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THREE-TIER VERMICULTURE BIOTECHNOLOGY TO TREAT BIO- SOLID WASTES INTO BIO-CLEAN PROBIOTICS FOR AGRICULTURE

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Abstract

The Biotechnology Resource Centre of India has developed and pioneered a prototype model of 'Three tier vermiculture biotechnology' to convert organic solid wastes produced by industries into useful plant probiotics. Over the last fifteen years, this innovative biotechnology has been successfully implemented in industries like food processing, solvent extraction [soybean oil], gelatine, pulp and paper, textile, antibiotics, dairy, municipal sewage and slaughter houses. The protocol consists of combined use of specific earthworm sp., microorganisms and enzymes, which can treat and convert organic wastes into bio-clean plant probiotics. These bio-safe probiotics can be distributed by a concerned industry as bio-ethical soil conditioners to enrich the fertility.

In the first phase, solid wastes are subjected to 4-6 days of enzymatic and microbial treatment. Type of enzyme is selected on the basis of waste characteristics such as nitro, lipid or cellulose-based. Set of enzymes used are like, proteases, lipases, cellulases, xylanases, amylases etc. Microbial bio-cultures are brigades of degraders and deodorizers. Their tailor made combination will be selected for specific bio-solid wastes.

Thereafter, enzymatically biodegraded and deodorized product in its second phase is subjected to the earthworm degradation in series of smooth-walled rectangular tanks having capacity to accommodate daily generation of bio-solid wastes of a specific unit along with sterilized bedding material. The complete biodegradation of waste into humus-like biomass is obtained after 18-20 days of vermiculture treatment.

In the third phase, to this biomass, selected nitrogen fixers and phosphate solubilizers are inoculated and kept for 5 days. In fine, within 30-32 days obnoxious sludge gets converted into clean and pathogen free plant probiotics. All the three phases are semi-automated and hardly 4 operators are required to manage a plant. Such a plant requires an area of about 1000 m^2 to treat 10 tonnes of solid wastes every day. The set up cost is just about £38,000 to 40,000. This is $1/5^{th}$ of conventional chemical ETP. As the energy bill is bare minimum the running costs are almost negligible.

In fine, the paper describes detailed mathematical prototype model to convert organic bio-solids produced by industries into value added bio-safe probiotics for agriculture.

Key words

Bio-safe probiotics, deodorizers, earthworms, enzymes, microbial brigades, nitrogen fixers, phosphate solubilizers, three-tier vermiculture biotechnology (TTVB)

Introduction

Huge amounts of potentially toxic industrial effluents in all forms are being perpetually released into the surroundings triggering a catastrophic environmental imbalance the world over. In India, this effluent disposal is addressed as a serious concern and the Pollution Control Boards set up by various State governments have laid down stringent norms in an effort to curb this menace. Nevertheless, on the ground level, most of the industries find difficult to meet these standards. A cluster of industrial units in any industrial development corporation may alone generate approximately 500 thousand tonnes or more of bio-solid wastes everyday.

Effluent generation is an off shoot of any production unit and getting rid of these diverse effluents is never a smooth sail as it also imparts an additional financial burden. Thus such industries require innovative technology which can successfully help them in pollution abatement. Additionally, if it helps in conversion of effluents into value added marketable products; it would be a bonus for the industry, by generating an additional income.

In India, maximizing the food production along with maintaining the efficiency of reuse and recycling of industrial effluents (which are misplaced resources) still remains a dual challenge for most of the industries. Thus, the need of the hour is to identify potential means for sustainable development in agriculture as well as in industrial effluent management and resource recovery practices.

This paper illustrates a mathematical prototype model for '**Three-Tier Vermiculture Biotechnology' (TTVB),** indigenously developed by Biotechnology Resource Centre (BRC), Mumbai, India. It focuses on the resourceful conversion of organic bio-solid effluents produced by industries into valuable plant probiotics.

Characteristics of the industrial bio-solids

Every industry produces distinct effluent bio-solids which need to be carefully examined before their treatment.

Bio-solid sludge generated from any industry can be broadly generalized as:

1. Protein-based bio-solids: gelatine manufacturing plants, slaughter houses, tannery, dairy etc.

2. Lipid-based bio-solids: solvent (Soybean oil) extraction, edible oil refineries etc.

3. Cellulose-based bio-solids: paper-mills, textile mills, sugar mills etc.

4. Mixed bio-solids: These are usually a combination of proteins, lipids and cellulose e.g. effluents from antibiotics units, food processing units, municipal solid wastes etc.

