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Potential Utilization of Jatropha Oil Meal as Feed Substrate for Earthworm Eudrilus Eugeniae for Production of Vermicompost

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ABSTRACT

Jatropha curcas seeds have been in use for the production of biodiesel as an ecofriendly fuel. But during biodiesel production from 100 kgs of Jatropha seeds only 30 kgs of oil is produced, while 70 kgs of de-oiled cake is generated as a byproduct. This oil meal contains toxic substances like curcin, toxalbumin and phorebolic acid which makes it unsuitable as an animal feed. It can be either used for biogas production or vermicomposting as can be seen from volatile solids of total solids content of 87.93%. So feasibility of this oil meal as a substrate for vermicomposting using earthworm *Eudrilus eugeniae* has been studied in detail. Original C/N ratio of the jatropha oil meal was around 42.94 while the raw waste mixture was around 38.57% after mixing with cattle dung and soil, which reduced to 12.6 after vermicomposting, which is an indicator of efficient worm activity. Volatile Solids reduction of 66.19% was observed which indicates efficient worm activity. Results showed good quality vermicompost production with efficient water holding capacity of 72.4100% and porosity of 78.2191%. Bulk density depicted 1.1052 g/cm^3 indicating good quality suitable for efficient root penetration. Scanning Electron Microscopy and microbiological analysis confirmed the maturity of the vermicompost.

Introduction

Galloping speed with which the modern world is moving in all directions to achieve comfortable life style, is also creating numerous environmental and health problems. Major problem facing the world is shortage of fossil fuel, because of the agricultural demand and industrial demand due to uncontrolled population growth. To fulfill the agricultural demand, more amount of fertilizers are required. Due to this demand, financial burden on the government increases. Chemical fertilizers though help in increasing the yield also reduces the soil fertility in long term. There is an urgent need for exploring and exploiting new alternate sources of organic fertilizers. In rural areas of developing countries various degradable organic biomass residues are available in plenty, their disposal in an easier and ecofriendly technique becomes a great task. But these organic biomasses have a very good potential for vermicomposting.

Vermicomposting is a well-known composting technique for stabilizing different degradable organic wastes (Hanc and Vasak, 2015; Lim, et al, 2016; Wu et al, 2014). During vermicomposting, earthworms maintain an aerobic condition in the organic wastes, ingest solids and convert them into vermicast. Vermicomposting has an advantage of reducing the total volume and particle size of the biomass waste and side by side increases its relative manurial value. Furthermore, the availability of macronutrients and micronutrients is generally higher in vermicompost than in the traditional compost and inorganic fertilizer, indicating that vermicompost is a better supplement to improve and stimulate plant growth (Lim et al, 2015a).

Many waste biomasses have been successfully converted by vermicomposting. In early years, water hyacinth, a weed was used for vermicomposting. This weed is one of the most productive and hardy of all the weeds and no attempt to control this weed or destroy it by chemical, biological, mechanical or hybrid means have ever achieved total success (Reddy and Smith, 1987; Ramaswamy, 1997; Abbasi et al, 1997).

Vermicomposting is an ecofriendly and sustainable technique which helps in reducing tonnes of waste into beautiful nutrient rich manure. This is an attractive route to utilize the biomass which can provide good healthy environment and also waste disposal with value added byproduct recovery can be achieved.

Among many organic wastes, Jatropha oil cake has been identified as an efficient substrate for biogas production (Pal et al, 2015). Vermicomposting of this waste is also possible as it contains 87.93% volatile solids of total solids. Jatropha seeds have been in use for the production of biodiesel as an alternative fuel. But during biodiesel production from 100 kgs of Jatropha seeds only 30 kgs of oil is produced while 70 kgs of de-oiled cake is generated as byproduct (Sichuan, P. R., 1979). This crop grows healthily in barren lands with minimum water and can thrive on marginal agricultural land where many trees and crops will not grow or would produce only slow growth yields. Jatropha cultivation provides benefits to local communities. More and more farmers are now cultivating Jatropha to fulfil the increased market demand.

