
Agar	10g
Distilled water	1000ml
pH	7.4

Dispense 5ml in tubes and sterilize by autoclaving.

kovac's reagent

Amyl or isoamyl alcohol	150ml
p-dimethyl – amino benzaldehyde	10g
Conc. hydrochloric acid	50ml

18. Gelatin hydrolysis test

Inoculated gelatin deep tubes and incubated at R.T. for 24-48h. Following the growth the tubes were refrigerated for 30 minutes and the medium was observed. Liquid medium after refrigeration was positive. Un inoculated tube served as cntrol.

Nutrient broth	1000ml
Gelatin	150g
pH	7.4

Digest dispense in small suspension tubes and sterilize (5ml) by intermittent sterilization at 100 ^{0}c for ½ hr for 3 consecutive days.

Spot inoculated the gelatin agar plates incubated at R.T for 24-48 h. fallowing the incubation flood the plates with gelatin precipitating agent. A clear zone around the colony indicates positive result.

Nutrient agar	100ml
Gelatin	0.4%
Gelatin precipitating agent	15% $HgCl_2$ in 20% (vol/vol) concentrated HCl

19. Urease test

Inoculated test culture heavily over the entire slope surface of urea agar slant tubes and incubated at R.T. for 24-48h. Following the growth, a positive test indicated by change in color from yellow to purple. Un inoculated tube served as control.

Peptone	1g
Sodium chloride	5g
Potassium dihydroge3n phosphate	2g
Phenol red 0.2%	6ml
Glucose 10 %	10ml
Distilled water	1000ml
Agar	20g
ph	6.8-6.9

digest/ dispense in 5 ml in tubes sterelize by autoclaving
sterelize 10% glucose separately.

20. **The activity of a deaminase-phenylalanine deaminase test**

Inoculated **phenylalanine deaminase** deep slant tubes and incubated at R.T. for 24-48h. Following the growth, allow few drops of 10% ferric chloride solution to trickle down the surface of the slant. green color developed in the fluid in the sope is positive test activity of deaminase. Un inoculated tube served as control.

Medium composition

yeast extract	3g
dl- phenylalanine	2g
[or l- phenylalanine	1g
di sodium hydrogen phosphate	1g
Sodium chloride	5g
Agar	15 g

Distilled water 1000ml.adjust the ph to 7.4 distribute in test tubes and sterilize by autoclaving at 121 ^{0}c for 15 minutes. Allow to solidify in tubes as long slopes.

21. Maltonate utilization

Inoculated Sodium maltonate medium tubes by means of stab inoculation and incubated for 48 h at 'R.T. Fallowing the incubation, observed the culture positive results are indicated by change in color of the indicator from green to blue. Un inoculated tubes serves as control.

Yeast extract	1g
Ammonium sulphate	2g
Dipotassium hydrogen phosphate	0.6g
Potassium dihydrogen phosphate	0.4g
Sodium chloride	2g
Sodium maltonate	3g
bromothymol blue	0.025g
distilled water	1000ml.

23. Triple sugar iron agar medium composition

Inoculated the slants of TSI agar using a straight needle first stab the butt down to the bottom, then streak the surface of the slant. Incubate at 370 for 18-24 hrs. The Yello butt red slant shows glucose has fermented but not lactose and sucrose. Yellow butt –yellow slant is taken as lactose and/ or sucrose has been fermented. Red butt red slant –neither glucose, lactose or sucrose has been fermented. Bubbles in butt/broken agar/ indicated gas production. Blackening of the butt indicates Hydrogen sulphide production.

Beef extract	3g
East extracts	3g
Peptone	15g
Protease peptone	5g
Lactose	10g
Sucrose	10g
Glucose	1g
Ferrous sulphide	0.2g
Sodium chloride	5g
Sodium thiosulfate	0.3g
Agar	15 g
Distilled water	1000ml
pH	7.4

Adjust ph to 7.4 if necessary, sterilize by autoclaving at 121 ^{0}c for 15 minutes.

24. Methyl red test

Inoculated MR-VP broth and incubated at room temperature. Then added 5-6 drops of methyl red indicator and observed for the color change. A bright red color was indicative of a positive test. Un inoculated tubes served as control.

Peptone	5g
Di potassium hydrogen phosphate	5g
Glucose	5g
pH	7.0

Dispense 5ml in tubes sterilize glucose separately put 0.25 ml of 10% glucose in each tube.

25. Voges Proskauer

Inoculated MR-VP broth and incubated at R.T. for 24-48 hrs. Fallowing the incubation added 1 ml of 40% potassium hydroxide and 3 ml of 5%solution of alpha-napthol in absolute ethanol. A pink color was indicative of positive result. Un inoculated tubes served as control.

Peptone	5g
Di-potassium hydrogen phosphate	5g
Glucose 10% solution (sterilize separately)	50 ml
Distilled water	1000 ml

26. Mortality medium

Inoculated nutrient broth and incubated at R.T for 24-48 hrs. Fallowing incubation Motile cultures showed diffused growth where as non motile cultures grew only along the line.

Peptone	10g
Beef extract	3 g
Sodium chloride	5g
Distilled water	1000ml
Agar	4g

Dispense 5ml in tubes and sterilize.

27. Nitrate reduction test

Inoculated nitrate broth and incubated at R.T for 24-48 hrs. Fallowing incubation, added 5 drops of sulfanilic acid and then 5 drops of alpha- napthylamine. Red coloration indicated a positive test while in negative test, red color observed after addition of 5mg of Zinc. Un inoculated tubes served as control.

Beef extract	3 g
Peptone	5 g
sodium chloride	5 g
Potassium nitrate	1 g
Distilled water	1000 ml
pH	7 . 2

Dispense in tubes (5 ml) of autoclave

28. Grams staining:

Reagents

Crystal violet solution:	Crystal violet	5g
	Distilled water	100 ml
Grams iodine solution:	Iodine	1g
	Potassium Iodide	2g
	Distilled water	300ml
Counter stain	Safranin 2.5% (wt/vol) in 95% Vol/Vol ethanol. 10 ml Distilled water 1000ml, Filtered through Whatman filter paper No.1.	
Decolorizing agent	Ethanol 95% vol/ vol.	

Procedure

1. Prepare a heat fixed smear from an 18-24 hour culture.
2. Stain with crystal violet solution for 1-2 minutes.
3. wash the smear in gentle and direct stream of tap water for 2 seconds
4. Rinse with Grams iodine solution and allow the iodine to set for 1 minute.
5. Wash the smear in gentle and indirect stream of tap water for 2 seconds.
6. Blot the smear dry with absorbent paper.
7. Flood with 95% ethanol for 30 seconds with gentle agitation.
8. Counter stain with safranin for 2 minutes
9. Wash the smear until in gentle and indirect stream of water until no color appears.
10. Blot or air dry the slide.
11. Observe / examine under the microscope.

29 Endo-spore staining(Schaeffer and Fulton's method)

1. Air dry or heat fix the bacterial smear with minimum flaming.
2. Place the slide over a beaker of boiling water with the bacterial film on the upper side.
3. Flood the slide with 5% aqueous solution of malachite green and leave it to act for 1 minute, while the water continues to boil.
4. Wash in cold water.

5. Treat with 0.5% safranin.

Endospres appeared green with cells colored red

Enzyme activities

30: Amylase :

The Amylase activity is determined by using starch agar plates. The microorganisms inoculated aseptically kept for incubation for 24 hrs. Following incubation the plates are flooded with grams iodine. The organism showing clear zones were recorded along with zone diameter.

Grams Iodine

31. Protease:

The protease activity is determined by using skimmed milk agar plates. The organisms inoculated aseptically kept for 24 hr incubation. Following the incubation, the plates observed for clear zone formation and the zone diameter recorded.

32 Cellulase:

The cellulose activity is determined by using carboxyl methylcellulose agar plates; the organisms were inoculated aseptically kept for 24 hrs incubation. The plates were flooded with cango-red and the observation for clear zones and the zone diameter were recorded.

Cango red	1g
Distilled water	100ml

33 Lipase:

The lipase activity is determines by using the coconut oil agar plates. Fallowing the incubation the plates observed for halos, the diameters were recorded.

34 Gelatinase :

The gelatinase activity is determined by using 12.5% gelatin in nutrient agar media.

This preparation is autoclaved successively for three consecutive days and then the plates are poured. The organisms are inoculated aseptically , hallowing the incubatin for 24 hrs the plates were observed for clear zones by adding gelatin precipitatin agent.

i.e. 15 .5 Hg Cl_2 in 20 % (vol/vol) concentrated HCl

35 : Chitinase:

10 g of Crude chitin is kept in 50 ml of concentrated HCL filtered through glass wool and over night in the refrigerator. This preparation centrifuged at 7000 rpm for 10 minutes. The pallet formed is purified by centrifuging with distilled water .Out of this 1g is taken and added to the 100ml media preparation as detailed in appendix V. Fallowing incubation for 24 hrs the clear zones are recorded for their diameters. (Kannan, 1996; Wilkie, 1998 ; Plummer, 1988)

CHAPTER IX

BIBLIOGRAPHY

BIBLIOGRAPHY

Abbasi S A & Ramaswamy E V, Solid waste management with earthworms, Discvery Pub House New Delhi, 2001:10-65.