All these bio-solids when left untreated turn into breeding grounds for pathogens and emanate an obnoxious odour causing serious environmental hazards. These effluents have to be subjected to primary and secondary treatments in the Effluent Treatment Plant (ETP) before they are released into the environment. Even these partially degraded residues released near water bodies and dumping sites cause immense pollution. They even damage the public sewer system by their corrosive action and silting. (Kavian and Ghatnekar 1992).

'Three-Tier Vermiculture Biotechnology' (TTVB)

With an objective to address this grave environmental issue, BRC has devised TTVB. It is a novel technology of treatment of bio-solid effluents from diverse industries by subjecting them to a synergistic activity of specific types of microbial enzymes, select species of earthworms and a brigade of specific engineered microorganisms. This treatment initiates a series of alternate aerobic and anaerobic microbial reactions, which degrade the complex materials in the effluent into simpler forms, which are bio-safe, and highly useful plant probiotics and soil conditioners.

Treatment requirements

Bio-Solid effluent: Industrial bio-solid sludge is analyzed for its physical and chemical constituents using standard methods. This is necessary to ascertain the treatment protocol, based on the characteristics of bio-solid effluent i.e, its pH, salinity, solid contents etc. The optimum values (ranges) of its various vital characteristics are mentioned in Table 1.

Table 1 **Optimum values (ranges) of vital characteristics of bio-solid effluents**

	Characteristics of the bio-solid effluents	***Optimum value/Range***
1.	pH of the bio-solid sludge :	5-10
2.	Moisture content :	Upto 40%
3.	Solid content in bio-solid sludge:	30-40%. Where the solid content is less, in-house bio-solids such as sawdust, fly-ash, lime sludge, paper clippings or even canteen/kitchen debris are used as additives. (Ghatnekar *et al.* 1998)

Enzymes - Enzymes selected for bio-solid effluent treatment are on the basis of its composition namely; proteins, lipids or cellulose-based. Combinations of extracellular and intracellular enzymes are employed for the degradation process (Table 2).

Table 2 **Degrading enzymes employed depending on the types of bio-solids**

Type of Bio-solids	***Degrading enzymes***
Nitro/Protein-based	Proteases
Oil/Lipid-based	Lipases
Cellulose-based	Cellulases, α-Amylases, Xylases
Lignin-based	Lignocellulases
Lactose-based	Lactases

Earthworm cultures - Breeders and capsules of select species of earthworms are employed for specific type of bio-solid effluents to be treated. Usually, earthworms need to be acclimatized to a particular effluent in various stages. At times, it may require 4-8 months before the earthworms actually start digesting the effluents. However, later the retention time is reduced to 40-45 days (Ghatnekar 1999).

Essentially the earthworms required for treatment must be of a very superior strain and should remain in the confined area only.

BRC maintains a rich vermiculture bank containing robust species of earthworms like *Lumbricus rubellus, L. terrestris, Eisenia foetida, Pheretima asiatica, Pheretima posthuma, Eudrilus eugeniae* etc. which are capable of digesting most of the bio-solids.

Microbial bio-cultures - The key to the success of this treatment lies in the selection of an appropriate combination of microbial consortia. General categorization of micro-organisms according to their specific function is shown in Table 3.

Table 3 Categorization of micro-organisms

Category of micro-organism	*Function*
Deodorizers	Eliminate the abhorrent odour
Degraders	Decompose the complex bio-solids into simpler form
Enrichers	Catalyses the humification process

BRC maintains a state-of-the-art fermentation plant at Badlapur (suburb near Mumbai). This plant comprises of a rich microbial culture bank where pure cultures of various species of microorganisms including deodorizers, degraders, phosphate solubilizers, nitrogen fixers etc., are maintained and multiplied, and even engineered. BRC is actively involved in isolation, characterization and culturing of newer microorganisms from diverse sources. Thereafter, their engineered isolates are prepared by acclimatizing them in specific bio-solid effluents and multiplied in this fermentation plants. These isolates potentially enhance the degradation process.

The culture bank contains collection of species/strains of bacteria, fungi and actinomycetes such as, cellulose, protein and lipid degraders, deodorizers, nitrogen fixers, phosphate solubilizers, humus enrichers etc.

Sterilized standard bedding material - The industrial bio-solid effluents are mixed with a standard bedding material formulated by BRC. It comprises of pre-sterilized sawdust, bovine dung, bovine urine and foliage of *Leucaena leucocephala* plant (Kavian *et al.* 1997). The bedding material facilitates the acclimatization of the earthworms and microbes with the selected effluent and enhances the degradation.

Smooth Walled Rectangular tanks (Degradation tanks) - The degradation process is carried out in series of smooth-walled cemented rectangular tanks with a capacity to accommodate daily generation of effluents of a specific industrial unit.

