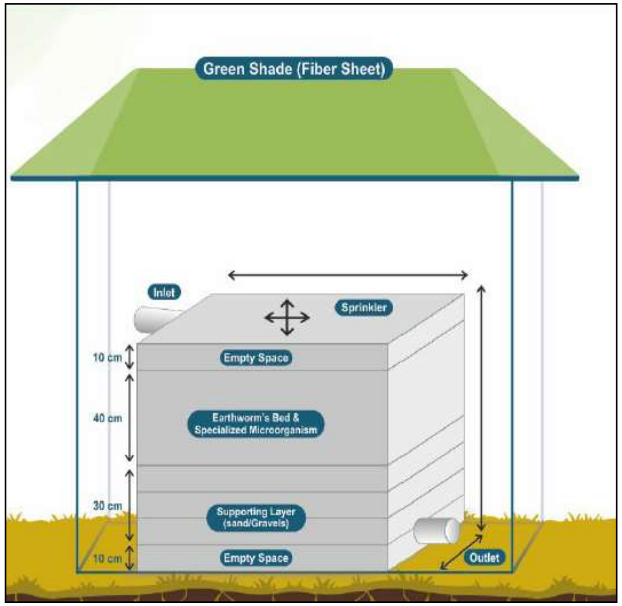
Design, Performance Evaluation, and Investigations of the dynamic mechanisms of Earthworms- Microorganisms interactions for wastewater treatment by Vermifiltration technology



SUDIPTI ARORA, Ph. D (Environmental Engineering), IIT Roorkee

Research Scientist,
Dr. B. Lal Institute of Biotechnology, Jaipur (India)

Date : 15/10/2020

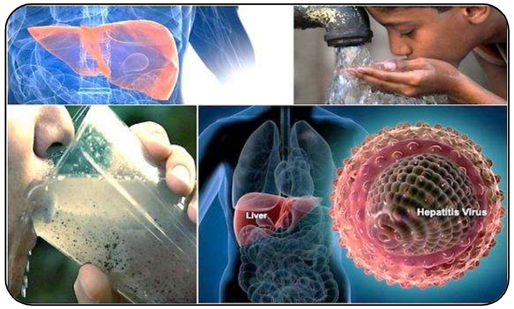
Content

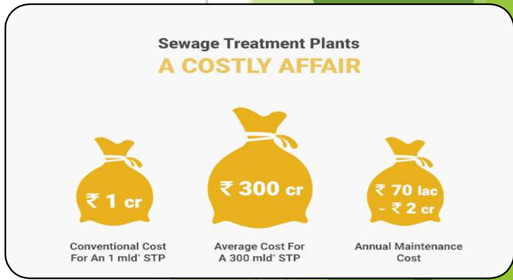

It’s most unfortunate
but its true

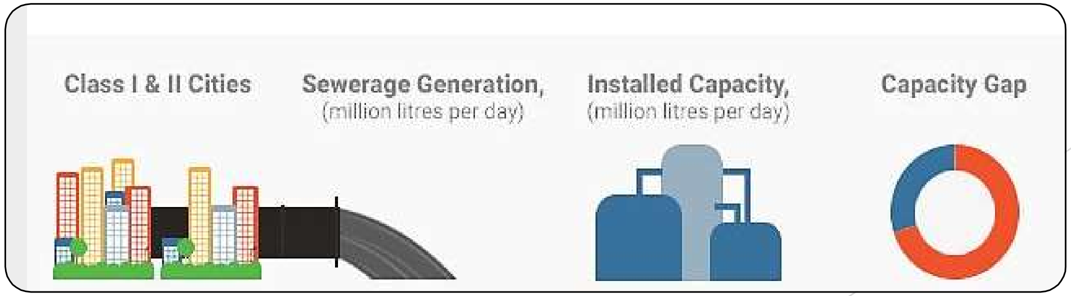
PROBLEM & NEED

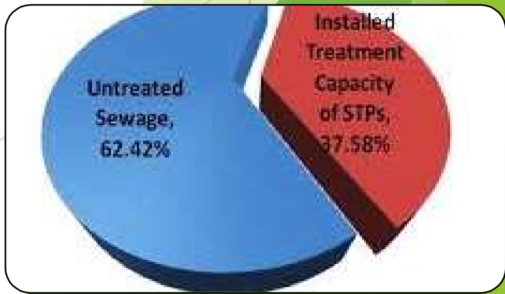
The Sad Reality

There is an urgent need to develop ***Affordable, Easy, Natural, sustainable and decentralised*** *solution for wastewater and sludge treatment*









OPPORTUNITY

VERMIFILTRATION TECHNOLOGY

Vermifiltration technology

- *Vermifiltration* is a process that adapts traditional Vermicomposting into a passive wastewater treatment process by using potential of earthworms.
- A novel technology that involves the synergistic and symbiotic earthworms and microorganisms interactions to process organically polluted wastewater.
- The body of the earthworms act as a biofilter, and allows mixing and aeration for the biodegradation of the organic matter, through their enzymatic activity and removal of contaminants.
- The central concept behind Vermifiltration is that microorganisms perform biochemical degradation of waste material, while earthworms regulate microbial biomass and activity by providing aeration through their burrowing activity, and directly/indirectly grazing on microorganisms.