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This cake is toxic in nature as it contains few toxic materials like curcin, toxalbumin and phorebolic acid which makes it unsuitable as an animal feed (Amaral et al, 1988).

One dangerous weed – parthenium also contains toxic substances like parthenin and phenol. This weed has been successfully vermicomposted. It is also further reported that the toxin contents are also eradicated during vermicomposting (Rajiv, et al, 2013). Most unexpected waste like night soil could be vermicomposted in combination with cattle dung and soil (Yadav et al, 2010). It gives an insight and also helps in searching for degradable and at the same time problematic organic matters. Almost any agricultural, urban or industrial organic materials can be used for vermicomposting provided it does not contain any serious toxic material that can harm the earthworms as per the literature. But it has been proved beyond doubt that combination of organic matter with cattle dung, soil and nitrogen rich materials can be vermicomposted without any problem. Various studies have shown that vermicomposting of organic wastes enhance organic matter stabilization (Neuhauser, et al, 1988; Frederickson, et al, 1997) and results in a value added product rich in nutrients in easily available forms for plants.

Very sturdy and hard leaves of coconut tree could be vermicomposted, coconut leaves contain high percentage of lignin thus making it hard and sturdy (CPCRI Report, 2009). Coconut leaves contain high lignin concentration of 31.0% and resists natural decomposition. But it has been efficiently vermicomposted. Highly cellulosic organic waste biomass like banana tree peels and paper & pulp industry sludge have been vermicomposted by mixing it with high nitrogen content wastes (Kibatu et al, 2015; Kavitha et al, 2010).

Combined vermicomposting of crop residues and cattle dung with *Eisenia foetida* has been reported (Bansal and Kapoor, 2000). A nitrogen deficient pulp and paper industry sludge was vermicomposted by mixing it with nitrogen rich wastes like pig manure, poultry droppings and sewage sludge (Elvira et al, 1997). It is further reported that individually the sludge from pulp and paper industry does not support earthworm growth and activity, but nitrogen rich waste amendment with the waste results in better bioconversion.

Vermicomposting process also depends on the earthworm species utilized. Weed parthenium is very dangerous and creates severe allergy in humans and cannot be treated easily. This weed grows rampantly everywhere and creates health problems. Its disposal poses great problems. Report on its use in biogas plant has been reported (Banerjee, 1987). But recently, this weed has been vermicomposted using earthworms *Eudrilus eugeniae*. This is one of the best solutions to get rid of this notorious weed which crept into India from USA. Problem with this weed is that it contains a toxin called parthenin along with phenols. This toxin can be eradicated by vermicomposting technique when mixed with optimum quantity of cattle dung. Report further says that around 30 – 35% organic carbon and 32 – 48% phenol contents are reduced (Rajiv et al, 2013). Vermicompost plays a major role in improving the growth and yield of different agricultural products. Vermicomposting is an appropriate alternative for the safe, hygienic and cost effective disposal of many degradable waste materials. Cellulose rich banana tree peels have been effectively vermicomposted. In addition *Eudrilus eugeniae*, was used successfully in vermicomposting of various agricultural wastes, such as soy bean husk (Lim et al., 2011), rice residues (Shak et al., 2014), palm oil mill effluent (Lim et al., 2012), empty fruit bunches (Lim et al., 2015b) and other wastes.

Many animal wastes like pig, cattle dung, poultry droppings and even human wastes are reported to have been converted successfully into nutrient rich manure by vermicomposting (Chan, 1988; Manuel Aira et al, 2002; Giraddi and Meenatchi, 2008; Yadav Kunwar, 2010). Thus, disease creating pathogens are also eradicated.

Some special wastes like arcanut and coconut leaves, which are very hard and rich in lignin have been tried for vermicomposting. It is reported that pre-decomposed arcanut and coconut leaves in combination with cattle dung could be efficiently vermicomposted (Chaudappa et al, 1999 and CPCRI report, 2009). It was also found that vermicomposting could be used as an efficient technology to convert empty fruit bunches into nutrient rich organic fertilizers, if the wastes were mixed with cattle dung in an appropriate ratio. Guar gum industry waste also had the potential utilization in vermicomposting (Suthar, 2006). Rubber tree leaves, which are very thick and contains sticky latex has also been used for vermicomposting (Chaudhari et al, 2001). Some plants which grow only in some specialized areas like cocoa plants, its leaves have also been utilized for vermicomposting.