Abbott J & Atkins I, Problems with vermiculture- where to for the industry. in Compost 97: green putreciiable waste management beyond 2000, Paper presented in Proc Compost Conf 14-15 July 1997.

Achithan Nair G, Muftah A, Martia J I & Abdegader K ,Earthworm resources of Benghazi, Libya J Environ. Biol, 26(2) (2005):175-178.

Adjuyigbe C O, Tian G, & Adeye G O, Microcosmic study of soil cosmic soil microorthropods and earthworm interaction in litter decomposition and Nutrient turnover' Nutrient cycling in Agroecosystem, 75(2006): 47-55.

Agenda Notes, 'Meeting of the National Waste Management Council MOEF, Government of India New Delhi,1992.

Aina P O, Contribution of earthworms to porosity and water infiltration in a tropical soil under forest and long-term cultivation. *Pedobiologia.* **26** (1984):131-136.

Albanell E, Plaixats J,& Caberero T, Chemical changes during vermicomposting Eisenia fetida of sheep manure mixed with cotton industrial wastes,J Biology and Fertility of soils, 63(1988):226-269.

Allen O N, Experiments in soil bacteriology, Burges publishing Co. Minneapolice, Minn, 15, (1957):177.

Allevi L, Citteerio B & Ferrovi A, Vermicomposting of Rabit Manure :Modification of microdflora, In Proc Symp of Compost Production quality and use, Organized by the commission of the European Communities, Udine, Italy, 17-18 April, 1987: 115-126.

Alone B Z & Bhide A D, Standard for composts from Municipal Solid Wastes, in proc. of Nat. Sem. on solid waste management held at Bangalore University, 12-14 Dec, (2002):115-119.

Anand Kumar S, Nandini N & Yaasin Md, Ecofriendly aleernative and Organic approach, Kisan World, April, 97 24(4) (1997): 54-56.

Anonymous, Special feature on Clean Technologies', Tech- Monitor, March April (1994):3.

Applehof M, Webster K & Buckerfield J,Vermicomposting in Austerelia and Newzeland, Biocycle, 37(6) (1996): 63-66.

Aquino A M, Almeda D L,Freire L R and Polli H, Earthworm reproduction in manure and sugarcane bagasse, Pesquisa Agropecuria-brasileria,(1994)29:161-168.

Arrhenus O,Influence of soil reaction on Earthworms, Ecology, 2 (1921) :255-7.

Asha Gupta, Evaluation of the waste composting potential of earthworm Octochaetona serrata, J Ecotoxical and Environmental Monitoring, 7(1997) 39-44.

Aston R J,The case for temperature control in vermi culture, Earthworm in Waste Management. The Mague, Netherlands, SPB, Academic publishing (1998):135-43.

Athanasopoulos N, Use of earthworm biotechnology for the management of anaerobically stabilized effluents of dried vine fruit industry, Biotechniology letters, 15 (12) (1993):1281-1286.

Athani S I, Hulamani N C & Shirol A M, Effect of vermicompost on maturity and yield of banana Rajapuri [Musa AB] south Indian Hoticulture, 47(1-6) (1999): 4-7.

Athani S I, Hulamani N C, Hanumanashetti S I, Shirol A M, & Patil S N, Effect of vermicompost on Nutrient status of leaf and yield in banana Cv. Rajapuri [Musa AB], Advances in Plant Science Research 12 (2000): 105-106.

Atlavinyte O & Lugaoskas A, The effect of lumbricidae on soil micro-organism Ann. Zool. Ecol. Anim. Special publ, 4 (1972): 73-80.

Bahl K N, The Indian Zoological memories I. Pheritima ,4th Edition Lucknow Publishing House. Lucknow (1950).

Baig M V V, Amoji S D, Shagoti U M & Birader, V A, Seasonal Variation in Compostabiity and production of vermi protein by *Eisenia fetida,* J of Environ Biol 24(2) (2003):165-171.

Bakhlin P U & Polosky M N, The role of earthworms in structure formations of sood Padolized soils, Pochvovedenie, (1950): 487-491.

Balaji S, Studies on vermicomposting and its influence on plant growth, Masters Dissertation Submitted to Anna University (1994).

Bano K, Kale R.D & Gajanan G N,Culturing of Earthworms, *Eudrilus eugenae* for cast production and assessment of vermicast as biofertiliser, Jr of soil Biol, (2) (1987a) :98-104.

Bano K, Kale R.D & Vijayalakshmi D, production and reproduction trend in a tropical earthworm, *Eudrilus eugenae*, In: Proc.of 5th Indian Symposium of invertebrate reproduction Ed.Palanichami,(1987b): 210-218.

Bareley K Pand Klenning C R, The ccupation of new irrigated lands by Earthworms. Aus Jr of Sci, 26(1994):200.

Barely K P & Jennings A C, earthworms in soil fertility, The influence of earthworms on the available Nitrogen. Aus jr of Ag Res, 10(1961):364-370.

Barely K P & Klenning C R, The Occupation of New Irrigated lands by Earthworms, Aus Jr of Ag Sci, 26 (1964): 290.

Barely K P, Abundunce of earthworms in agricultural lands and their Possible significance in agriculturen: Adv in Agron, 13 (1961) :249-269.

Barley K P, Abundance of earthworms in agriculture lands and their possible significance in agriculture, Adv in Agron, 13(1961):249-269.

Barve J V, Vermiculture in grape cultivation, In Congress Traditional Technology, Key note Paper, I I T Mumbai, Extended Abstracts, 4(1992):10.11 to 10.13.

Bassalik K On silicate decomposition by soil bacteria, Z Grungs-Phyiol, 2(1913):1-32.

Baver L D, Am Soil survey association. Bull. XVI (1935):55.

Bhandari G S, Randhwa M S & Naskina M S, Polysaccharide content of earthworm cast, Curr Sci, 36 (1967): 519:520.

Bhattacharya KK, Mukhopadhyay N, Mukharjee, D & Das S K, Comparative efficiency of improved compst techniques,In Proc int conf.Waste recycling and resource management in developing World, Ed: Jana B B Kalyani University (2002):219-224.

Bhattacharya P, Prospects of organic nutrient resources utilization in India, Indian J of fertilizer, 3(1) (2007): 93-107.

Bhawalkar S V, A promising source of Biofertilizers, A paper read at National Seminar on agricultural biotechnology at Gujrat Agri, Univ. Navasari 7-8 March, (1989).

Bhawalkar U S, Chemical Forming to natural farming : a quick changeover withut loss of yield. L L Somani, K.L Totwal & B.L Basser(eds) proc. Nat. sem. Natural Farming. Rajasthan College of Agriculture, Udaipur,(1992) :79-88.

Bhawalkar V & Bhawalkar U, Vermiculture Biotechnology, BERI Pune, (1992):1-60.

Bhawalkar V S, Vermiculture bioconversion of organic residues, Ph.D Thesis Sub IIT Mumbai, 1995:10-45.

Bhiday M R, Earthworm in Agriculture' Indian Farming, March, (1994):32.

Bhide A D & Sunderesan B B, Solid waste management in Developing Countries INSDOC State of the Art Report Series 2, New Delhi, 1983.

Biawas D, Management of municipal solid status and options, CPCB pub, cups/39/1994-95,(1995).

Bird S & Hale I M, Development of vermicomposting operations at prototype plant, Cunsaltants report to A.E Burges, Waste Control Division, Environment, Canada, (1982) :175.

Black C A, Methods of soil Analysis. Am.Soc. Agron. Madison, Wiscnsin, U.S.A. (1965): 1572.

Bohar R V & Bhide A D, Studies on Amonification during Acrobic Composting' Indian J. of Env. Health, Vol 25(3) (1983): 200-7.

Bouche M B, Strategies lombriciennes, Ecol Bull,(Stockhome)25(1977):122-132.

Bouche M B, Valorisation des dechets organiques parles lombricians. In a Docunentation Franchise, Collection Recherche of Environment 11(1979):384.

Brady N C, The Nature and properties of soil, Ehrasic Publishing House Pvt. Ltd., New Delhi,1988.

Buchanan R E & Gibbons N E, Bergy,s Manuel of dterminative Bacteriology *th Edtion Waverly Press, Inc., Baltimore,1975.

Butt K R, Utilisation of solid paper mill sludge and spent brewery yeast feed for soil dwelling earthworms. Bioresource-Technology, 44(1993):105-107.

Butt R R, The effect of Temperature on the intensive production of *L terristris*, Pedobilologies, 35(4) (1991):257-64.

Chan, K Y and Heena, D P, Occurrence of enchytraeid worms and some properties of their casts I Australian soils under cropping, Aus jr of Res, 33(1995):651-657.