Treatment protocol

Semi-solid effluents (sludge) obtained from an industrial ETP are subjected to the treatment protocol of TTVB (Figure 1) which consists of following three phases.

First phase – The sludge is inoculated with waste-specific enzymes and tailor made combination microbial degraders and deodorizers. The incubation period is usually 4-6 days.

Second Phase – In this phase the enzymatically treated biomass is mixed with the sterilized standard bedding material in a series of degradation tanks. Carefully selected combinations of earthworm spp. which are earlier acclimatized in the specific effluent are inoculated into these tanks. Various actinomycetes inhabiting the earthworm's gut also trigger the degradation process of the bio-solids. This phase lasts for 18-20 days where the obnoxious effluent is converted into humus-like biomass.

Third phase - In this phase, this humus-like biomass is inoculated with selected nitrogen fixers and phosphate solubilizers. This humification process is aided by a prolific increase in these microbes and acclimatized in 4-5 days.

Permutation and combination of specific type of enzymes, select earthworm spp. and degrading brigade of selected microbial species used in TTVB is the key for this total biodegradation process. It is important to mention here that, the TTVB-based ETP must be "tailor-made" to suit the specific properties of the solid effluents.

In fine, within 30-32 days, a TTVB-based ETP converts the bio-solid sludge with an obnoxious odour into odourless bio-safe, bio-clean and bio-ethical probiotics and soil conditioner.

Mathematical prototype model of 'TTVB'

Safe disposal of hazardous bio-solid effluent imposes an additional financial burden on an industry. Moreover it involves a serious ethical concern for an industry related to its disposal. Recently, more stringent regulations are being increasingly imposed on the industries leading to voluntary and sometimes even forced shutting down of some of the units for not complying with the law. Keeping an eye on the prevailing strict environmental norms and to provide respite to such industries, BRC has developed a low investment and cost effective mathematical prototype model of this biotechnology [Figure 1]. Since last fifteen years, BRC has successfully commissioned the 'Three tier vermiculture biotechnology' in several industries across India and has provided unique balance of economy with environment.

The mathematical model of TTVB (Figure 2) illustrates the advantageous cost-benefit ratio in the following factors, achieved by an industry by adopting this technology.

i] Space: A typical conventional chemical-based ETP setup requires an area of 5,000-7,000 m^2 to treat about 10 tonnes of bio-solids per day. The vital decisive factor for all industries undertaking this top-of-the-line biotechnology is lesser space requirement, since a TTVB based ETP requires an area of about 1000 m^2 which is $1/5^{th}$ to $1/7^{th}$ of the space needed for a conventional ETP.

ii] Setup: The processes involved in TTVB can be carried out in a series of degradation tanks. Unlike other conventional ETPs, there is no need of a complex infrastructure. Forty five tanks of dimensions 450cm x 300cm x 80cm would suffice the treatment of 10 tonnes of bio-solids per day.

iii] Cost: The initial cost for commissioning this technology for a treatment of 10 tonnes of bio-solids per day is approximately £ 38,000 to 40,000 that is merely $1/5^{th}$ of the cost incurred for setting up of a conventional ETP. The degradation tanks are perpetual and hence the only further expenditure incurred is for governance of the earthworms and microbial cultures.

iv] Conservation of Electric power and water: Running cost of TTVB-based ETP is almost negligible due to negligible electricity consumption. This technology also consumes less water as the effluents to be treated are in semi-solid state with 40-50% moisture.

v] Labour: All the three phases in this treatment procedure are natural and semi-automated and hence only 4-5 semi-skilled operators under a part-time supervisor can easily manage the setup.

vi] Cost-effective end product: The application of TTVB can be a lucrative venture for an industry as it offers an environment friendly technique for the conversion of bio-solid wastes into easily saleable soil nourishers. The quantity of final humified product is 25% more than the actual quantity of the treated bio-solids. This is due to the additions made in the form of bedding material during the treatment. The final products of TTVB-based ETP are cost effective as the cost incurred in their production and packaging is £ 37.5-62.5/ton, while it fetches a price of £ 125- 150/ton.

Commercial application of TTVB

BRC has successfully commissioned TTVB on a commercial scale for a wide array of Indian industries including food processing, soybean oil mill, pulp and paper, dairy, gelatine manufacturing units etc. (Ghatnekar *et. al.* 1995 and Ghatnekar 1999).