Based on the detailed literature survey it was decided to study the feasibility of vermicomposting of Jatropha curcas oil cake in combination with cattle dung and soil.

Materials and Method

Required Jatropha oil cake was brought from an unit at Chattisgarh State, India, where biodiesel is being manufactured. Raw material procured was subjected to routine physico-chemical parameters and heavy metal analysis as per the standard methods (Clesceri et al, 2012).

Cattle dung from a local cattle shed was brought and dried in shade before its use. The reason for drying the cattle dung is to prevent any harm to earthworms due to the heat generation in the fresh cattle dung. Moreover, fresh dung invite termites, worms and insects. Required soil sample was brought from a nursery. Table 1 depicts the physico – chemical characteristics of raw Jatropha oil meal. Figure 1 shows the raw Jatropha oil meal. Initially only Jatropha oil meal was tried for vermicomposting but it did not support earthworm activity. So cattle dung and soil amendment were done and it was pre-decomposed and then was subjected to vermicomposting.

Table 1. Characteristics of Raw Jatropha Oil Meal.

Parameters	Values
pH	5.64 - 6.0
Conductivity (μs/cm^2)	568
Alkalinity/Acidity as $CaCO_3$	2300
Chloride as Cl (mg/l)	19.63
Sodium as Na (mg/l)	1.4
Potassium as K (mg/l)	0.60
Sulphate as SO_4(mg/l)	77.4
% Organic carbon	47.2
% Nitrogen as N	1.102

% Phosphate as PO_4	0.54
% Total Solids	92.8
% Total Volatile Solids	81.6
% Volatile Solids of Total Solids	87.93
% Moisture	7.2
C/N Ratio	42.94
heavy metals (in mg/l)	
Cadmium	0.0001
Cobalt	0.0116
Chromium	0.0433
Copper	0.5161
Iron	10.9026
Manganese	1.2513
Nickel	0.1084
Lead	0.0299
Zinc	2.0439

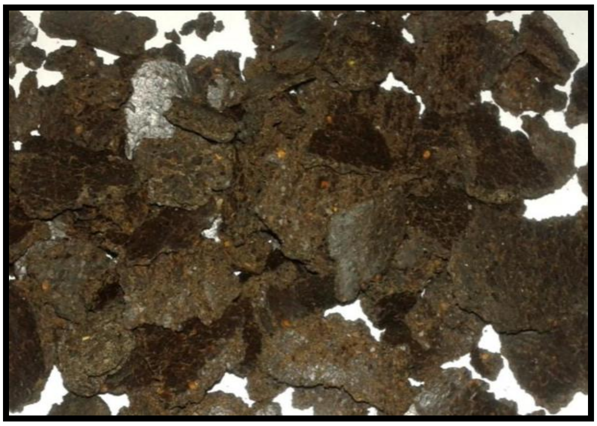

Figure 1. Raw Jatropha Oil Meal.

Earthworm species of *Eudrilus eugeniae* required for the experimental work was procured from vermiculture centre of Science for Villages at Wardha, Maharashtra, India. Jatropha oil meal, soil and dried cattle dung were mixed in 1:1:1 ratio and left for predecomposing. Table 2 shows the characteristics of this mixture and Table 3 shows the characteristics of shade dried cattle dung. After pre-decomposing the mixture for approximately ten days, the mixture was then transferred to earthenware vermi beds and a total of fifteen earthworm species of *Eudrilus eugeniae* were introduced into the experimental pots having an average weight of 3-5 gms. Beds were covered with mulch and daily water was sprinkled to keep it moist and maintain the moisture content in the required level. The vermibeds were kept in shade preventing direct sunlight and rain water entry. A total of 70 days later, good granular, dark brown fluffy vermicompost was noticed with an earthy smell indicating complete vermicomposting of the Jatropha oil meal. (Figure 2)

Table 2. Characteristics of the raw substrate mixture (jatropha : cattle dung : soil).