Chang S T, Microbial biotechnology — Integrated studies on utilization of solid organic wastes,Waste Management, Volume 25, 9(2005): 928-939.

Chopra S L & Kanwar J S, Analytical Agricultural Chemistry, Kalyani Publishers, New Delhi. India, 1991:309-310.

Chowdhary P C, Das P K, Ghosh A, Mishra R K & Datta R.K, Recycling technology for sericulture waste as compost, Indian Silk, 32(1993): 28-30.

Constant M, Nareav H, Ferrero G L, & Nyns E J, Biogas use in European Community, London Elsevier applied science publishers 1989.

CPCB, 'Status of solid waste generation and disposal in metro coties (Central urban pollution studies) Cups/pub/1999-2000, 2000.

Crossely D a, Richie D E and Edwards C AIntake and turnover of radioactive cesium By earthworm Lumbricidae, Pedobiologia, 11(1971): 71-76.

Daliwal G S, Sansha G S & Ralha P K , Solid wastes and hazardous substances, Fundamentals of environmental science, Kalyani Publishers New Delhi 1996, 145.

Darwin C R, The Formation of Vegetable Mould through the Action of Worms with Observations on their Habits." Murray, London, 1881.

Dash M C & Senapati B K, Potentiality of Indian earthworms for vermicomposting and Vermifeed, in proceedings of soil biology, symposium, Hissar, India, (1985):61-69.

Dash M C & Senapati B K, The earthworm resource and Vermiculture, zoological survey of India Culcutta. 4(1986):6-8.

Dash M C & Senapati B K, Vermitechnology, An option for organic waste managementin India. Proc of Nat Sem on organic Waste utilization and vermicomposting part, B verms and Vermicomposting, Dash M. C, Senapati B K & Mishra P C (Eds),(1986a) :157-172

Dash M C, Behera N & Dash H K, Earthworm and micro fungal interaction in soil. Proc. soil. Biol. Symp. Hissar. (1985): 77-83.

Dash M C, Senapati B K, Mishra P C , 1980, Oikos.34: 322-325.

Dawsan R C, Earthworm microbiology and formation of water stable aggregates, Proc. Soil,Sci, Soc Am. 12 (1948): 512-16.

Delvi & Seenappa, 2000, 'worm + Waste = Weath', Meantime, April 2000: 34.

Deolkar A V, Mitra A, Bhatachryee P and Chakraborty S, Effects of vermicomposting process on metal content of paper mill solid waste, J Env Sci & Engg. 47 (2). (2005):81-84.

Doekson L & Van Wingerden C G, Notes on the activity of the earthworms in different types of Egyption soil, J App Eol, 1(1964):271-287.

Domínguez J, & Edwards CA,Vermi-composting organic wastes: a review. In: Hanna SHS, Mikhail WZA (eds) Soil zoology for sustainable development in the 21st century, Cairo, (2004):369–395.

Domínguez J, State of the art and new perspectives on vermicomposting research', In: Edwards CA (ed) Earthworm ecology, 2nd edn. CRC, Boca Raton. (2004):401–424.

DubeyV P, Singh S, Duggal B & Kaur, Ravindra, Problems of environmental pollution in Punjab," Man and Development. Vol (18) 3 (1996):37-58.

Dutt A K, Earthworm and soil aggregation, J.AM.Soc, agri,(1948) :40, 407.

Dutta & Mahendra, Waste generation and disposalon land, Engineered land fills, M Dutta (ed.)Nervosa Pub House, New Delhi.(1997):1-13.

***Duweini A K & Ghabbour S I,** Population density and bilmass of earthworms in different types of Egyption soil, J.app. Eol, 2 (1965): 271-287.

Edwards A P & Bremner J M, J of Soil Sci,18(1967): 45-64.

Edwards C A & Thomson A R, Pesticides and soil fauna residue reviews, 45 (1973): 1-79.

Edwards C A & Bater, The use of earthworm in environmental Mnagement, Soil BIol Biochem, vol (24) 12 (1992):1683-1689.

Edwards C A & Bohlen P J, Biology and ecology of earthworms 3rd edn. Chapman and Hill, London, 1996.

Edwards C A & Lofty R E, Biology of earthworms Chappman and Hill, London (1977): 129-47.

Edwards C a, Dominguez T and Neuravser E F, Growth and reproduction of Perionyx excavatus(Perv) (Megacolidae) as factor in organic waste management,Biology and fertility of Soils,27(1998):155-161.

Edwards C A, Earthworm ecology', 2nd edn. CRC Press, Boca Raton, 2004.

Edwards C A, Earthworm' Mc. Grow Hill Encyclopedia, (1995): 81-83.

Edwards C A, Historical Overview of vermicomposting, BIocycle. June (1995): 6-58.

El-Duweini A K & Ghabbour S I, Population density and biomass of earthworms in different types of Egyption soils. Jr of Appl Ecol. 2(1965): 271-287.

Elvira C, Dominguez J, Sampedro L & Mato S, Vermicompoting for paper-pulp industry. Biocycle, 36 (1995): 62-63.

Elvira C, Goicoechea M, Sampedrp L, Mato S & Noglales R, Bioconversion of solid paper mill sludge by earthworms. Biore Tech., 57 (1996): 173-177.

Elvira C, Sampedro L, Benitez E & Nogales R, Vermi-composting of sludges from paper mill and dairy industrie with *Eisenia Andrei* a pilot scale study, Biore Tech., 63 (1998): 205-211.

Elvira C, Sampedro L, Dominguez J & Mato S, Vermicomposting of waste water sludge from paper pulp industrywith nitrogen rich materials.Soil biology and biochemistry, 29 (1997): 759-762.

EPA, Compendium on Solid Waste Management by Vermicomposting [U.S.Env. Prot. Agency, Ohio] (1980): 45.

Fragoso C I, Barosis Z C, Ganjali C, Arteagar & J.C Patron, Relations between earthworms and Soil Organic matter levels in natural and managed ecosystems in the Mexican tropics, Soil organic matter dynamic and sustainability of tropical agriculture int. Ins. Trop. Agri. K.U.Lewen Awiley Sayee Co. Publication, 1993: 231-239.

Franz H & Leitenberger L, Biological Chemical Investigation in to formation of humus through soil animals, Ost Zool, z 1(15) (1948): 498-578.

Gandhi M, Sangawan V, Kapoor K K. & Dilbaghi N, Composting of house hpld waste with and without earthworms, Env and Ecol, 15(2) (1997): 432-434.

Garad N M & Magar S B, Environmental management in sugar – Distillary industry', In Proc. of Int. conf. Cleaner Technology anf Env. Mgt. PEC, Pondichery Jan (2007): 4-6.

Gates G E, Ecology og some earthworms with special reference to seasonal activit. Am.Midl.Nat.66(1961):61-86.

Gautam Bhattacharjee and P. S. Chaudhuri, Cocoon production, morphology, hatching pattern and fecundity in seven tropical earthworm species — a laboratory-based investigation, Jr of Biosci, 27, 3 2002: 283-294.

Geeta, K.S & Reddy J M, Energy transfer by earthworms', In Vermitch worm powered technology, Ins.of Research in Soil Biology and Biotechnology, The New Colege Chennai,(1997): 23-25.

Geset V C A M, Dirven V B E M & Baerselman, R, Influence of environmental conditions on the growth and reproduction of the earthwor Eisenia Andrei in an artificial soil substrate. Pedobiologia, 36 (1992):109-129.

Ghatenkar S D, Green 2000 for Industry through Biotechnoloy: Business opportunities' In Proc. Nat canf Ind and Env, Karad, 28-30 Dec (1999): 3-8.

Ghatenkar S D, Kavian M & Ghatnekar G, Biomanagement of waste through vermiculture, Ecology, Dec95, Vol(10) (1995): 1-7.

Ghilarov M S, On the Interrelations between soil dwelling invertebrates and soil micro organisms, In Soil Organisms, J Doekson and J Vandr Drifteds). North Holland Publishing Co., Holland Ansterdem, 1963:255-259.

Ghildyal B P & Gupta R P, 'Soil structure problems and management,ICAR New Delhi,2002:1-55.

Giraddi R S & Tippanavr, P.S., 'Techniques of biodegradation of organic wastes using earthworms. In: Proc of national seminar on Env Poll,UAS Dharwad. Nov, 2000: 24-25.

Giraddi R S & Kitturmath M S, Biodegradation of some industrial wastes Using Eudrilus eugeniae A MSc Thesis. Sub UAS Dharwad, (2002) :6-20.

Goivindan V S, Vermiculture and vermicomposting In: Ecotechnology for pollution control and environmental management (R.K Trivedy & Avavind Kumar eds.1998.

Gotass H B, Municipal solid waste disposal part V. American city, 77(6) (1962):190.

Grant W C , Status in moisture relationship in earthworm, Ecology, 30 (3) (1995):400-7.

Gubbuck H, Payadas S & Kasha N, Effect of Nitrogen and Farmyare Manure Levels on the stem and finger growth and direction of fruit development of Cavindish and Basari banana clones, Doga Turk Tarun ve armoncilik Degris, 17(1993): 239:253.