PROPOSED SOLUTION- VERMIFILTRATION TECHNOLOGY

Wastewater

Decomposition & degradation of organic matter by the enzymatic activity

Earthworms

Treated effluent for Non-potable reuse

Symbiotic & Synergistic interactions

High nutrient rich end products for recycle & reuse

OFMSW

Removal of pathogens by antimicrobial activity

Specialized Microorganisms

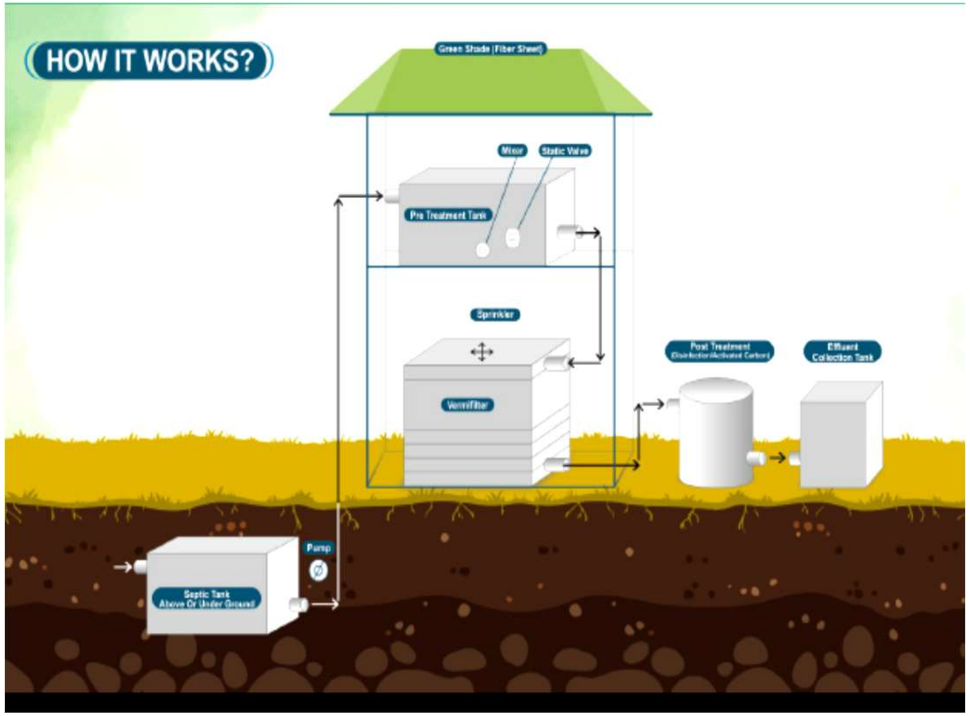
Vermicompost as manure

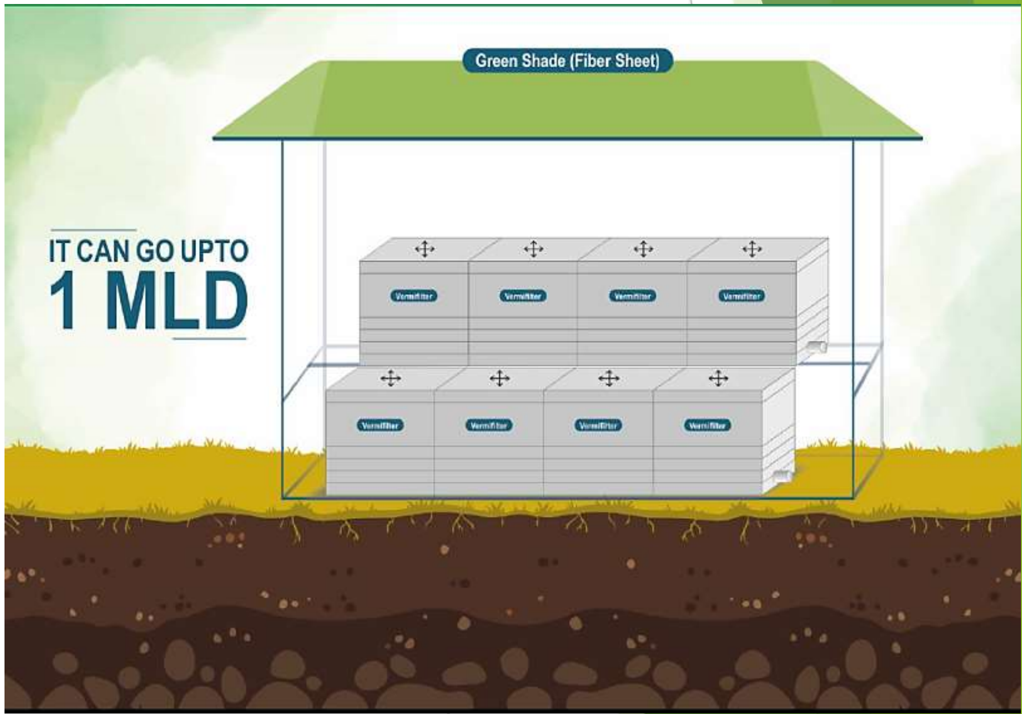
Design & features

- The design criteria includes: optimum Hydraulic loading rate, HRT, Earthworm's type and stocking density, optimum Organic loading rate, health & maturity of earthworms, filter media, appropriate environmental conditions.
- The effectiveness of Vermifilter (VFs) for wastewater treatment has been tested on a variety of wastewaters and sludge, such as domestic sewage, faecal sludge, industry wastewater like Herbal Pharmaceutical wastewater etc, urban run off, and livestock wastewater and at a range of scales in the recent years.
- The removal Efficacy of pollutants (BOD>90%, COD > 85%, pathogens 99.9 % removal) showed the effectiveness of the technology.
- Vermifiltration technology encompasses all forms of TREATMENT (Primary- removal of silt, grit, etc., Secondary- removal of organic matter and Tertiary- removal of pathogens/disinfection) into single unit. However, this technology can be a used as stand alone or in combinations with other technologies.
- After the treatment process, the treated effluent may be utilized for irrigation purposes, and sludge gets converted into Vermicompost which an be used as manure.

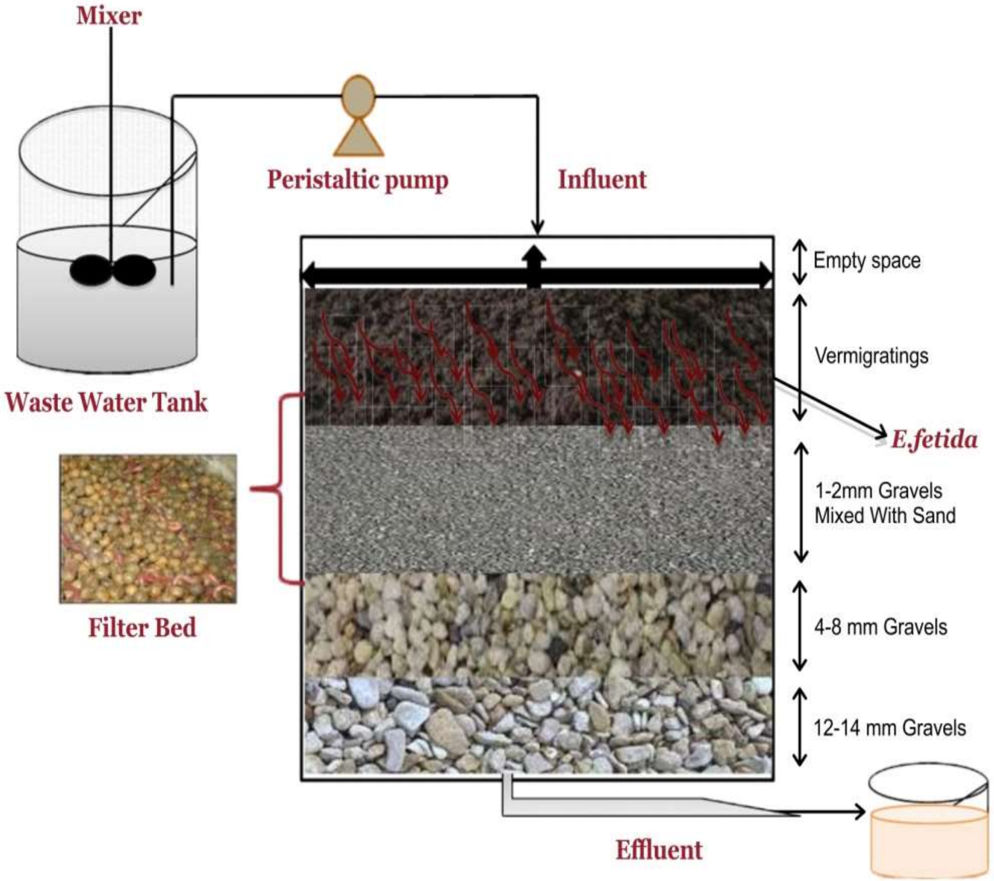
Design & features

An easy to construct, low carbon footprint, energy efficient, no chemical requiring, cost effective and high performance technology for wastewater treatment, for various applications/segments





Inside a Vermifilter Plant



- One of the promising decentralized, cost-effective and sustainable wastewater treatment technology is VERMIFILTRATION.
- It involves the symbiotic and synergistic interactions of earthworms with aerobic microorganisms, that provides mixing and aeration of the organic matter, to treat wastewater and solid waste.