Recently, BRC has set up a TTVB-based ETP in a gelatine manufacturing industry located in Vapi, Gujarat, India. This industry produces food and pharma grade gelatine which generates around 30 tonnes of proteinaceous bio-solid sludge everyday. The sludge consists of animal residue like bones, skin, hair and silica. Conventional physical, chemical as well as biochemical treatments had failed to contain the highly obnoxious odour emanated by the effluents. This proved to be a great hurdle for that industry in meeting the strict norms laid down by the State Pollution Control Board.

Methodology

The bio-solid sludge to be treated was analyzed for its physical and chemical constituents (Table 4) and was further subjected to extensive studies to determine the degrading microbial brigade of select spp. and for its standardization on Orbitek shakers (Scigenics Biotech Ltd.).

Table 4 **Composition of the bio-solid sludge generated in gelatine manufacturing process**

Constituents	***Description***
Colour	Off-white
Odour	Abhorrent
Texture	Fine
Moisture	50-60%
Nitrogen	2%
Calcium	9-10%
Phosphorus	2-3%
Silica	3-3.5%

Pure culture of *Aspergillus niger*, *A. flavus* and *Bacillus licheniformis* were obtained from the culture bank of BRC. The engineered effluent resistant isolates of these species were transferred to flask cultures and were maintained at 32-35^{0}C and 90 rpm in the rotary shakers (Orbitek) and later multiplied in the fermentor (BRC- Bio-Boom).The trials for designing the effluent-specific protocol for the proteinaceous sludge of the gelatine industry were conducted at BRC's research laboratory, Badlapur, Maharashtra, India.

As per the results of the above trials, the effluent sludge was later subjected to treatment as mentioned in the TTVB protocol using protease enzyme. Microbial strains of *Aspergillus niger* (BRC 135), A. *flavus*

(BRC 117), and *Bacillus licheniformis* (BRC 122) and earthworm sp. namely *Lumbricus rubellus* (American Red Wriggler).

Within 45 days the effluent was converted into humus-like plant probiotic of immense agricultural value.

Results

Microbial analyses of the samples (treated and control) collected at different intervals and at the end of the humification process exhibited the exponential increase in total C.F.U. in the treated set was 20 fold in relation to untreated control set. C.F.U of the selected micro-organisms also exhibited 17 fold increase in treated sets than in control (Ghatnekar *et al.* 2009).

Incorporation of BRC's TTVB-based ETP helped the gelatine manufacturing industry to curb perpetual pollution and also offered a value-added option by converting bio-solid effluent into bio-wealth.

Application of the products of TTVB in agriculture

India is primarily an agricultural economy as the GDP is mostly influenced by a good harvest. But the perpetual exploitation of agricultural land using chemical fertilizers and chemical pesticides, to meet the ever increasing demand of food has lead to detrimental decrease in soil fertility levels.

The soil conditioners and plant probiotics produced by the application of the TTVB have been successfully tested for their bio-safety on diverse groups of experimental plantations cultivated at BRC. Furthermore, BRC, in association with leading agricultural universities and horticultural departments from various states all over India, has conducted extensive trials for evaluating the bio-safety and bio-efficiency of these products on field level i.e., farms and forests.

The encouraging results of application of probiotics from typical coir industry wastes are demonstrated in various crops as mentioned in Figure 3. These probiotics are also found to be excellent potting mix for green house and tissue-cultured orchids and ornamental plants (Ghatnekar *et. al.* 2002).

Role of TTVB in Clean Development Mechanism (CDM) and carbon credits

Since last fifteen years, BRC's TTVB is successfully implemented in diverse industries. Because of the Clean Development Mechanism (CDM) involved in this biotechnology, almost all these industries are likely to be rewarded with Carbon Credits. One of the leading industries in India, Zoom Biofertilizers Pvt. Ltd., Kolhapur has been recently awarded these Carbon Credits after successful implementation of this state-of-the-art biotechnology by BRC.

Discussions

Biotechnologists are striving to address various problems of global proportion and magnitude of which, effluent management and increase in agricultural output are of prime importance to the society. The 'Three-Tier Vermiculture Biotechnology' provides a 'Golden means' to address both these vital aspects.

Karam and Nicell (1999) reported a large number of enzymes from a variety of plants and microorganisms playing an important role in an array of effluent treatment applications. Kavian *et al.* (1999) have carried out extensive studies on cellulases from *Lumbricus rubellus,* the earthworm species used more frequently in vermiculture based effluent treatment processes. Enzymes can act on specific recalcitrant pollutants to remove them by precipitation or transformation to other products. They can also change the characteristics of a given effluent to render it more amenable to treatment or aid in converting effluent material to value-added products.

Kale *et al. (*1991) deduced from their studies that vermicompost application on paddy fields enhanced the activity of selected microbes like nitrogen fixers, actinomycetes and spore formers and highlighted the four fold increase in percent micorrhizal colonization in vermi-composted plots.