Guild W F Mc L, Effecdt of soil type n population . Ann Appl Bil, Vol(35) 2(1948):182-92.

Guild W F Mc L, Earthworm in Agriculture, scot Agri, 30 (4) (1951): 220-230.

Guild W F Mc. L, Earthworm and soil structure In Soil Zoology, D. E. Mc. E Kevan (id) Butter worths, Lndon, (1955): 83-98.

Guild W J Mc L, Effect of soil type on populations, Annals of Applied Biology,1948.

Gunthilingaraj K & Ravignanam T, Vermicomposting of sericultural wastes. Madras Agri jr, 83 (7) (1996) :455-457.

Gupta K, Anamika Tewari, Richa Srivastava, R. C. Murth[1] and **Saurabh Chandra**' Potential of *Eisenia foetida* for Sustainable and Efficient vermicomposting of Fly Ash , Water, Air, & Soil PollutionVolume 163, 1-4 (2005) :293-302.

Haimi J and Hata, Comparision between conventional compost, Biol Fertls, soils, 12 (1987) 126-131.

Hallatt L, Vijoen S A & Reinecke A J, Moisture requirement in the life cycle of Perionyx exavatus. Soil Biol. Biochem, 24(1992) :1333-1340.

Hamilton W E and Dindal D L, Influence of earthworm and leaf litter on edaphic varables in sewage – sludge treated soil micrcosmos. Boil.Fertil. Soils, 7(1989):129-133.

Hartenstein R Neuhauser E F and Kaplan D l, A progress report on the potential use of earthworms in sludge management. In Proc. of 8th Nat Sludge Conf.Florida, Information Transfer Inc., Silver Sprind MD(1979).

Hastevistein R, Neuhauser E F & Kaplan D L, A progress report on the potential use of earthewrm in sludge management .In. Proc. Of the Nat sludge Conf, Florida, Information Transfer Inc, Silver Sprind MD, 1979.

Hemalatha B & Meenabal T, Vermicomposting – Ecofriendly disposal Method for MSW and Vegetable Waste Along with Industrial Effluents' Asian Jr of Microbiol Biotech. Env Sc, Vol 8 No (1) (2006):153-154.

Hopp H, What every gardener should know about earthworms, Garden Way Publishing Company, Vermont, USA 1973.

Hopp H & Hopkins H T, Earthworm as afactoe in the formation of water stable aggregates J.of Soil, Water conservation I (1946):11-13.

Indrabir Singh, Elimentary satstics for medical workers, Jaypee Brothers Medical Pub.Pvt ltd. New Delhi. (1990) 83-93.

IS:1948-1970, Classification and identificastion of soil for general engineering purposes,1970.

IS:2720(Part XVII)- 1986 , Indian Standard Methods of tests for soils,1986.

Ismail,S.A. & Sasi Ravanan , A compendium on Exnora International Chennai, 1993:42-64

Ismail,S.A. and Sasi ravanan , Vermicology (the biology of earthworms) orient lngman, Channai, 1997.

Iswaran, V, A labarotary Handbook for Agricultural Analysis Today and Tommorows Pub. New Delhi. Organic Manures, (1980): 208-217.

Jaashankar S 1994. Stidies of vermicomposting as a Method of Swage sludge Disposal M S Thesis Anna University Madras.

Jain S C,Garg K K & Sharma S, Solid waste management in Chandigarh9A stsus report) pub. NITTTR Chandigarh,(1996):1-40.

Jain S C and Garg K K, Current environmental issues, solid wastes characteristics, manageent and disposal, MOEF Pub, 1997, 75-100.

Jakshe & Jutta, Biological Waste Management, special feature, waste management, Tech Monitor, Jan-Feb, (1995): 25.

Jambekar H A, Use of earthworms as potential source to decompose organic waste, In proc. Nat. Seminar on Organic Farming, MPKV, Agri. College, Pune, (1992): 52-53.

Jamble S V & T Manivanan, Vermiculture Biotechnology for waste management, Green Buisness opportunities Jan- March 1996: 19-20.

Jayant Barve, Vermiculture experience in ngrape cultivation " congress on Traditional Science and Technology, I.I.I Bombay, 4 (1993): 10-13.

Jayesh kumar S, Studies of vermicomposting as a method of sewage sludge disposal, M.Sc. Thesis Anna University, Chennai 1994.

Jeevan Rao K & Ramolakshmi S, 'Vermiculture technology for effective urban waste management, pub in proc of Nat. Sem. on solid waste management held from 12-14, Dec 2002:317-324.

Jeyabal A, and Kuppuswamy G, Reccling of agricultural and agro industrial wastes for production of vermicompost snt its effect on soil fertility management, In Proc. of Sci conf.and general assembly on food security in Harmony with nature. UAS Bangalore December1-4,1998.

Jorge Dominguez, Clive A. Edwards and John Dominguez, The biology and population dynamics of Eudrilus eugeniae (Kinberg) (Oligochaeta) in cattle waste solids , Pedobiologia,45,(4),2001:341-353.

Joshi N V & Kelekar B B, The role of earthworms in soil fertility, Ind J. of Agri.Sci, 22, (1952)189-96.

Joshi S N, Worm composting Inora News letter, Pune, vol. 1(1) (1997) 2.

Jowell M.E and Lewis,H.B, The occurrence of lichenase in the digestive tract of the invertebrates, The J f Biological Chemistry, VOL.XXXIII NO 1(1917)161-167.

Julka J M, Earthworm resources in Indiaand their utilization in vermiculture, In: Earthworm resources and vermiculture Zoological survey of India,Culcutta,(1993): 51-56.

Kalam A, Tah J & Mukharjee A K, Pesticide effect on microbial Population and soil enzymes activities during vermicomposting of agricultural waste J. Environ. Biol, 25(2) (2004): 201-208.

Kale R D & Krishnamurthy R V, Distribution of earthworms in relation to soil conditions in Bangalore. In Soil Biology and Ecology in India(eds) Edwards C A & Veeresh G K, UAS, Tech Ser, 22 (1978): 63-69.

Kale R D, Bano K, Vinyak & Bhagyaraj D J, Suitability of neem cake as an additive in earthworm feed and its influence n the establishment of microflora. Jr of Soil Biol and Ecol, 6 (1986): 98-103.

Kale R D, Vermiculture Scope for the New Biotechnology, In Earthworm Resoure\ces and Vermiculture Zoological Survey of India, Culcutta, 1993.

Kale R D & Bano K, Earthworm cultivation and culturing techniques for production of vee comp. 83 E UAS Mysore Jrl Agri Sci, 22 (1988):339-344.

Kale R D & Krishnamoorthy R V, Enrichmnt of soil fertility by earthworm's activity, In: progress in soil Biology and Ecology in India Ed. Veeresh, G.K., UAS Tech series, 37(1981): 64-68.

Kale R D, Bano K & Selcia J and Bhagyaraj D.J., Do earthworms cause damage to paddy crops?Mysore J of Agri Sci,23 (1989):370-373.

Kale R D, Bano K, Srinivas M N & Bhagyaraj D J, influence of worm Cast (Vermicmcomp E. USA 83) on the growth and Micro Colonization of two ornamental plants, South Indian Horticulture, 35(5) (1987): 433-437.

Kale R D, Mallesh B C, Kubra Bano, Bhagyara D J, Influence of vermicompost application on available micronutrients and selected microbial populations in paddy field, Soil Biol Biochem, 24(12) (1992):1317-1320.

Kale R D, Vermicomposting of waste materials, Earthworm Cindrella of Organic Farming, Prism Book Pvt Ltd, (1994): 47.

Kale, R.D. and Sunitha, N.S. (1992), 'Utilization of earthworms in recycling of hosehold refuse, Proceedings of workshop on biogas slurry utilization, October 1992 CORT.

Kalpana D L, The biochemistry of sludge decomposition ,sludge stabilization and humification, In: Utilisation of soil organismsin sludge management. Ed. Hartenstein, R, National Technical Information Services, Springfield, Virginia, (1978):76-78.

Kamala Bai S & Suresh K T, Organic industry – History and its perspective' Kisan World June (2000): 44.

Kannan N, Laborartary Manuel in General Microbiology, Palani Paramount Pub Palani India 1996: 30 – 90.

Karmarz P, Cadmium and Zinc accumulationand its demographic effects in invertebrates, In Kammenga J, Laskowski R E(eds) Demography in ecotoxicology. Wiley, New York, (2000):91-111.

Katharisasn M K, Varsatality of press mud which boosts the sugar cane, Sista Sugar Journal, (1991) :17- 23.

Kaushal B R and S. P. S. Bisht Growth and cocoon production of *Drawidia nepalensis* (Oligochaeta Biology and Fertility of Soils 14, (1992): 205-212.

Kavian M F. & Ghatnekar S D, Biomanagement of dairy effluent using culture of red earthworms (*L rubellus*), IJEP,11(9) (1996) :680-682.