Parameters	**Values**
pH	6.89
Conductivity (μs/cm^2)	1260
Alkalinity/Acidity as $CaCO_3$	2560
Chloride as Cl (mg/l)	156
Sodium as Na (mg/l)	4.1
Potassium as K (mg/l)	0.62
Sulphate as SO_4(mg/l)	36.0
% Organic Carbon	33.17
% Nitrogen as N	0.86
% Phosphate as PO_4	0.67
% Total Solids	88.8
% Total Volatile Solids	57.2
% Volatile Solids of Total Solids	64.41
% Moisture	11.2
% Ash content	42.8
C/N Ratio	38.57
Heavy Metals (in mg/l)	
Cadmium	0.0365
Cobalt	0.942
Chromium	0.6926
Copper	3.282
Iron	319.00
Manganese	4.262
Nickel	0.0630
Lead	0.0282
Zinc	1.3162

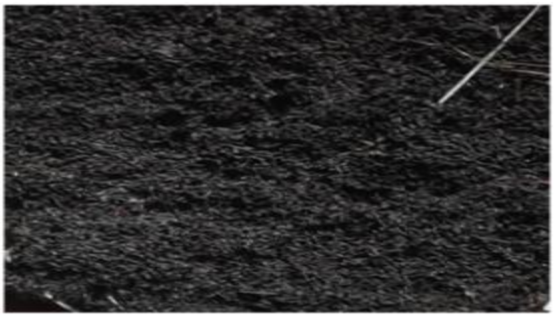

Figure 2. General Morphology of Vermicompost of Jatropha Oil Meal.

Table 3. Characteristics of the Shade Dried Cowdung

Parameters	**Values**
pH	7.5
Conductivity (μs/cm^2)	2200
Alkalinity/Acidity as $CaCO_3$	4382
Chloride as Cl (mg/l)	236
Sodium as Na (mg/l)	5.2
Potassium as K (mg/l)	0.29
Sulphate as SO_4(mg/l)	16.20
% Organic Carbon	39.353
% Nitrogen as N	0.92
% Phosphate as PO_4	0.63
% Total Solids	93.0
% Total Volatile Solids	67.85
% Volatile Solids of Total Solids	72.96
% Moisture	7.0
% Ash content	27.04
C/N Ratio	42.77
heavy metals (in mg/l)	
Cadmium	1.28
Cobalt	0.032
Chromium	18.00
Copper	35.35
Iron	1284.00
Manganese	116.00
Nickel	0.005
Lead	2.2
Zinc	141.6

Figure 3. Healthy Earthworm species *Eudrilus eugeniae.*

This vermicompost was also subjected to routine physico chemical parameters. Few important physical parameters of importance in vermicomposting viz porosity, bulk density and water holding capacity were also estimated to assess the quality of vermicompost (Mckenizie et al, 2004). Detail physico chemical parameters of vermicompost including heavy metals are indicated in Table 4 while Table 5 indicates important, physical parameters and their comparison with the soil sample. Volatile Solids reduction was also calculated as per the Standard Method (Anaerobic Sludge Digestion, 1986).

Table 4. Characteristics of vermicompost.

Parameters	**Values**
pH	7.09
Conductivity (μs/cm^2)	1665
Alkalinity/Acidity as $CaCO_3$	1310
Chloride as Cl (mg/l)	186
Sodium as Na (mg/l)	11.64
Potassium as K (mg/l)	1.26
Sulphate as SO_4(mg/l)	16.2
% Organic Carbon	14.25
% Nitrogen as N	1.5
% Phosphate as PO_4	0.81
% Total Solids	85.86
% Total Volatile Solids	32.6
% Volatile Solids of Total Solids	37.96
% Moisture	14.14
% Ash content	53.26
C/N Ratio	12.6
Heavy Metals (in mg/l)	
Cadmium	0.0475
Cobalt	1.1054
Chromium	0.8590
Copper	4.7720
Iron	453.6726
Manganese	24.2503
Nickel	1.1722
Lead	0.05288
Zinc	2.4249