Kavian (1994) studied the effect of vermicomposted biofertilizer on *Lycopersicon esculentum* Mill. (Rajani) and *Hibiscus esculentus* L. (Parbhani Kranti) cultivated in succession with respect to ideal dosage of chemical fertilizer and reported that chemical fertilizers could be safely replaced by the vermi-composted probiotics.

Kavian and Ghatnekar (1991) conducted the studies on the biomanagement of dairy effluents using culture of *Lumbricus rubellus* and concluded that sludge cake could support the growth of earthworms without processing. Kavian *et al.* (1996) studied biomanagement of paper mill sludge using vermiculture biotechnology. The red American earthworms (*Lumbricus rubellus*) were employed to treat approximately 1.5 tonnes of the sludge generated by the mill daily which was converted into biofertilizer and plant tonics.

Ghatnekar (1994), Ghatnekar and Kavian (1995, 2001) and Ghatnekar *et al.* (2002) described application of vermiculture in Indian industries and were successful in treating massive quantities of solid and liquid effluents generated from the diverse industries. Kavian and Ghatnekar (1999) and Ghatnekar *et al.* (2008) stated that there is a wide scope of vermiculture biotechnology in agriculture. Their study revealed that fertility of soil can be increased through the application of vermi-compost and can sustain India's overgrowing population by incremental productivity.

Ghatnekar *et al.* (1995, 1998) suggested that the successful composting of solid waste is dependent on microorganisms producing specific enzymes. He also emphasized the role of microorganisms in composting. Likewise, Atkinson *et al.* (1997) made efforts to degrade complex molecules by microbial consortia.

Present paper highlights the role of enzymes, earthworms and microorganisms working in tandem to give the best results in minimal time and minimum expenditure than most of the conventional effluent treatment processes. This biotechnology provides a sustainable, environment-friendly bio-sludge management.

Conclusions

The prototype model of 'Three Tier Vermiculture Biotechnology' stands out to be the best possible solution to dispose off the diverse industrial effluents.

TTVB is advantageous over complex and high cost conventional ETPs due to its cost-cutting features and bio-safe and commercially viable end products like soil conditioners and plant probiotics.

Agricultural field trials of these products have proven results on its bio-safety on the variety of crops. These probiotics also has high market value as it finds application even in horticulture, floriculture and silviculture..

A successful application of this innovative biotechnology can also help industries to earn valuable 'Carbon Credits'.

In fine, "Three-tier vermiculture biotechnology" is the wholesome 'Green Technology' and no longer a utopian dream but possible reality.

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References

Atkinson, C.F., Jones D.D. and Gauthier J.J. (1997) Microbial activities during composting of pulp and paper-mill primary solids. *World Journal of Microbiol. & Biotech.,* **13**, 519-525.

Ghatnekar S.D. and Kavian M.F. (1992) New spin-offs of biotechnology after the RIO-Earth Summit, *In Proceedings of Inaugural Session of BIOTECH INDIA* 1992, pp. 10-14. Bangalore.

Ghatnekar S.D. (1994) Industrial Waste Treatment with Worms, *Indian Industrial Sources*, 37-40.

Ghatnekar, S.D., and Kavian, M.F. (1995) The applications of vermiculture in Indian industries. *In* Sudhir Chandra, K.K. Khanna and H.K. Kehri (eds) *Microbes and Man*, Published by BSMPS, Dehra Dun, India. pp 137-149.

Ghatnekar, S.D., Kavian, M.F., Ghatnekar, G.S., (1995). Vermiculutre-based Effluent Treatment plants in Diverse Industries. *In* Ray S. K. (ed) *Proceedings of Biotechnology Strategy for Development.*, *Biotek South Asia, '94*, pp 167-169. New Delhi.

Ghatnekar, S.D., Ghatnekar, M.S. and Kavian, M.F. (1998) Biotechnological innovations in industrial waste treatment, *In Proceedings of the national conference on plant biotechnology* 1998, pp 107-114. Mumbai.

Ghatnekar S.D. (1999) *Green 2000 for industry through biotechnology – Business opportunities, Keynote address delivered in National Conference on Industry and Environment – Challenges and Solutions in the New Millenium* – CIE 99, Karad.

Ghatnekar, S.D., and Kavian, M.F. (2001) *Innovative biotechnological products for agriculture,* Green Channel, 18-21.

Ghatnekar, S.D., and Kavian, M.F., and Ghatnekar, S.S. (2002) Biotechnological developments to convert flyash from thermal power plants into value added products. *Indian J. Env. Prot.,* **22** (8): 922-926.