Fabrication of model

Filter media for different layers

Eisenia fetida

Treated water for reuse

OFMSW

Vermicompost

OUR RESEARCH FINDINGS

IN LAST 8 YEARS

2012 - 2015

Lab scale & pilot scale researches on optimizing the vermifiltration technology

2016

Field scale vermifilter and investigations on microbial community dynamics inside the vermifilter & earthworms for the treatment process

2017

Post treatment option for vermifilter treated wastewater to remove pathogens for safe end use by hybrid disinfection model

2017

Investigations on earthworms coelomic fluid & gut microflora and its enzymatic activity & antimicrobial activity

2018

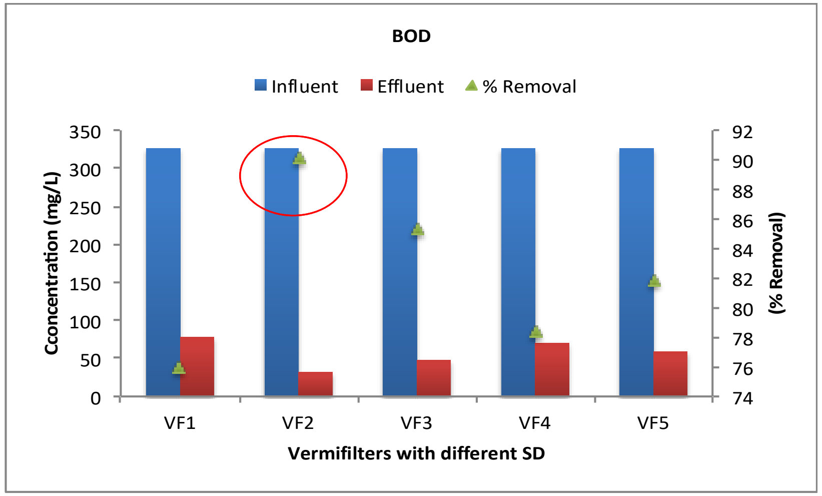
Prevention of prevalence and spread of antimicrobial resistant genes and microorganisms

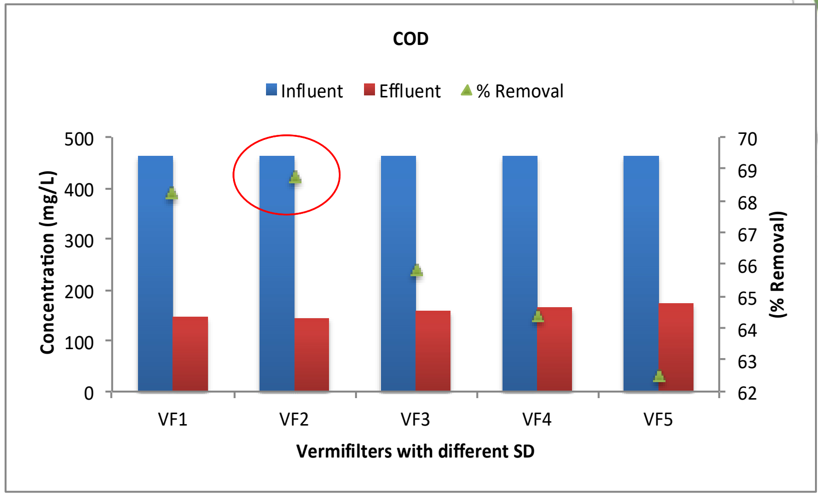
2019

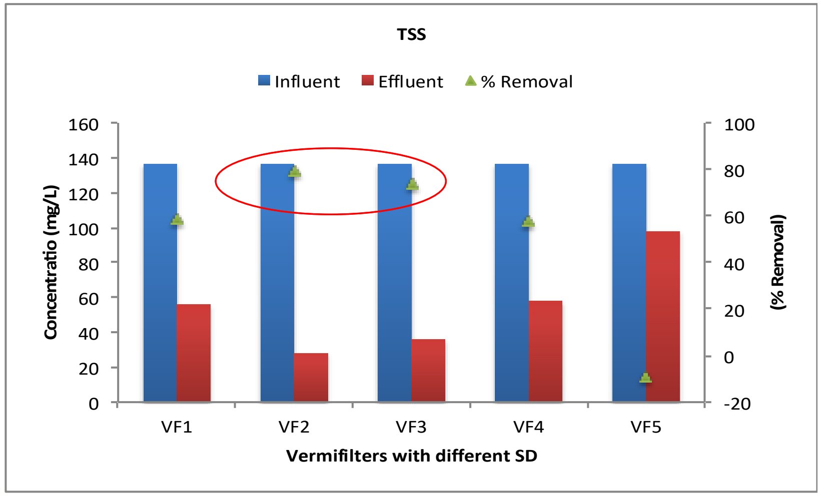
Evaluating the effect of irrigation by treated effluent on plants growth & properties.

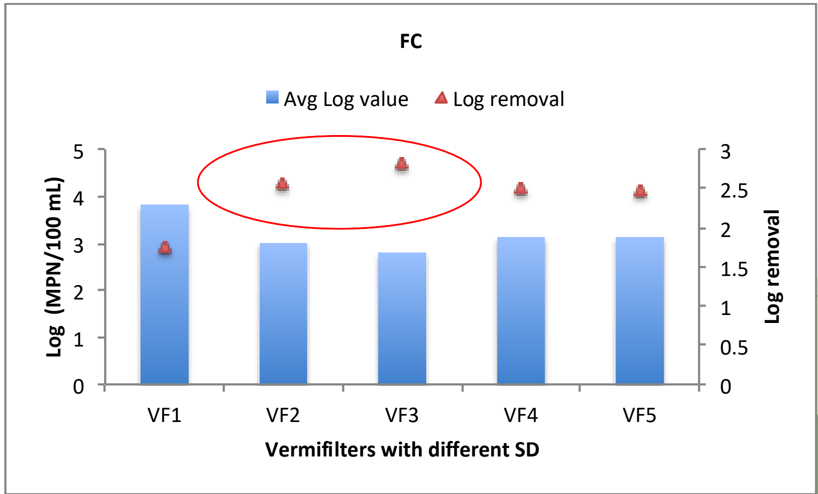
Laboratory scale Experimental set up to develop design criteria for vermifiltration

Run 1: Performance of VFs with different stocking density









VF1= 8,000; VF2= 10,000; VF3= 15,000, VF4= 20,000; VF5= 25,000 worms/m^3

OPTIMUM SD = 10,000 worms/m^3

Different filter media used for experiments

a) Different media sterilized before experiments

b) gravels (6-8 mm)

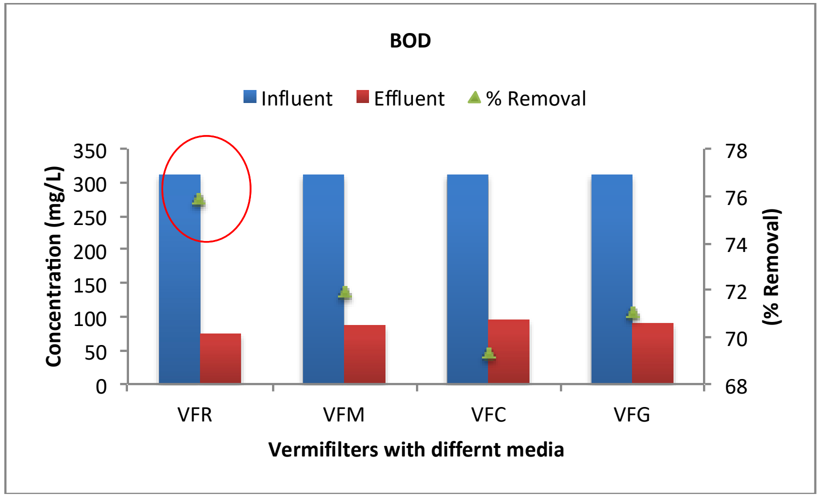
c) gravel (10-12 mm)