Kevin R, Mervi V & Tiasto Siren, Population and behaviourlevel responses of arable soil earthworms to boardmill sludge application, Biol Fert Soils, 42(2005):163-167.

Khalil M A, Abdel-Latief H M , Bayoumi B M & Van Straalen,N M,Analysis of separate and combined effects of heavy metal on growth of Aporrectodea caliginosa using the toxic approach. Appl Soil Ecol, 4(1996): 213-219.

Khanna P and S N Kaul, Industrial effluent treatment, Jr IAEM 23(1996):153-155.

Kitturmath M S, Shankarmath M & Narayan, Vermicompost, Kisan world Jan (2007):26.

Kolher R C, Effects of in-situ vermiculture on Mulberry & cacoon yeeld. Ms Thesis sub to UASD No. 3559, 1995.

***Kollmansperger F**, Lumbricidae of humid and arid regions there effects on soil fertility VI cong. Int. Sci, Rapp. C,(1956) :293-7.

Kozolovskaya I S & Zhdannikova E N, Joint action of earthworm and microflora in forest soils, Dokl. Akad.Nauk.SSSR.139 (1961):470-473.

Kretzschmar A, Burrowing ability of the earthworm Aporrectodea longa limited by soil compaction and water potential, Biology and Fertility of Soils, 11(1991): 48-51.

Kretzschmar A. (ed.) Proceedings of 4th International Symposium on Earthworm Ecology. Soil Biol Biochem, 24(12) (1992) :1193-1773.

Kuhad R.C. & Singh A, Ligno cellulose Bio Technology: Current & Future Prospects in Critical Reviews in Bio technology, 13(2) (1993) :151-172.

Kumar P K R, Singh A, & Schugerl K, Formation of acetivc acid from cellulse materials by Fusarium oxysporum, Appl. Microbial Biotechnol, 34 (1991): 570.

Lane A G, Anaerobic digestion of organic peel. Food Techn Aust, 36 (1984) 125.

Lane A G, Methane from an aerobic digestion of fruit and vegetable processing wastes' Food Tech Aust, 30 (1979): 201.

Laxmanan P S G, Vermi-compost increase yield of sugarcane, Kisan World, Feb,2006:19

Lee K E, Earthworms - Their Ecology and Relationships with Soils and Land Use, Academic Press, Sydney, (1985): 411.

Lee K E & Foster R C, Soil fauna and soil structure, Aust Jr Soil Sci, 29 (1991): 745-775.

Levele J M, Sow B and Schaefer R,.The geophagus earthworm community in the Lampto Savanna: Nitche part timing utilization of soil nutritive resources. In Dindahd(Ed), Syracuse, Washington DC, (1980):653-672.

Loquet M, Batnagar J, Bouch M.B & Ruvelle J, Estimation of ecological influence by earthworms on microorganisms, 17 (1977): 400-417.

***Lunt H A & Jacobson G M**, The chemical Composition of earthworm casts , Soil Sci , 58 (1994): 367.

Madge D S, Field and labarotary studies on the activities of two species of tropical earthworms, Pedabiologia, (1969): 188-214.

Madhu Arora, Biological control of environmental pollution, Anmol pub Pvt ltd, New Delhi,(1998):200-208.

Madhukeshwar S S, Anil K N,Andani Gowda, Laxminarayan M T & Shreeram shtty T.A, Organic farming ans sustainable agriculture, A paper presented at National Seminar UAE Dharwad. October 1996: 9-11.

Mahajan B B, Methods in biostastics, Japee Brothers Medical Pub pvt ltd,New Delhi Ed,2004:130-156.

Male S R , Revathi G and Soappan A R , Waste to wealth through sugar industry , Co-operative sugar,29(1998):623-624.

Marinissen,J.C.Y and Ruiter,P.C.D., Consortium of earthworms to carbon and nitrogen ccling in agro ecosystems, AGrill.Ecosyst.Environ,47(1993)::59-74.

Marinssen J C Y and Boch F V D, Colonization of New Habitats of earthworms.Oecologia.91(1992):371-376.

Martin J P & Waksman S A, Soil Sci, 50 (1940) 29.

McColl, H.P., Hart, P.B.S. and Cook, F.J. Influence of earthworms on some soil chemical and physical properties, and the growth of ryegrass on a soil after topsoil stripping - a pot experiment. *New Zealand Journal of Agricultural Research.* **25** (1982): 239-243.

Meenatchi R Kambekar D N, Earthworms for pest and disease management, Agrobios news letter Vol VI No 1 (2007):17.

Mitchell M J & Horner S C, Decomposition process in sewage sludge and sludge amended soils in soil biology as related to land use practices, Proc. 7th Intn. Colloq, Soil. Zool, New York, (Ed.) D.L. Dindal, office of pesticides and Toxic substances, EPA, Washington D C, (1980): 129-138.

Mitchell M J,Role of invertebrates and microorganisms in sludge decomposition. In Utilisation of soil organisms in sludge management(ed) R. Hartenstein. Nat Tech. Inf. service Pb 286932, Springefield Virginia. ,(1978):35-38

.**Miyo B and Giranval P H,** Earthworms as indicators of forest site quality Soil Biol.Biochem.29(1997):323-328.

Mohammad S J, Earthworms and vermiculture, A introduction ,Pub Zoological survey of India, in earthworms resources and Vermiculture, (1993):1-5.

Mohan Rajesh V & Vignesh M, Co generation of Electricity, A boon to sugar factories Kisan World Feb 2006: 20.

Monson C C, Damodharan G, Senthilkumar & Kanakasbai V, Compoting of vegetable waste using In vessel and vermibeds',Proc of Int conf on cleaner tech and Env Mgt,PEC Pondichery, India Jan 4-6,2007.

Moody S A, Briones M J I, Pierce T G & Dighton J, Selective consumption of decomposing wheat straw by earthworms. Soil Biol Biochem 28(1995): 533–537.

Moore A W, Azolla: Biology and agronomic Significance Biol Rev, (35) (1969): 17-34.

Morgan J E & A J Morgan, Ingesta and feaces of ecophysiologically different earthworm species, Soil Biol Biochem, 24(3) (1992): 1691-1697.

Morne R, Benitez E, Melgar R, Polo A, Gomez M & Nogales R, Vermicomposting an alternative for reusing byproducts from the olive industry, Fresnius Enviromnmental Bulletin, 9 (2000): 1-8.

Munnoli P M , Arora J K & Sharma S K, Impact of vermi processing on soil characteristics' Jr. Ind. Pollution Control 18(1): (2002) 87-92.

Munnoli P M , Arora J K & Sharma S K, Organic waste management through vermiculture: A case study of Pepsi Foods Channoo Punjab. In waste resource recycling in the developing world Ed. Jana.BB, Banarjee R.D, Guterstam Band Heeb J,(2000) :203-208.

Muthukumarswamy R, Revathi G, Murthy V, Mala S R, Vedivelu M & Solayappan A R, An alternative carrier material for bio fertilizers, Co-operative sugar, 28 (1997): 677-680.

Muyima N, Renecke A J & Viljoen S A, Moisture requirement for *D veneta*, A candidate for vermi-composting, Soil Biol, Biochem, vol 26 (8) (1994) 973-76.

Nagaraj H C, studies on the use of organic wastes in cultivation of azolla (azolla pinnate R.Br.) M Sc. thesis, sub to UAS Dharwad , no. 1459, 1981.

Nahamani J & Lavelle P, Effect of metal pollution on soil macro invertebrate burrow systems, Biol Fertil Soils ,42(2005): 31-39.

Nahamani J , Copowiez Y & Lavelle P, Effect of metal pollution on soil macro faunain a grass land of Northern France, Eur J Soil Biol, 38 (2005): 297-300.

Nair P M , Future of Biomethanogenesis and Bioconversion, Encology, Vol 9,No 9,Feb (1995):19-20.

Nair P M, Future of Biomethanisation and Bio- conversion, Encology, vol.9, NO.9, Feb 1995:18-20.

Nand K, Evaluation of fruit & vegetable processing wastes for bioconversion to methane by an aerobic digestion, wealth from waste, TERI pub, (1995): 227-43.

Nand Kishore T C, Studies on urban solid wastes composting, MSc Thesis Sub to UAS Dharwad. No 1465 (1980).

Narayan J, Vermicomposting of Biodegradable wastes collected from Kuvempu University campus using local and exotic species of earthworm, proc Nat Cont Ind and Env, Karad, Dec 28-30 1999: 417-419.

National Waste Management council,[NWMC]–Meeting of NWMC MOEF Agenda notes GOI, (1992).

National Waste Management council,[NWMC]–Report of the sub group II (1990), Industrial waste management, MOEF, 1990: 1-5.

Nayak & Rath L K, Vermiculture and its Application, Kisan World, Dec 1996: 61-2.

Needham A E, Composnents of Nitrogeneous extreta in the earthworms. Lumbricus terristris and Eisenia fetida Savingl) J of Exp. Bio. 34(1957):425-446.