Ghatnekar S.D. Kavian M.F., Ghatnekar S.S. and Singh N. (2002) Biotechnological Developments to Convert Solid and Liquid Effluents into Value Added Products in Diverse Industries. *In National Conference on Appropriate Technologies for Industrial Pollution Control and Environmental Management,* pp. 52-56. Tirupati.

Ghatnekar, S.D., Ghalsasi, D.S., Ghatnekar S.S. and Tamhane B.M. (2008) Vermiculture based solid waste treatment plants for biosafe vermicompost. *Green Farming*, **1** (10-11), 73-77.

Ghatnekar, S.D., Ghalsasi, D.S., Tamhane B.M. (2009) The Novel Three-Tier Biotechnology to Convert Solid Waste of Gelatine Manufacturing Unit into Useful Plant Probiotics. *Indian J. Env. Prot.,* **29**(9)

Kale R.D., Bano K., Sreenivasa M.N., Vinayak K., and Bagyaraj D.J. *(*1991) Incidence of cellulytic and lignolytic organisms in the earthworm worked soils. *In* Veeresh G. K., Rajagopal D.and viraktamath C. A. (eds) *Advances in management and conservation of soil fauna.* Oxford and IBH Publishing Co. Pvt. Ltd., pp 599-604. Bombay.

Karam J. and Nicell J.A. (1999) Potential Applications of Enzymes in Waste Treatment. *Journal of Chemical Technology & Biotechnology,* **69** (2), 141-153.

Kavian, M.F. and Ghatnekar S.D. (1991) Biomanagement of dairy effluents using Red earthworms (*Lumbricus rubellus*). *Indian J. Env. Prot.,* **11** (9), 680-682.

Kavian, M.F. (1994) *Biotechnological and Microbiological aspects of Vermiculture*. M Sc. (Research) Thesis, University of Mumbai, India, 173pp.

Kavian, M.F., Ghatnekar S.D. and Kulkarni P.R. (1996) Biomanagement of paper mill sludge using culture of Red American earthworms (*Lumbricus rubellus*). *Indian J. Env. Prot.,* **16** (5), 330-333.

Kavian, M.F., Ghatnekar S.D. and Kulkarni P.R. (1997) Biodegradation studies of soybean oil extraction sludge waste by *Lumbricus rubellus. Jr. of Industrial Pollution Control,* **13** (2), 177-183.

Kavian, M.F., and Ghatnekar, S.D. (1999) Scope of Vermiculture Biotechnology in Agriculture. *In* D.J. Bagyaraj, Ajit Verma, K.K.Khanna and H.K. Kehri (eds) *Modern Approaches and Innovations in Soil Management*. Rastogi Publications, pp 151-161. Meerut.

Kavian M.F. Ghatnekar S.D.and Kulkarni P.R. (1999) Studies on cellulase of Lumbricus rubellus (USA). *Bioresource technology,* **69** (2): 161-165.