*Volatile Solids Reduction (in %) - - - 66.19

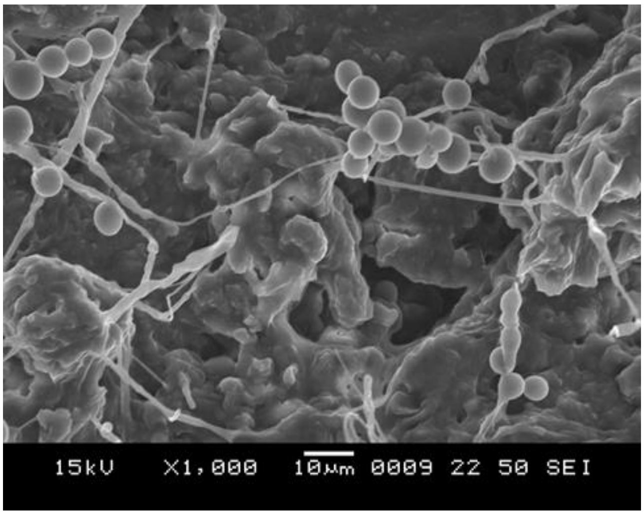

Figure 4. SEM of Raw Jatropha Oil Meal.

Table 5. Physical parameters of vermicompost and soil.

Parameters	Sample	
	Soil	Compost
Bulk density (in g/cm^3)	1.6966	1.1052
Porosity (in %)	46.8815	78.2191
Water Holding Capacity (in %)	38.7447	72.4100

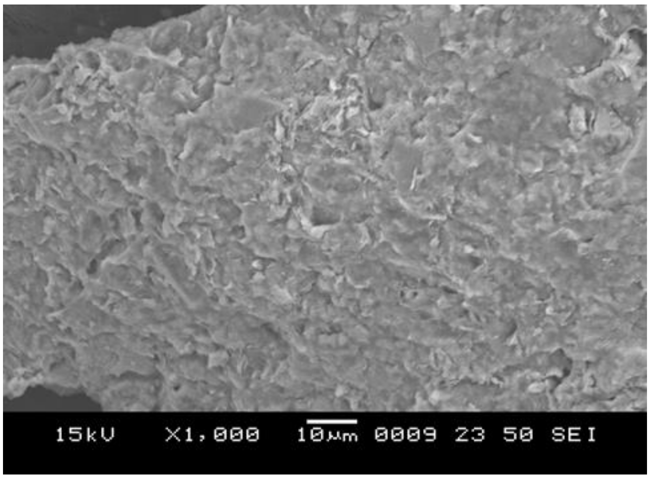

Figure 5. SEM of Vermicomposted Jatropha Oil Meal.

SEM studies of vermicompost were carried out to evaluate the maturity of the compost and its granulation pattern. Scanning Electron Microscopy [Model, JEOL, JSM 6380A, USA] was used. Samples were prepared by standard procedure (Bond et al, 1995), where a drop of sterile distilled water on a clean sterile glass coverslip was taken and then a smear was prepared on the coverslip and fixed with the help of flame. After fixing the coverslip was dipped overnight in Millonig's buffer. The cover slip was later removed from the buffer solution and dehydrated in different grades of alcohol. Coverslip was then air dried and cleaned with tissue paper. Finally the samples were ultra sound de-agglomerated in etalon for ten minutes gold splattered and then subjected to SEM analysis.

To further evaluate the extent of maturation of vermicompost, an aqueous suspension of the vermicompost was prepared for microbial analysis. It was allowed to stand for 24 hours and then it was filtered through Whatman Filter Paper No. 42. Filtered sample solution was subjected to routine microbial test. [Table 6]

Table 6.Microbial Quality of the vermicompost.

S. No.	Microbes	c.f.u/ml
1.	Total count of bacteria	0.01 x 10^8
2.	Total count of fungi	Nil
3.	Total Actinomycetes	0.01 x 10^7

* No Pathogenic bacteria found.