Needhran A E, Components of the nitrogeneous excreta in the Lubricus terristris and *Eisinea foetida* (savigny), J of Exp Biol, 34 (1957): 425-446.

Neeraj Kumar, Singh R, Misra B N & Chandra K.G, Influence of organic manure, Bi fertilizer and nitrogen levels on Nutrient Uptake, Soil properties and yield of Rice, Indian J of Fertilizer, 3(7)2007): 45-50.

Nijhawan S D & Kanwar J S, Physico chemical properties of earthworm castings and there effect on the soil, Indian J agri Sci, 22 (1952): 357-73.

Nijhawan S D & Kanwar J S, Physico chemical properties f earthworm castings and their effect on productivity of soil, I J Agr Sci,22 (1952):357-373.

Nobel J C, Garden W T & Kieing C R, The influence of earthworm on development of mats of organic matter under irrigated pasture in southern Australia in plant nutrients and soil fertility proceedings of international Gresol congress in Queenland, Australia, 1970.

Nowak W Kozlowska & Kotowski, Comparative study f effectsof mineral fertilizers and bio-humas on yield and quality of raddish (Raphanus sativus). In Science for horticulture practice, ed. A Filipowicz, Proc. conf. Lubin, Poland, (1995): 595-599.

Orozco F H, Cegara J,Trujillo L M & Roig A, Vermicomposting of coffee pulpusing the earthworm, *Eisenia fetida* effects on C and N contents and the availability of nutrients, Biology and fertility iof soils,22 (1996):162-166.

Osamu K & Carl W H, Bio mass Hand Book Composting, Garden and Breach Science Pub, 1987: 302-306.

Pagaria, P & Totwat, K.L, Effects of pressmud and spent wash in integratin with spent wash with phosphogypsum on metallic cation build up in the calcareous sodic soils, J ind society of soil science 55(1),(2007):52-57

Parle J N, A microbiological study of earthworm casts, Journal of general microbiology, 31(1963): 13-22.

Parle J N , Activities of micro organisms in soil and influence of these on the soil fauna' Ph.D Thesis London (1959).

Parthasarathi K & L S Ranganathan , Aging effect of microbial populations, enzyme activities and N P K contents in the soil in the soil wormcasts of *Lampito mauriti*(Kinberg) and *Eudrilus eugeniae*(Kinberg)- Pollution Research, 20 (1) (2001):53-57.

Parthasarathi K, Aging of pressmud vermicast of *Lampito mauritti* (KInberg) and *Eudrilus eugeniae* (Kinberg) –Reduction in microbial Population and activity, J Environ Biol, 27(2) (2006): 221-223.

Patnaik B R, Performance of bio-fertilizers from food wastes, Congress on Traditinal Science and Technology of India, IIT Mumbai, 14 (1992): 10-26.

Patra A K, Wastes as pollution environmental studies(man and environment). Kalyani publishers, New Delhi, 1997: 234.

Pawar R B, Dynamics of earthworm-soil-plant-relationship in semi arid tropics. Ph>D University of Agricultural Sciences, UAS Dharwad 1996.

Payal Garg, Asha Gupta and Santosh Satya' Vermicomposting* of different types of waste using *Eisenia fetida*: A comparative study, Bioresource Technology ,97, (3) (2006):391-395.

***Pearlkamp P K,** Trans, 4th Int Congr Soil Sci,(1950) 2:50.

Pelczar M.J, E.C.S Chanm and N.R. Krieg ., 'Microbiology', Microbiology of foods,Tata Mc Graw Hill Pub Co.Ltd., New Delhi, India, 1993:618-642,

Perdersen J C & Hendriksen, Effect of passage through the intestine tract of dteritivore earthworms (*lumbricus spp*)on the number of selective gram negative and total bacteria, Biol Fertil Soils, 16 (1993): 227-232.

Petrov B C, The active reaction of soil pH as a factor, in the distribution of earthworms, Zool. Jour, 25(1) (1946) 107-10.

Pevey H S, Rowe D R & Tchabonoglous G, Environmental Engineering Mc Graw Hill International Edition ,(1985) :573-593.

Phillips E P, Earthworms Plants and Soil reaction, Ecology, (1923):4-89.

Piccone G, B Biosoil, G Deluca & Minelli L, Vermicomposting of different organic wastes' Compost production and use symposium, udine Italy, 17-19, April 1986, 818-21.

Plummer T D, An Introduction to Practical Biochemistry, Tata Mc Geaw Hill Pub Co. New Delhi,1988.

***Ponomareva S I,** The Influence of the earthworms in the creation of stable structure in a sad pedolised soil, Ztrudy po, chu Inst Dokuchaeva, 41(1953): 304-78.

***Ponomareva S I** , Soil macro and micro organisms and there role in increasing fertility, vitroya, zoologischoskaa,Konferrenceiva litvskri, SSR, (1962): 97-98.

Prabhakar K, Shaik Alawdeen, Srinivasan K & Mohamad Shamsudin, Enrichment of soil by the activities of earthworm Octochaetona serrata J.Ecobiol,18(2) (2006):121-125.

Prabodhini J, Recycling of Kitchen waste into vermicompost, Indian Farming, 213 (12) (1994): 34.

Pramanik, P G.K. Ghosh P.K. Ghosa and P. Banik Changes in organic – C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants ,Bioresource Technology ,Volume 98, (13), September 2007: 2485-2494.

Pscst report, Solid waste management, Chandigarh vermiculture biotechnology' Punjab state council for science snd Technology pub 1993.

Pulakeshi M B, Amoji S, D Shagoti U. M, Influence f organic wastes and Seasonal environmental factors of growth and reproduction of *Eisinea fetida*, J.Environ. Biol, 24(1) (2003): 81-89.

Pulakeshi M B, Amoji S D, Shagoti U M & Biradar VA, Seasonal Variatins in compostability and production of Vermiprotien By *Eisenia fetida,* J Environ Biol, 24(2) (2003):165-171.

Pumnia B C, Siol mechanics and foundation Engineering Standard Book House New Delhi, 2001: 1-121.

Raghunandan S, Vinod Singh A, Ravi Teja P & Avinash M, Bioconversion of organic wastes in to high value chemicals',In Proc. of Int. conf. Cleaner Technology anf Env. Mgt. PEC, Pondichery, Jan 4-6:(2007).

Rajeshwaran, K, Food processing Industries In India opportunities and Challenges, Kisan Word July, 2006: 9.

Rajeshwari K V, Pant D C, Lata K, Ram Lata K Raman P, Biomethanisation of vegetable market waste. In Proc of Int Symp Entrnet APRON Bandung Indonasia, 23-24, March 1998.

Rajiv K. Sinha, Sunil Herat, Sunita Agarwal, Ravi Asadi and Emilio Carretero, 2002),Vermiculture and waste management: study of action of earthworms *Eisenia fetida, Eudrilus euginae* and *Perionyx excavatus* on biodegradation of some community wastes in India and Australia, The Environmentalist, 22, (3), 2002:371-376.

Ramachandran C, Food preferences by earthworm's In: Vermitech worm powered technology, Institute of research in soil biology, The New College, Chennai, (1997): 20-22.

Ramachndran S , Lignin Bio conversion, in proc. Nat. Sem. On Biotechnology of Lignin Degradation, I.V.R.I. Izatnagar, 1989.

Ramalingam R & Thilagar M, Bio-Conversion of Agro waste sugarcane trash using an Indian Epigeic earthworm *Perionyx excavatus* (perier) Ind J Environ & Ecoplan, 3(3) (2000): 447-452.

Ramanathan K M, Organic farming for sustanabiity, J.Of Indian Society of soil Science 54(4) (2006),418-425.

Ramaswamy P P, Recycling of wastes as manures-potentials and prospects : In summer school on Productivity management for sustainable agriculture. ICAR New Delhi, 1998:83-91.

Ramesh Babu G, Vermiculture for soil enrichment, Ksan World, 23(1995): 40.

Ranade D R & R V Gadre, Microbilogical aspectsof anaerobic digestion labarotary Manuel, Maharashtra association for the cultivation, Science Research Unstitute, Pune, (1988).

Ranganath Reddy R L, Ramakrishnan Parama V R & Bhargana M M V & Radha Kale, Vermicomposting a method of urban Solid waste management ' Pub proc. Of Of Sem on Solid waste management held from, 12-14 Dec 2002: 98-99.

Ranganathan V Christopher, Vermicomposting, Kisan World, Sep 1996: 17.

Rangaswami G, Diseases of crop lants in India, Third edition Prentice Hall of India pub 1988: 518.

Rao C S, Environmental pollution control Engineering, Wiley Eastern Ltd, 1991:24-47.

Raut R S, Mane S S & Kohite O D, Seminar paer highlights Research, Vertmiculture Technology and Utility of vermicompost, Inora News letter, June 1997, vol(3) (1997): 2.

Ravichandran C, Edwin Chandrashekharan & Christy Priyadarshi F, Vermicompost from different solid wastes using treated dairy effent IJEP Vol 21, No 6 (2001): 530-542.