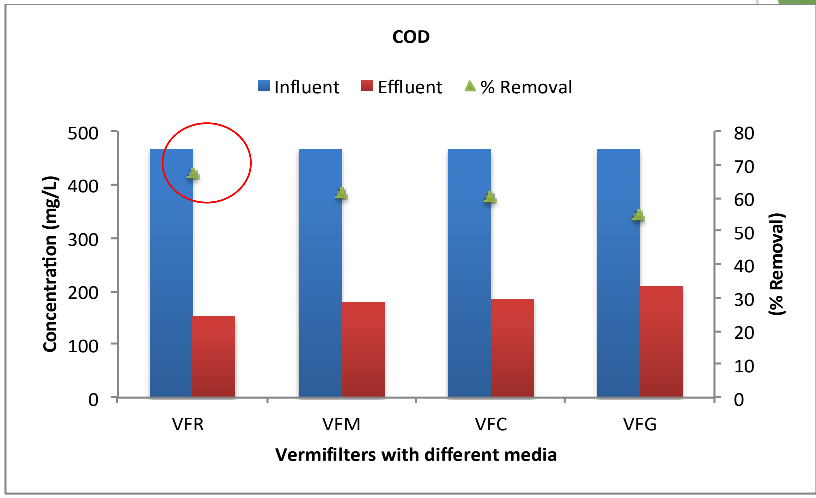
d) Coal

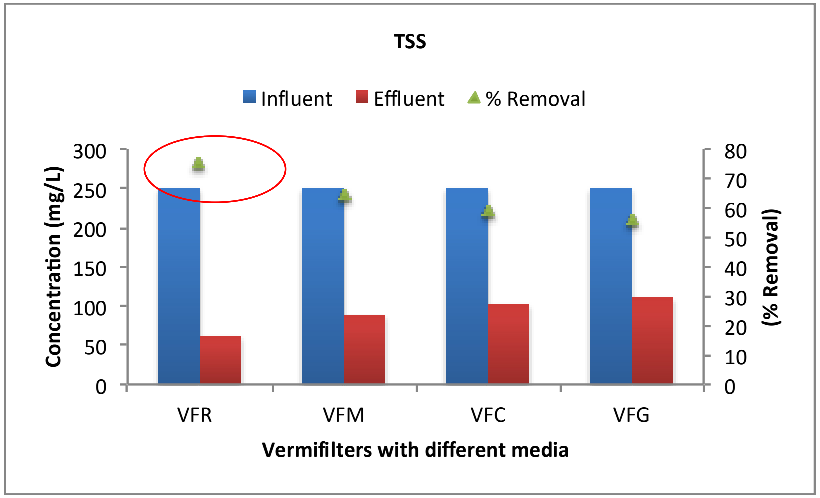
e) Glass balls

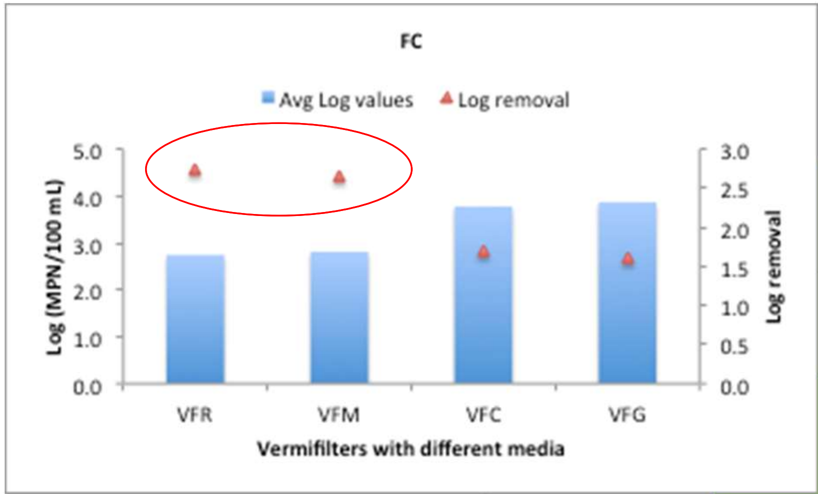
f) Mud balls

Run 2: Performance of VFs with different Filter media





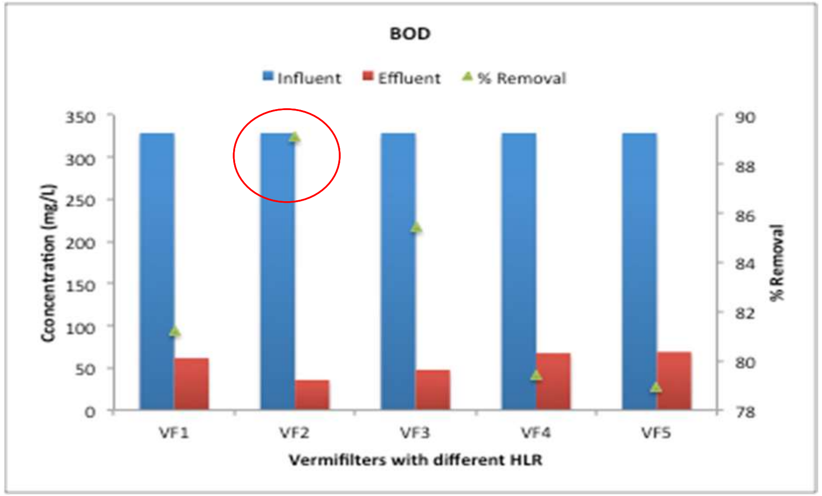


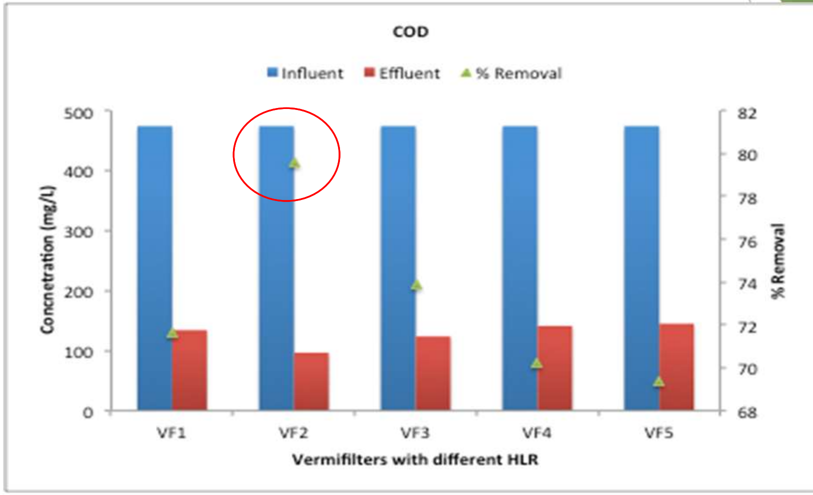


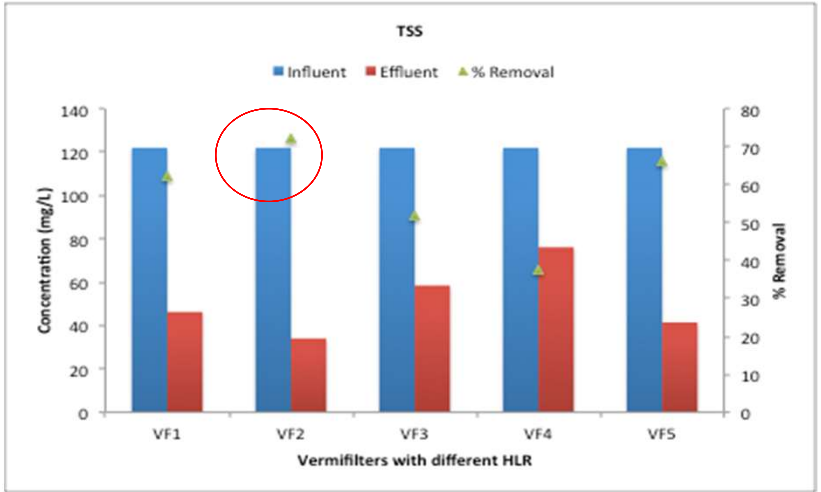
VFR= River- bed
VFM= Mud balls media
VFC = coal
VFG= Glass balls