Results and Discussion

Jatropha oil meal is an unconventional solid waste, which is generated during biodiesel production. Management of the solid waste has become a major problem. This cake is toxic in nature as it contains few toxic substances like curcin, toxalbumin and phorebolic acid which makes it unsuitable as an animal feed. If this waste is disposed on land then it may lead to ground and surface water pollution, pathogens and odour nuisance etc. unscientific disposal of large quantity of jatropha oil meal may cause energy, economical and environmental problems.

As this waste is rich in organic matter to the tune of 81.6% and also mineral elements, it can potentially be utilized in restoring soil fertility. Recycling of jatropha oil meal by vermicomposting can mitigate environmental hazards resulting from simple disposal on land and also value added byproduct recovery rich in plant nutrients can be harvested. This solid waste needs an eco-friendly disposal method. On analysis of this solid waste as indicated in Table 1, the presence of very efficient quantities of volatile solids of total solids i.e. around 87.93% indicates that this waste oil meal is highly biodegradable in nature and suitable for both biogas production and vermicomposting.

The original pH which was in acidic range became almost neutral after addition of dried cattle dung and soil. Increase in pH during vermicomposting was due to mineralization of nitrogen and phosphorus into nitrites / nitrates and orthophosphates. If original pH is on higher side say 10.0 or so, then there appears a decrease in the pH during vermicomposting which may be due to CO_2 and organic acid production by microbial metabolism.

During vermicomposting C/N ratio of the original substrate decreased from 38.57 to 12.6. This indicates efficient bioconversion. Standard C/N ratio of 12 - 18 is generally considered as "A" grade compost (Phirke et al, 2004). In this study, the C/N ratio falls well within this ratio. It clearly indicates that Jatropha Oil Cake is a very good substrate for vermicomposting except that it required longer period of seventy days apart from initial ten days of pre-decomposing. Reductions in C/N ratio was not in accordance with the reduction in carbon, it may be due to the loss of nitrogen from the vermibed during the degradation process.

A wide range of C/N ratio for mature compost has been reported in the literature (Suthar, 2008). This value depends upon the type of waste and its degradation rate and the fate of carbon and nitrogen during the vermicomposting. This indicates that C/N ratio cannot be used as maturity criterion for the vermicomposting. In the initial stages of vermicomposting, nitrogen might be lost in the form of ammonia, so initial stages of vermicomposting nitrogen concentration reduces. It has been reported in literature that microorganisms during composting require 30 parts of carbon and one part of nitrogen in the balanced substrate (Suthar, et al, 2012). Organic carbon content of the vermicomposted Jatropha Oil meal was around 18.9% indicating efficient quality of manure.

If the nitrogen concentration is on very higher side then excess nitrogen will be lost in the form of odourous ammonia and at low C/N ratio, nitrogen loss would be high and it can be up to 60%. The loss of carbon as CO_2 through microbial respiration and simultaneously addition of nitrogen by worms in the form of mucus and nitrogenous excretory material lowers the C/N ratio of the substrate (Senesei, 1989). C/N ratio is considered as a parameter to establish the degree of maturity of compost and its agronomic quality (Joseph, 1999). Decline of C/N ratio to less than 20 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes (Hait et al, 2011).

But on the contrary some literature reports 1.8 - 2.5 times higher nitrogen content during vermicomposting of the waste organics (Garg et al, 2006; Kaushik et al, 2004). This situation was encountered in our earlier studies on nitrogen rich organic waste - soya sludge, wherein we observed nitrogen increase from original 2.8% to 4.2% in the final vermicompost (Das et al, 2015). It is likely that the nitrogen content in the final vermicompost depends upon the amount of nitrogen and C/N ratio of the original feed material.