Ravichandran C, yeshoniketan S, Nagarajan G & Shaikh Kadhan J, Vermicomposting of Municipal Solid wastes in tiruchinapalli City' Pub. In Proc. Of Sem on Solid waste management held from 12-14 Dec 2002:100-103.

Reddy M V & Pasha M, Influence of rainfall,temperature and some soil physico chemical variables on seasonal population structureand vertical distribution of earthwormsin two semi arid tropical grasslands soils,International J Biomet,37 (1993):19-26.

Richerdson H C, The nitrogen cycle ingrassland soils with reference of Rothmstad Park Expt, J of agri Sci, Comb,28 (1938): 73-121.

Riggle D, Vermicompost research and education. Biocycle,39 (5) (1998): 54-56.

Rodale J I and Staff of Organic Gardening and Farming Magzine, the complete book of composting Rodale Books, inc Emmaus, PENNA, (1967): 874-923.

Roig M G, Martin Rodrigues M J & Cachazal M, Principles of biotechnology treatment of industrial wastes, in Cri Res of biotech, 13(2) (1993): 99-115.

Ross K J & Cairns A, Effect of earthworms and rye grass on respiratory and enzyme activitiesof soil. Soil Biol, Biochem, 14 (1982): 583-586.

Roy G K, Biogas from solid wastes, Science Reporter, June,(1989): 268.

Roy D K & Sngh B P, Effect of level and time of nitrogen application with or without vermicompost on yield, yield attributes and quality of malt barley, Indian Journal of Agronomy 51(1) (2006): 40-42.

Roy G K, Biogas from solid wastes. Science reporter, June 1989: 268.

Roy L Danahue, Raymond W Miller & John C. Shickluna, An introduction to soil and plants growth, pub. Prentice Hall of India Pvt Ltd, New Delhi, 1990: 117-169.

Ruikar S K, Some useful information about vermicompost, Inora, oct 97, vol (4) (1997):2.

Russel E J ,Soil conditions and plant growth ' Lngman 8 th Edition 1950.

Saha N, Bhatacharya K K & Mukhopadhya N, Two step composting for the management of lignocellulosic materials,In Waste recycling R esource Management in the developing world,Ed.Jana,B.BKalyani University India international ecological society , Switzerland ,(2000): 213-218.

Sahu S S, Mukharjee D & Panda R N, Improvement of soil environment by vermicompost' In proc. Of int. conf. Waste Recycling and Resource Management in the Developing Wrld, Ecological Engineering approach Edited by B.B. Jana University of Kalyani, (2002) :209-212.

Sannigrahi A K, Vermicomposting f Biodegradable House Refuses for Reducing Municipallty Soild Waste load Pub in Proc of Nat. sem on solid waste management held from 12-14 Dec 2002: 107-111.

Satchell J E, Earthworm Microbiology, In earthworm Ecology . Chappman and Hill London, (1983): 351-364.

Satchell J E, (ed) (1983), Earthworm Ecology: From Darwin to Vermiculture, Chapman and Hall, London 1983.

Satish Gupte, Short Textbook of Medical labarotary for Technicians, Japee Brothers Medical Pub. New Delhi, 1998: 83-122.

Scheu S, Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). Biol Fertl Soils 3(1987) :230-234.

Selviraj N Anitha P & Mohan L, Modern organic farming for horticulture crops, Horticulture Research Station, TNAU, Ooty, (2005).

Senapati B K, Earthworm Resources and Vermiculture, Zoological Survey of India, (1993): 97-99.

Senapati B K,Vermi Technology An Option for Recycling of Cellulose Waste in India, In New Trends in Biotechnology,(1989) 347-357.

Senappa C, Jaganath Rao C B & Radha D Kale, Conversion of distillery waste into organic manure using earthworm *Eudrilus eugenae* (kinb) 3rd. int. conf, Appropriate waste, EERI, management technology for developing countries, Nagapur, Feb 25-26, (1995) :1165-1170.

Sengar R S & Salini Gupta, Biofertilisers: Boon for Farmers" Kisan World, July2006: 47.

Senthil Kumar M, Sivalingam PN & Sungathi, A, Vermitech' Kisan world, March 2000: 57-58.

Sevenson J E, The distribution of Lumbricidae in an area of pennine Mooveland, J.Animal Ecol, 26(2) 1957: 409.

Shahul hameed P, Gokulakrishnan K, Rajasekaran M, Thangavel K & Raja P,Vermicomposting of solid wastes from tanning Industry, Pub in Proc. of Nat. Sem on Solid waste management held from 12-14 Dec 2002:104-106.

Sharma S K, Application of Vermiculture in the management of Municipal waste and agro residues, A paper pub in proc of Nal. Sem on SWM held in Bangalore University 13-14 Dec 2002: 112-114.

Shaw C & Pawluk S, The development of soil structure by Octolasion tyrtaeum, *Aporrectodea turgida* and *Lumbricus terrestris* in parent materials belonging to difficult textural classes, Pedobiologia, 29(1986) :327-339.

Shrikhande J C & pathak A N, A comparative study of Physico chemical characters of the castings of different insects, Indian J.of Agri Sci, 21(1951): 401-7.

Shweta & Mamata, Qualitative variation of vermicompost prepared from different substrates and by different earthworms, VII national Symposium of Soil Biology and Ecology, GKVK Bangalore,November 2001: 7-9.

Singh J, Habitat pretturner of selected Indian earthworm species and their efficiency in reduction of organic material, Soil Biol Biochem, 29(1997): 585-588

Singh S & Sinha S KFarmers frien gives rich manure Science Reporter, 38 (2001):28-29.

Singh, N.B., Dissertation on Development of process package for organic solid waste management through vermiculture Biotechnology, in organic waste generating industries in Punjab (1997) :91-93.

Sinha N B, Hajra J N & Nandi N, Use of white Rot fungi for rapid composting of soft, hard wood dusts and rice straw in the single step composting system' pub. in Int. Conf Proc Waste Recycling and Resource Mgt, Iin the developing World, (2000): 234-243.

Sneh Goyal S.K. Dhull and K.K. Kapoor, Chemical and biological changes during composting of different organic wastes and assessment of compost maturity, Biores Techn, 96(14) Sept(2005) :1584-1591

Spinks, 1980.[Sighted in Roig, 1993]

Springett, J.A., Gray, R.A.J. and Reid, J.B. (1992). Effect of introducing earthworms into horticultural land previously denuded of earthworms. Soil Biol Biochem. **24(12):** 1615-1622.

Staff H, Foliage litter turnover and earthworm populations in three beech forest of contrasting soil and vegetation types, Oecologia,72 (1987):58-64.

***Stephenson J**, The Oligochaeta, Oxford University Press, 1930: 978.

Stewart V I, Scullion J, Salih R O & Al-Bakri K H, Earthworms and Structure Rehabilitation in Subsoils and in Topsoils Affected by Opencast Mining for Coal, Biological Agriculture and Horticulture, 5(1988): 325-338.

Stockdill S J & Lossens G G,The role of earthworms in pasture production and moisture conservation proceedings of Newzeland Grassland Association, (1966) :168-183.

Subaiah B V & Asija G L, A rapid procedure for the estimation of available Ntrogen in soils, Curr Sci, (25) (1959): 259-260.

Subba Rao N S, Soil Microbiology, fourth Ed. Oxford and IBH Pub House New Delhi, India, 1999: 252-270.

Sudhakar V Kulkarni, Methods of vermicomposting under open field conditions and management of natural enemies of esarthworms. Nov 1997, UAS Dharwad. MSc. TH. 5326 ,1997:10-45.

Sundararajan R, Jayanthi S, Sadasivan P, Anaerobic Digestion for the recovery of energy from Kitchen refuse, in IJEH, Vol.38 (1) (1996): 7-12.

Sundarvadivel S, Sultan Ismail & Ismail S, Efficiency of a biological filter unit in the treatment of distillery effluent, Journal of ecotoxicology and environmental monitoring, 5(1995):125-129.

Surekha R & Mahadev Kumar R, Organic and potential organic sources for system of rice cultivation, Kisan world August (2007):26.

Surya Prasad S & Raghavendra Rao B, Industrial Environmental Management –New Directions, In Proc Int Conf On Cleaner Technologies and Env Management PEC, Puduchery, India Jan 4-6,2007: 542-547.

***Tamil Selvi** ,Vermicomposting of municipal soild waste with coir pith and pngamea leaf letter . M.Sc Dissertation Sub Bharatidasan University Tiruchanapalli, 1996.

Tandon H L S, Methods of Analysis of soils, plants, water and fertilizers , Fertiliser development, 1993.

Tchobanoglous G, Theisen H and Vigil S A, Intergated solid waste management, Tata Mc Graw Hill Int Eds. New Delhi, India, (1993): 611-705.

Teotia S P, Dubey F L & McCalla T M, Effect of soluble mulching on number and activity of earthworms, Nebraska Agric, Exp Sta Res Bull,(20) (1950):165.