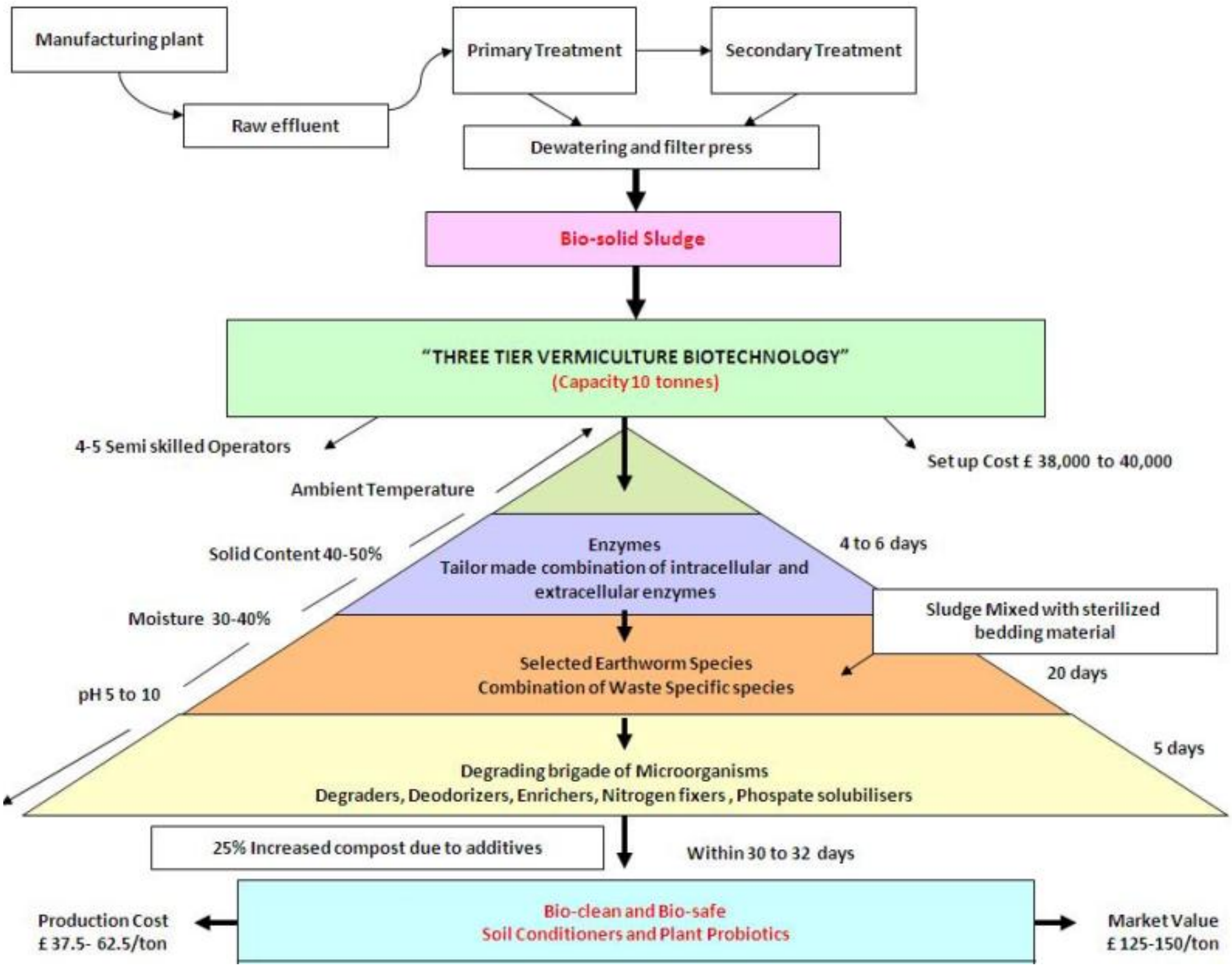

Figure 1 **Prototype Mathematical model for "Three-Tier Vermiculture Biotechnology"**