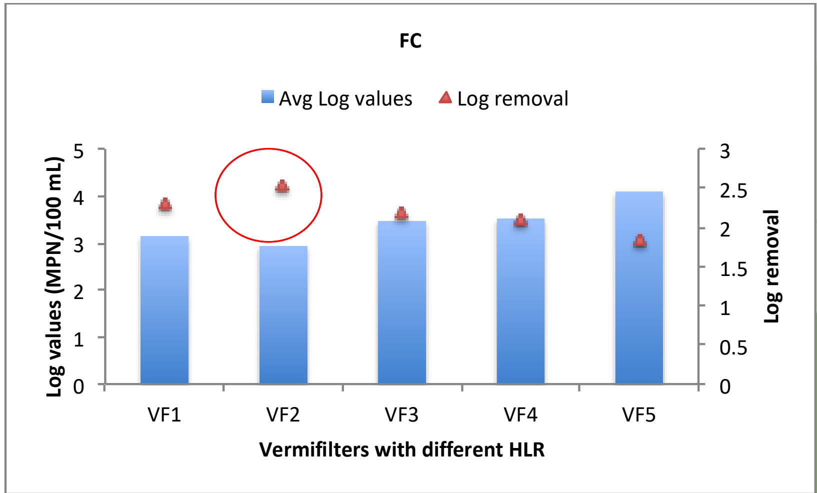
OPTIMUM Filter media = River bed material, Overall performance VFR> VFM> VFG > VFC

Run 3: Performance of VFs with different Hydraulic Loading Rate (HLR)









VF1= 0.5; VF2= 1.0; VF3= 1.5, VF4= 2.0; VF5= 2.5 $m^3/m^2/day$

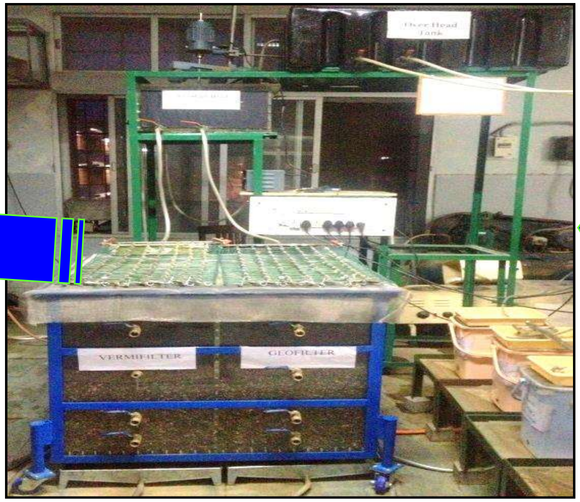
OPTIMUM HLR = 1.0 $m^3/m^2/day$

Outcome of the Design Criteria

Optimizing the Design Criteria

- Run 1: Stocking density (number) of earthworms → Optimum Stocking density = 10,000 worms/m^3
- Run 2: Filter media → Optimum Filter media = River bed material
- Run 3: Hydraulic loading rate → Optimum HLR= 1.0 $m^3/m^2/day$

Phase II: Installing a pilot scale Vermifilter



RESULTS

Performance Evaluation of Vermifilter for continuous wastewater treatment (120 days)

Parameter	Influent (Avg ± SD)	Effluent VF (Avg ± SD)	% Reduction (R)	Effluent GF (Avg ± SD)	% Reduction (R)
pH	7.97 ± 0.1	7.23 ± 0.1	-	7.89 ± 0.2	-
Temperature	27.4 ± 1.5	27.2 ± 0.5	-	27.3 ± 1.5	-
DO (mg/L)	0.05 ± 0.01	5.4 ± 1.5	-	4.45 ± 1.9	-
BOD (mg/L)	235.7 ± 30.4	15 ± 9.9	93.6%	25.5 ± 8.8	89.2%
COD (mg/L)	463 ± 28	125 ± 25	73.1%	137 ± 28	70.4%