Nitrogen content in the vermicompost of Jatropha curcas was around 1.5% compared to original of 0.86% in the raw feed mixture. It is generally observed that during bioconversion / biodegradation process nitrogen content and other nutrients like phosphorus and potassium show increasing trend due to mineralization of the organic waste. Though nutrients are required for assimilation of earthworms during the vermicomposting, the quantity required is very minimum as compared to the total quantity present in the original feed substrate. But, if expressed on a dry weight basis (Total Solids) the nutrients concentration showed an increase due to oxidation of organic matter during vermicomposting. Optimal C/N ratio is often dependent on the initial feed stock. Similar trend was also reported in literature (Martin et al, 1992; Nogales et al, 1999). But in general during vermicomposting of organic wastes increase in nitrogen content is observed.

Electrical Conductivity of raw jatropha oil meal is around 568 $\mu S/cm^2$. Literature also indicates increase in Electrical Conductivity during vermicomposting. Increase in electrical conductivity to the tune of 1665 $\mu S/cm^2$ is due to the soluble salt levels resulting from mineralization action of earthworms and microorganisms present in the gut of earthworms. Also because of those microorganisms which are already present in the original organic substrate. Gradual decrease in the organic carbon content in the raw material also seems to be responsible for the increase in the Electrical Conductivity.

Percent volatile solids reduction observed is around 66.19% in the final vermicompost. This indicates good consumption of the substrate and its conversion by the earthworms. Increase in heavy metal concentration was also observed. Figure 3 shows healthy and mature earthworm *Eudrilus eugeniae*.

Final vermicompost was subjected to Scanning Electron Microscopy (SEM) studies to evaluate the maturity. For comparative studies, raw Jatropha oil meal was also subjected to SEM. Figure 4 shows the SEM of raw while the Figure 5 shows the final vermicompost prepared out of Jatropha oil meal. It is clearly seen in the SEM of raw jatropha oil meal, haphazardly arranged cellulosic fibres with yeast cells having no binary fusion property and are just budding. pH on acidic side in Jatropha oil meal is very congineal / favourable for multiplication of yeast cells and other soil microorganisms. Moreover, sugar needed as a substrate for yeast cell is present in Jatropha oil seed kernel in enough quantity. These yeast cells encourage aerobic fermentation process which is good for vermicomposting activity. This activity is a combination of soil based beneficial microorganisms including phototrophic bacteria, yeast cells and naturally occurring soil and cattle dung bacteria.

While SEM of vermicompost prepared out of Jatropha oil meal (Figure 5) shows uniformly digested cellulosic fibres expressing good uniform matrix. Earthworms with the help of microorganisms present in the gut gradually degrade the raw material. In this vermiconversion process, microorganisms including yeast cells present in the raw substrate also work hand in hand with the gut bacteria. Final vermicomopst depicts more uniform matrix depicting that the substrate has been efficiently vermicomposted and resulted in a manure with good porosity and water holding capacity indicating increase in surface area. Final vermicompost had 14.14% moisture, which is an indicator of good quality.

Important physical parameters in vermicomposting are depicted in Table 5. Bulk density is normal and is around $1.1052 g/cm^3$, which indicates that root growth and penetration into soil would be very good. When bulk density of soil increases to around 1.6 g/cm^3 and more, it tends to restrict the root growth. But in the present situation, the bulk density of the vermicompost remained well within congineal range. Likewise, porosity and water holding capacity also showed an increase and were 78.2191% and 72.4100%, respectively.

To further confirm the quality of final compost, it was subjected to microbial analysis. The results are indicated in Table 6. It is very clear from the results that vermicomposting has helped in the complete eradication of pathogenic bacteria. Thus, it helps in easier handling of the final compost.

Conclusion

The experiments conducted have indicated the potential of vermicomposting technology for processing of Jatropha oil meal. Pre-decomposing in combination with dry cattle dung is necessary to make the substrate palatable to earthworms. Vermicomposting has also resulted in complete eradication of pathogenic organisms. Original C/N ratio of 38.57 decreased to 12.6 indicating good bioconversion of Jatropha oil meal. Percent volatile solids reduction observed is around 66.19% which is a very good reduction. Organic carbon reduced to less than 20% indicating good bioconversion.

Scanning electron microscopy showed good uniform matrix resulting in large surface area thereby water holding capacity and porosity also increased. Final vermicompost depicted dark brown colored uniform granules with pleasant earthy smell.

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