Tewatia R K, Kalve S P & Choudhary, R.S, Role of Biofertilizers in Indian Agriculture, Ind J of Fert, 3(1) (2007):111-118.

Thomas Sonia and Trivedy R.K., 2002 'Earthworm Biotechnology for waste management and crop improvement A review of research ' Pub. in proc. of nat. Sem on soild waste management held on 12-14 Dec 2002:120-123.

Tiwari S C , Tiwari B K, and Mishra R R, Microbial populations,enzyme activitiesand nitrogen phosphorus-potassium enrichment in earthworm castsand in the surrounding soilof apineapple plantation, Biol Fertl.Soils, 8(1989):178-182.

Tracey MV, Cellulase and Chitinase of earthworms. Nature Lond, 167 (1951) :776.

Trivedi R P & Raj G,199 Solid waste pollution ,Encyclopedia of environmental Science, Vol –24, Akash deep Publishing House, New Delhi 1992.

Trivedy,R K (2002) National seminar on solid waste management Held from 13-15 Dec. 2002

Ushakumari K, Prabhukumari P & Padmaja P, Efficiency of vermicomposrt on Yield and quality of banana (AB) Cv Njalipooran, South Indian Hortculture, 45 (3-4) (1997):158 -160.

Uthai P A, Acceleration of press mud decomposition by microbial inoculation fr quality product, M.Sc Thesis Sub to UASB No 6263, 1997.

Van Rhee J A, A study on effect of earthworms on orchard productivity, *Pedobiologia.* 17(1977):107-114.

Varamali Patel, Vermiculture expression insugar cane cultivation congress on Traditional Science and Technology, II.T Bombay, 4(1993) 10-13.

Venkatesh Patil P.B, Athani, S.I., Reddy P.N, Patil M P, & Dharmatti P R, quality Characteristics of grapes berries as influenced by insitu vermiculture and vermicompost, Advances in Agri Z Research In India, 10 (1998) :129-132.

Venkatesh, Effect of vermicompost on Soil Composition, growth, Yield and Quality of Thomson Seedless grapes (*Vitis Vinifera L*)M.Sc Thesis Sub To UAE Dharwad,1995.

Venkatesh, Patil P.B, Sudhirkumar K & Kotikal, Y K, Influencce of in situ vermiculture and vermicompost on yield and yield attributes of grapes, Advances in Agriculture Research in India, 8 (1997) :53-56.

Venkateswarah D, composting the decomposed, Indian Silk, 34 (1995): 5-6.

Virendra Kumar & Mishra B, Effect of two types of pressmud cake ongrowth of rice maize and soil properties, journal Indian Soc Soil Sciences, 39(1991): 109-113.

Viswanath P, Sunitradevi S & Nand K 'Anaerobic digestion of fruit & vegetable wastes for biogas production, Biores Tech,(1992): 40-43

Wallwork J A, Earthworms Biology' 1st edition. Arnold – Heinemann, London, 1984

Weiss B and Trespendorfer I, Influence of earthworm on microbial activity- labarotary and outdoor experiments, Mitteilungender Deutschen Bodenkundlichen, Gesellschaft, 69 (1993):155-158.

Whiston R A & Seal K J, The occurrence of cellulose ih the earthworm *Eisenia fetida*',Biological wastes, In Gtrren tecnoogy unit (1988): 85.

White S, Vermiculture Bioconversion in India worm Digest, June 1996: 65.

Wilkie F Harrigan, Labarotatry methods in food microbiology, 3rd ed. Acad press pub.Londn, 1998.

***World Health Organisastion** , 1971 Slolid waste disposal and control report, Geneva.

Yaduvanshi N P S, Yadav & Singh, Economy in Fertiliser Nitrogen by its integrated application with sulphitation filter cake in sugar cane Biological wastes, 32(1) (1990) :75-79.

Zajonc I & Sidar, 'use of some wastes for vermin compost preparation and their influence on growth and reproduction of the earthworm *Eisenia fetida.* Polnohospodarstvo,36 (1990):742-752.

Zende G K, Problems of organic farming and utility of vermicompost INORA News Letter Jan 1997, 1(2)1.

Zhang B G, Rouland C,Lattaud G and Levelle P, Activities of digestve enzymes in gut of the tropical earthwormPontoscolex corethrurus, Eur J Sil Biol 29(1) (1993): 7-11.

Zhang H & Schrader S, Earthworm effects on selected physical and chemical properties of soil aggregates, Biol and Fert of Soils, 15(1993): 229-234.

Zucconi F & Betroldi M De, Compost specification for the production and characterization of compost, From MSW, proceedings of Symposium organized by Europian Community R & D, Italy 17-19 April 1986:31-51.

*-Refferences not seen in original

Presentations and publications:

Relevant presentations:

1. **Munnoli, P M and Saroj Bhosle**, Solid Waste Disposal in Goa, National Seminar on Solid Waste Management organized by Department of Environmental Science Bangalore University 12th to 14th December 2002. pp. 9-13.

2. **Munnoli, P M and Saroj Bhosle** , Solid Waste: Problems and Recovery Options, presented at the state level Seminar on Goa's Urban scenario organized by Department of Geography Smt. Parvatibai Chowgule Cultural Foundation College of Arts and Science. Margao Goa on 19th April 2003 P-24.

3. **Munnoli, P M and Saroj Bhosle** , Rural Waste Management: A case study of Marcornem Village" presented at the National Seminar on New Horizons in Environmental Science and Engineering, organized by Department of Environmental Science Bangalore University, Bangalore from 17th to 19th November 2004.

4. **Munnoli, P M and Saroj Bhosle**, Implementation of Vermi culture Bio – Technology in Goa for Rural up liftment", Prsented at the UGC National Conference on Bio -Technology for Agriculture and Rural Development held at Matoshri Bayabai Shripatrao Kadam Kanya Mahavidyalaya, Kadegaon, District – Sangli, Maharastra. From 29th to 30th December 2004.

5. **Munnoli, P M and Saroj Bhosle**,Bio fertilizer and community development A case study of Majorda village at the National Seminar on Recent trends in Plant Sciences' Sponsored by UGC Organised by Smt. Parvatibai Chowgule Cultural Foundation College of Arts and ScienceMargao Goa from 23 to 24 th Feb,2005.

6. **Munnoli, P M and Saroj Bhosle,** Rural waste management through training in vermi compost technology: a case study of Malcornem village; Goa', National Meeting on Vocational Education and Training for sustainable rural livelihood conducted jointly by Dr. Harisingh gaur university, Sagar Madhya Pradesh, and Pandit Sundarlal Sharma Institute of Vocational Education, Bhopal. (A constituent of NCERT New Delhi) at Dr. Harisingh Gaur University, Sagar Madhya Pradesh from 23rd to 25th February 2005.

7. **Munnoli, P M and Saroj Bhosle,** Vermi-composting A sustainable Technology for Rural Development " At the National Seminar On Fronteers in Bioferrtilisers and Bio Pesticides , Organised By the P.G Center of Babasaheb Ambedkar University Osmanabad From February 19 to 21st 2006.

8. **Munnoli, P M and Saroj Bhosle,** Vermi-composting : A Clean Technology for the treatment of press mud: A case study of Sanjeevani sugar factory Dayanand Nagar Goa, Presented at the International Conference On Cleaner Technologies and Environmental Management. Organised by Pondichery Engineering College Puduchery, INDIA.Held from 4 – 6th Janaury (2007): 247-253.

9. **Munnoli, P M and Saroj Bhosle,** Stainable industrial development through microbial technology, (2008) Accepted for presentation in the National Convention of Environmental Engineers Institution of Engineers Ranchi Jarkhand, from 12-14 (2008).

Published in the proceedings of National and International Seminars/Conferences

1. **Munnoli P M and Saroj Bhosle**,(2002). Solid Waste Disposal in Goa. In proc. Nat. Sem. on Solid Waste Management organized by Department of Environmental Science Bangalore University December 2002. pp. 9-13

2. **Munnoli, P. M., and Saroj Bhosle**.,2007. Vermicomposting a clean technology for the treatment of pressmud: A case study of Sanjeevani co operative sugar facftory dayanand Nagar Goa. In Proc of Int. conf on cleaner Technologies and Environmentalmanagement, Organised b pondichery Engg College Puducheri, India from Jan 4 to 6 2007.pp.247-253.

3. **Munnoli,P M, Saroj Bhosle**., 2008. Sustainable industrial development through microbial technology, IN 23rd Nat. Conv. Of Env Engg Nat Sem.Env Economics and Clean Technologies, January 2008.pp 49-63.

Published in Journals

1. **Munnoli, P.M and Saroj Bhosle**, (2008). Soil aggregation by vermicompost of press mud. Curr Sci. 95(11):1533-1535.

2. **Munnoli P M and Saroj Bhosle**, (2009). Effect of soil cow dung proportion of vermi-composting, Journal of Scientific and Industrial Research, (68):57-60.