Neutralizing pH, Buffering capability

Aerobic conditions, Maintains DO naturally

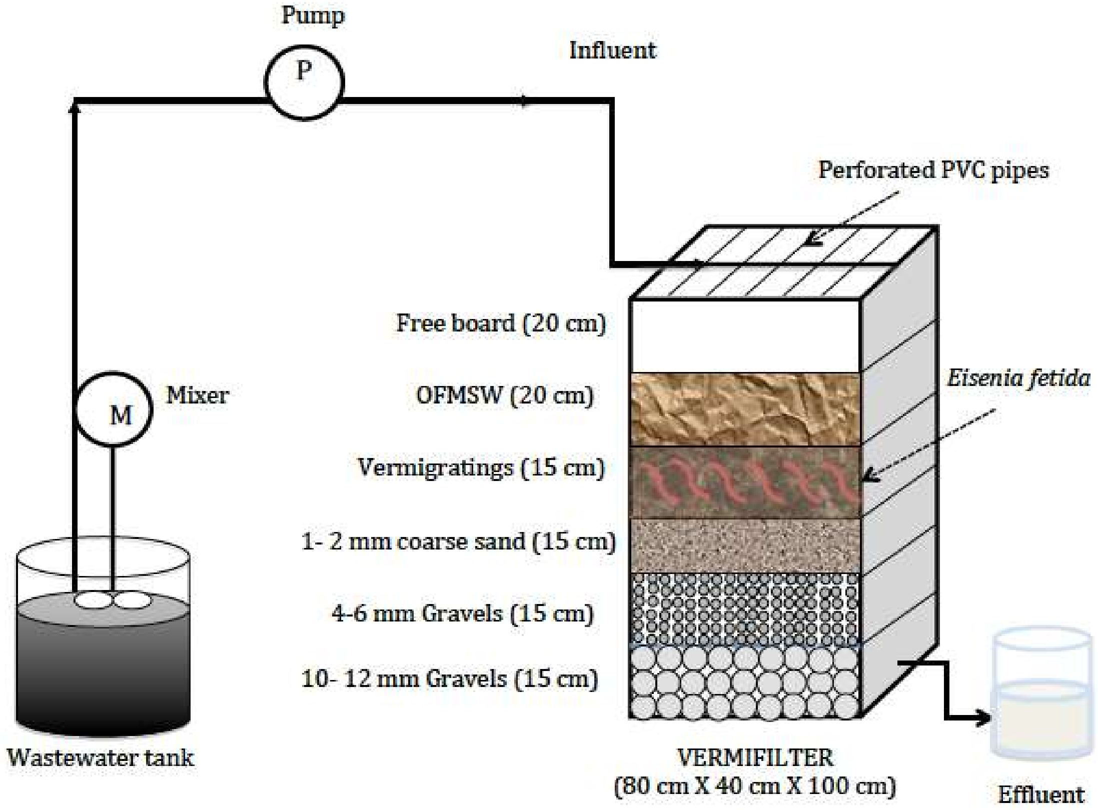
Significant BOD & COD Removal

RESULTS

Organisms	Influent	Effluent VF	Log removal (K)	Effluent GF	Log removal (K)
Total coliforms (MPN/100 mL)	3.5 X 10^8	2.5 X 10^5	3.15	2.0 X 10^6	2.24
Fecal coliforms (MPN/100 mL)	2.0 X 10^6	8.3 X $10^{2.5}$	2.88	6.5 x 10^4	1.49
Fecal streptococci (MPN/100 mL)	3.3 X 10^6	1.9 X $10^{2.5}$	3.74	3.0 X 10^4	2.04
Total Heterotrophic Bacteria (CFU/mL)	1.4 X 10^9	2.0 X 10^5	3.85	6.8 X 10^6	2.32
Total fungi (CFU/mL)	2.2 X 10^6	7.5 X 10^2	3.46	1.6 X 10^4	2.14
Actinomycetes (CFU/mL)	6.5 X 10^5	5.2 X 10^4	1.09	2.5 X 10^5	0.4
Salmonella (CFU/mL)	1.2 X 10^6	1.5 X 10^2	3.9	1.2 X 10^4	2.0
Escherichia coli (CFU/mL)	1.5 X 10^6	1.4 X 10^4	2.03	1.5 X 10^5	1.0

Considerable removal of pathogens, within the WHO standards (10^3 MPN/100 mL) of water fit for reuse

Phase III: Combined Treatment of Biodegradable solid waste and wastewater



RESULTS

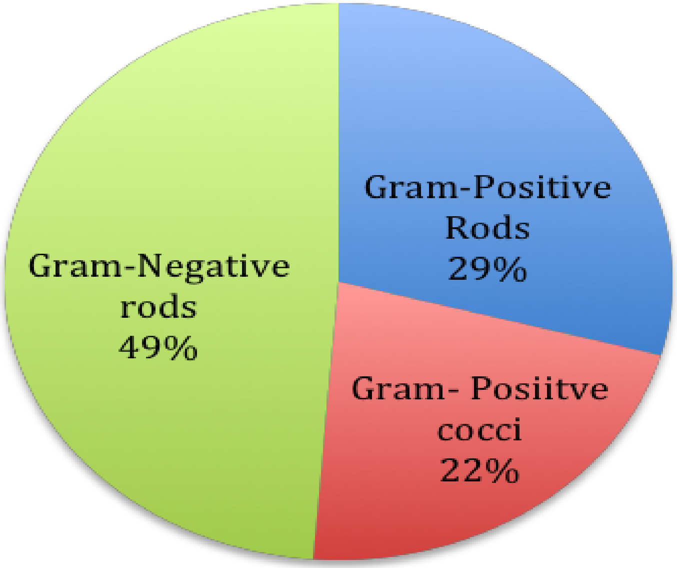
Performance evaluation of a vermifilter for combined treatment of solid waste and wastewater (90 days)

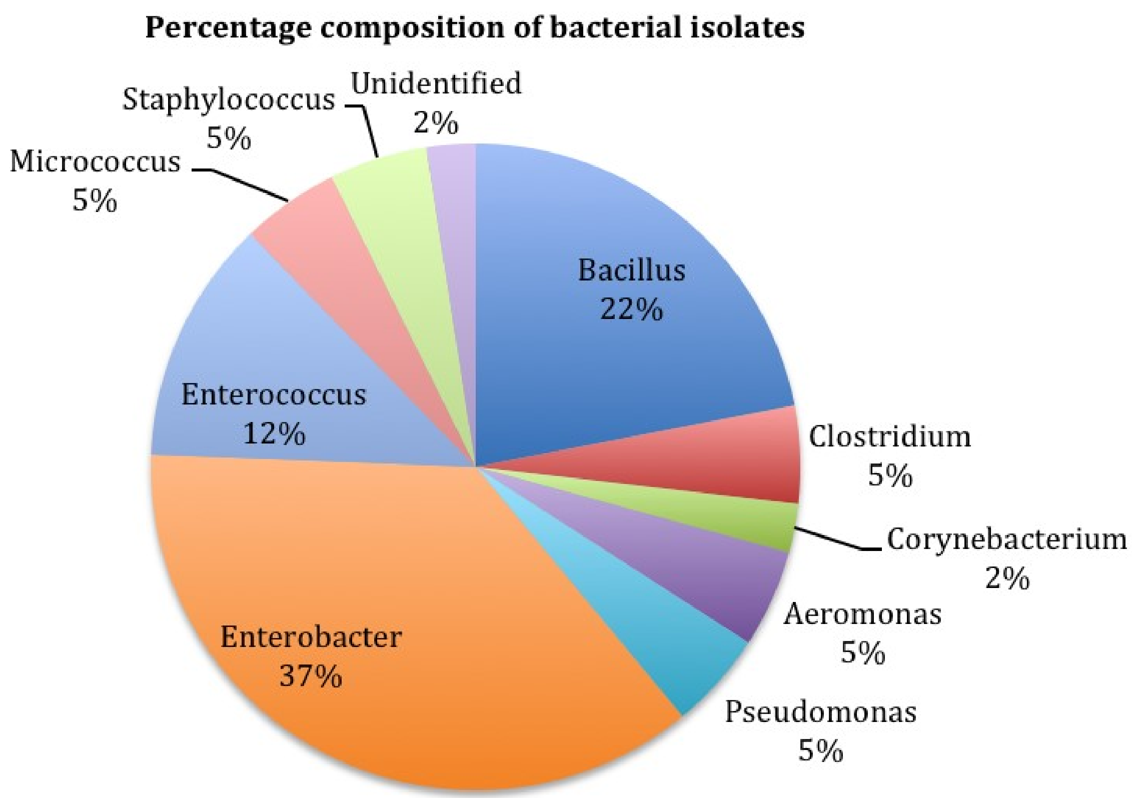
Parameters	0th day	30th day	60th day	90th day
pH	8.6 ± 0.2	8.5 ± 0.3	7.9 ± 0.3	7.5 ± 0.1
Ammonia- N (%)	0.4 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.1 ± 0.1
Nitrate- N (%)	0 .3 ± 0.2	0.9 ± 0.1	1.5 ± 0.2	1.7 ± 0.2
TOC (%)	58.3 ± 0.2	48.4 ± 0.9	39.6 ± 0.8	32.0 ± 1.1
TN (%)	0.97 ± 0.1	1.0 ± 0.2	1.5 ± 0.3	1.9 ± 0.1
C/N ratio	60.10	48.40	26.40	16.84
TP (%)	0.96 ± 0.2	1.2 ± 0.2	1.4 ± 0.1	1.6 ± 0.5
C/P ratio	60.73	40.33	28.28	20.00
TC (MPN/g)	6.68 ± 0.5	4.86 ± 0.3	3.72 ± 0.2	2.60 ± 0.2
FC (MPN/g)	4.73 ± 0.6	4.23 ± 0.2	3.32 ± 0.2	2.50 ± 0.3
FS (MPN/g)	3.56 ± 0.4	2.98 ± 0.5	2.2 ± 0.6	1.90 ± 0.2
Salmonella (CFU/g)	2.97 ± 0.2	2.25 ± 0.2	1.47 ± 0.3	1.25 ± 0.2
Escherichia coli	2.90 ± 0.5	1.97 ± 0.2	1.50 ± 0.4	1.33 ± 0.2