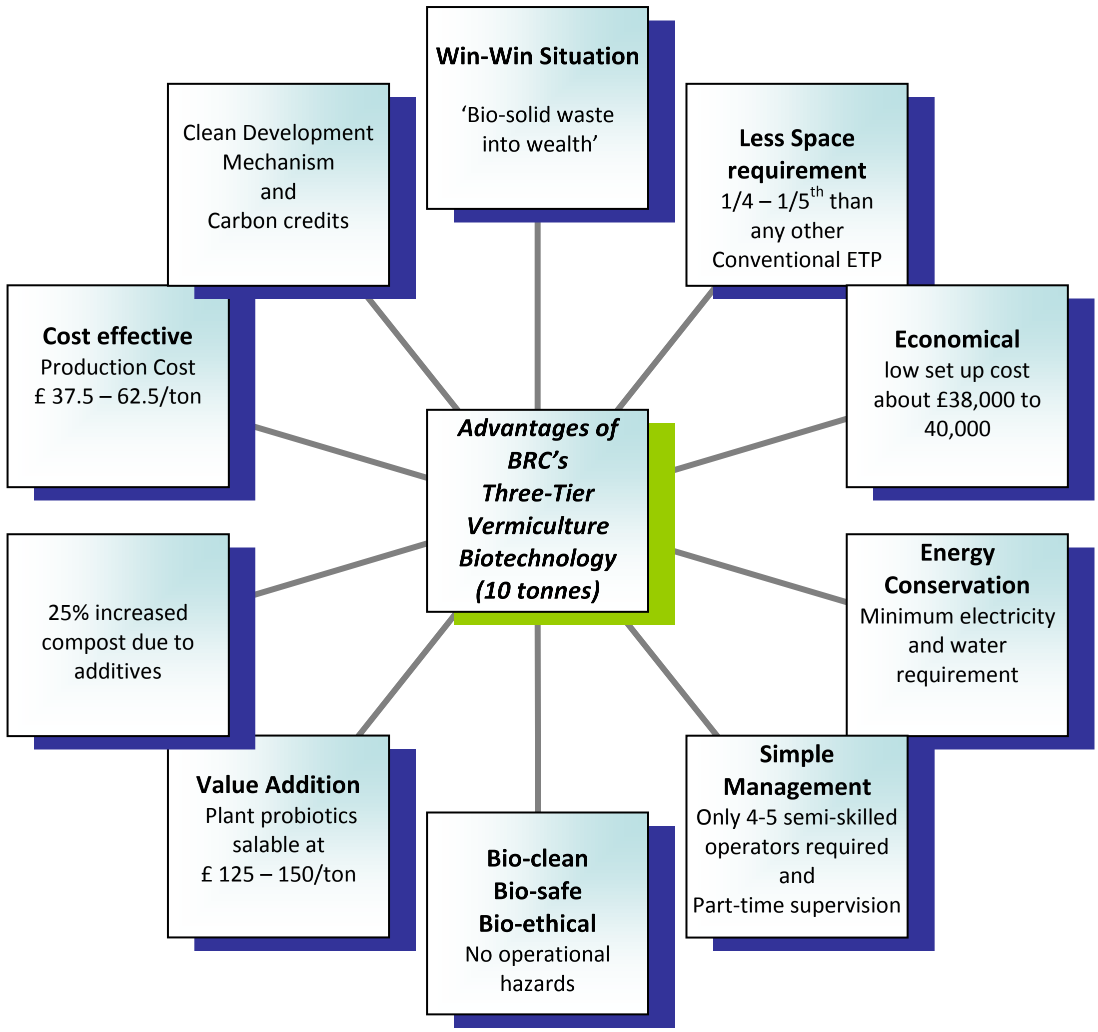

Figure 2 **Advantages of BRC's "Three-Tier Vermiculture Biotechnology"**

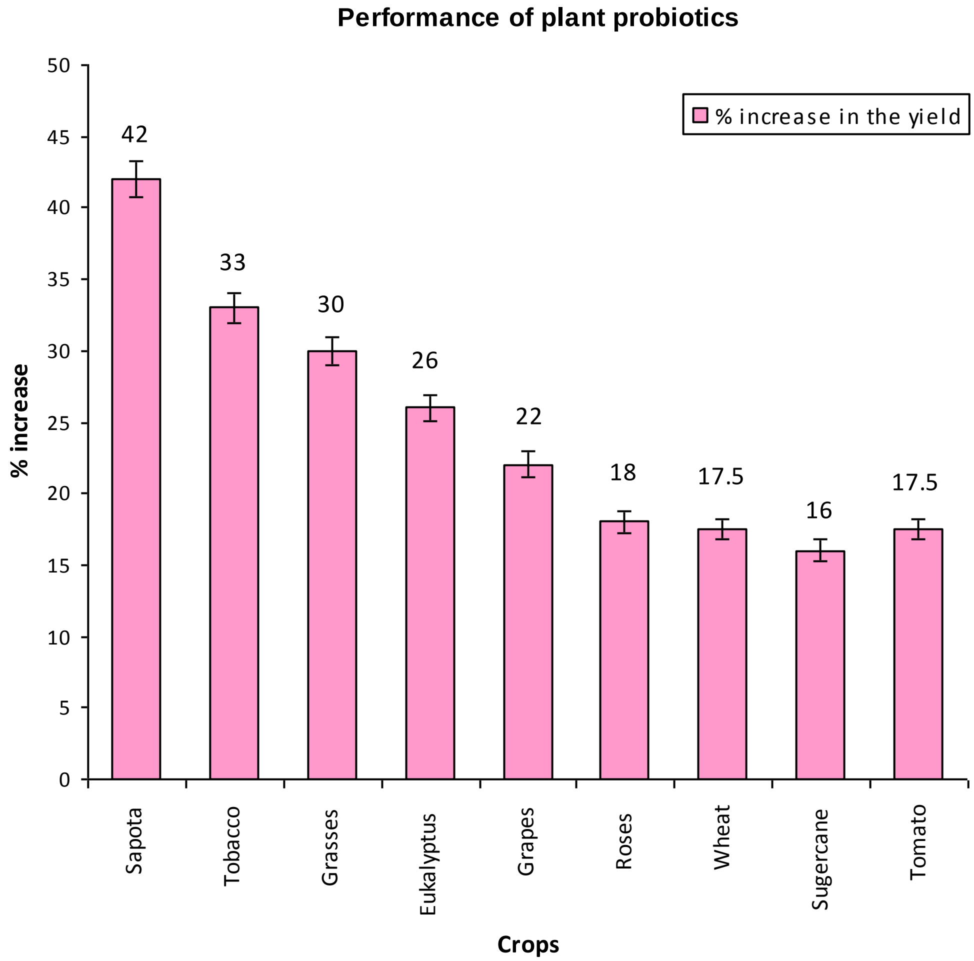

Figure 3 **Percentage increase in the yield of diverse crops treated with the plant probiotics developed using Three-Tier Vermiculture Biotechnology.**