RESULTS

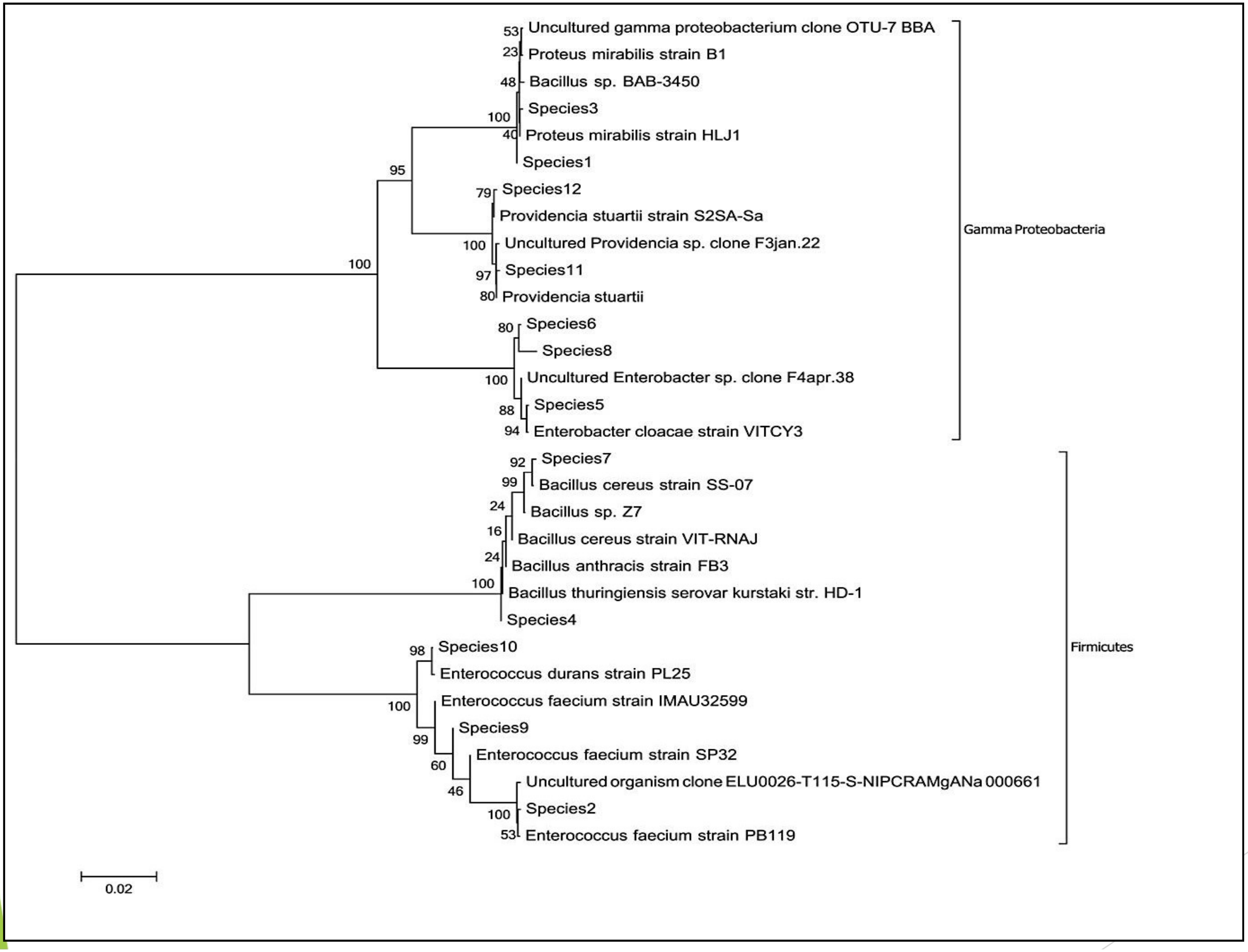
Parameters	Influent	Final effluent	Removal (%)
TC (MPN/100 mL)	6.63 ± 0.60	2.72 ± 1.60	98.89
FC (MPN/100 mL)	5.48 ± 0.37	2.66 ± 0.30	97.12
FS (MPN/100 mL)	5.45 ± 0.66	2.80 ± 0.50	99.29
E. coli (CFU/mL)	4.50 ± 0.42	1.99 ± 0.10	99.99
Salmonella (CFU/mL)	3.87 ± 0.94	1.67 ± 0.92	96.81
Total bacteria (CFU/mL)	6.89 ± 0.25	3.54 ± 0.14	89.61
Total fungi (CFU/mL)	4.61 ± 0.60	3.81 ± 0.72	99.9
Actinomycetes (CFU/mL)	5.63 ± 0.15	3.72 ± 0.50	98.89

Microbial diversity by culture dependent approach





PHYLOGENETIC ANALYSIS



The 16 S rRNA analysis of the 12 active strains having were clustered into 2 phylogenetic groups

- Gamma-proteobacteria
- Firmicutes

Phase IV: Field Scale run for one year

Two Pilot vermifilters Installed at 1 KLD capacity for Institutional Wastewater & Clinical laboratory wastewater treatment, operating since 2016 (June)

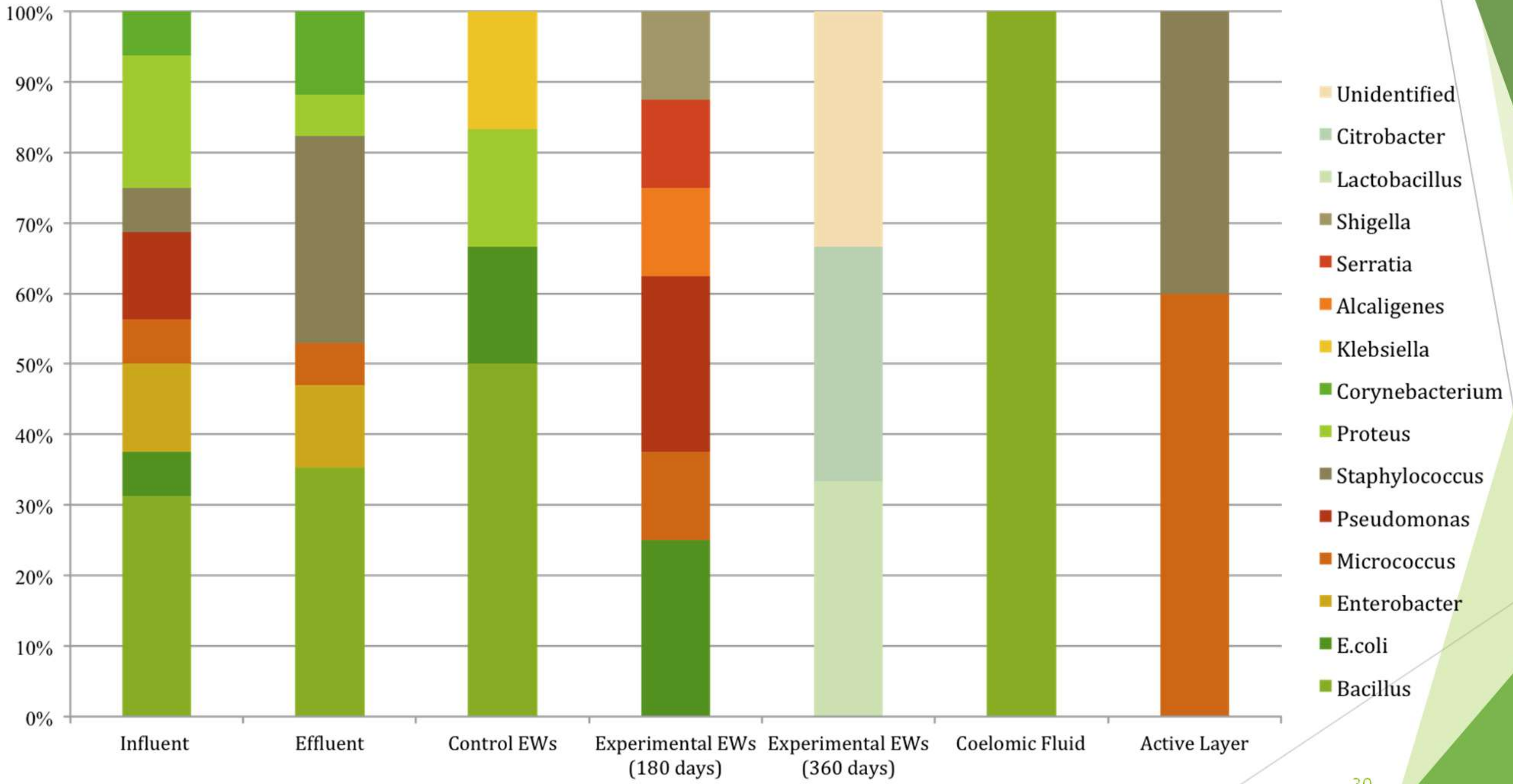
Results of Performance Analysis for Institutional wastewater treatment

Parameters	Influent	Final effluent
pH	7.0-7.8	7.0-7.5
Temperature (^oC)	25-30	25-30
Turbidity (NTU)	40-100	<5
DO (mg/L)	0-0.5	3-4
Color	Grey or Dark Grey	Slightly pale yellow
Odor	Strong	Odorless
BOD (mg/L)	100-200	<5
COD (mg/L)	200-400	<30
TDS (mg/L)	1000-1500	50-100
TSS (mg/L)	250-300	20-30
NO_3^- (mg/L)	30-50	10 ± 2.7
PO_4^{2-} (mg/L)	1-5	<3
SO_4^{2-} (mg/L)	100- 200	<50
Fecal coliforms (MPN/100 mL)	3-4 X 10^3	Below Detection Limit

Results of Performance Analysis for Clinical Laboratory wastewater treatment

Parameters	Influent	Final effluent
pH	6-8	7.0-7.5
Temperature (^oC)	25-30	25-30
Turbidity (NTU)	100-200	<10
DO (mg/L)	0-0.5	3-4
Color	Grey or Dark Grey	Slightly pale yellow
Odor	Strong	Odorless
BOD (mg/L)	200-250	<15
COD (mg/L)	350-400	<30
TDS (mg/L)	1000-1500	50-100
TSS (mg/L)	250-300	20-30
NO_3^- (mg/L)	30-50	10 ± 2.7
PO_4^{2-} (mg/L)	1-5	<3
SO_4^{2-} (mg/L)	100- 200	<50
Fecal coliforms (MPN/100 mL)	3-4 X 10^3	Below Detection Limit

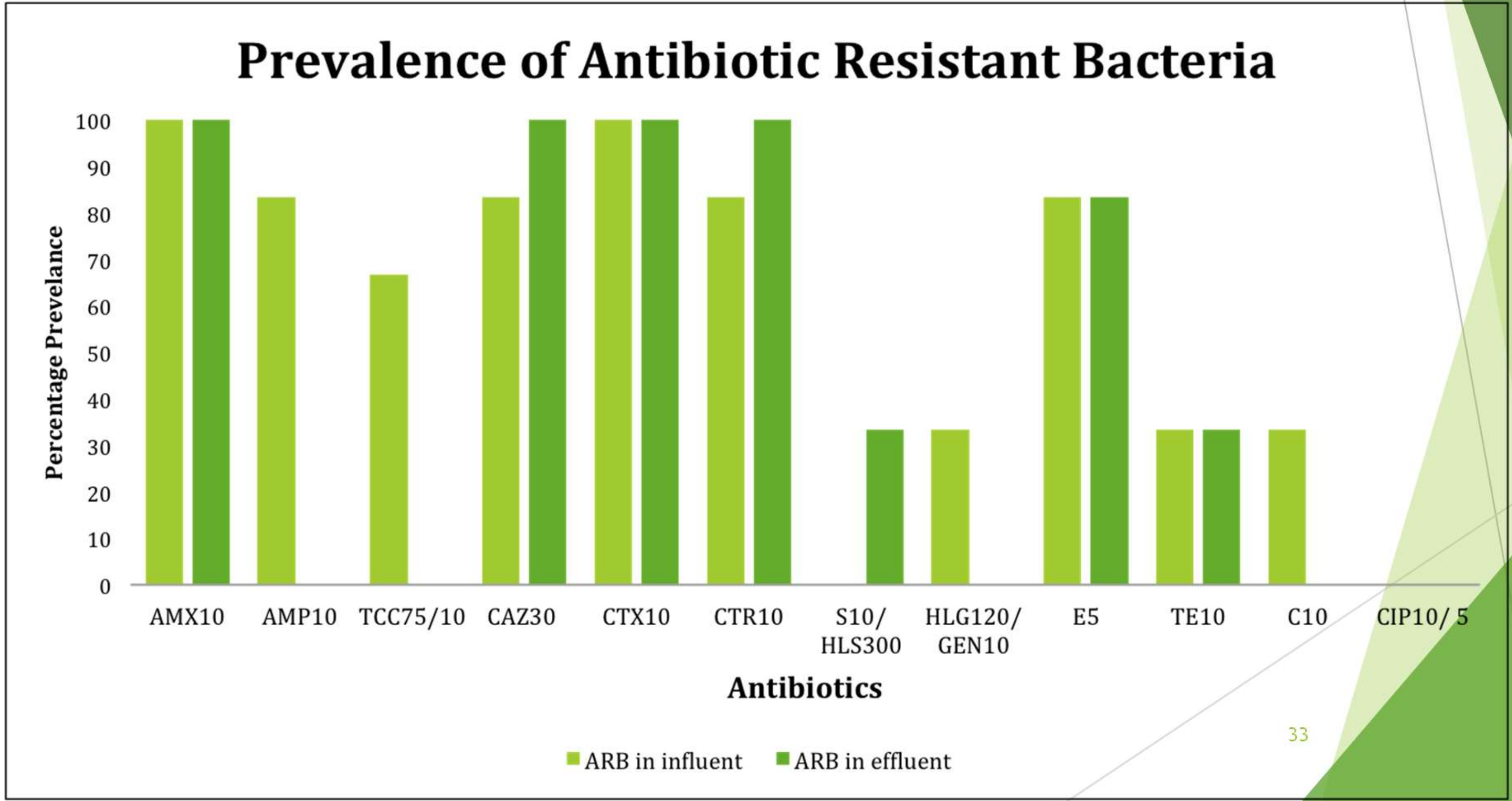
Diversity of microbes

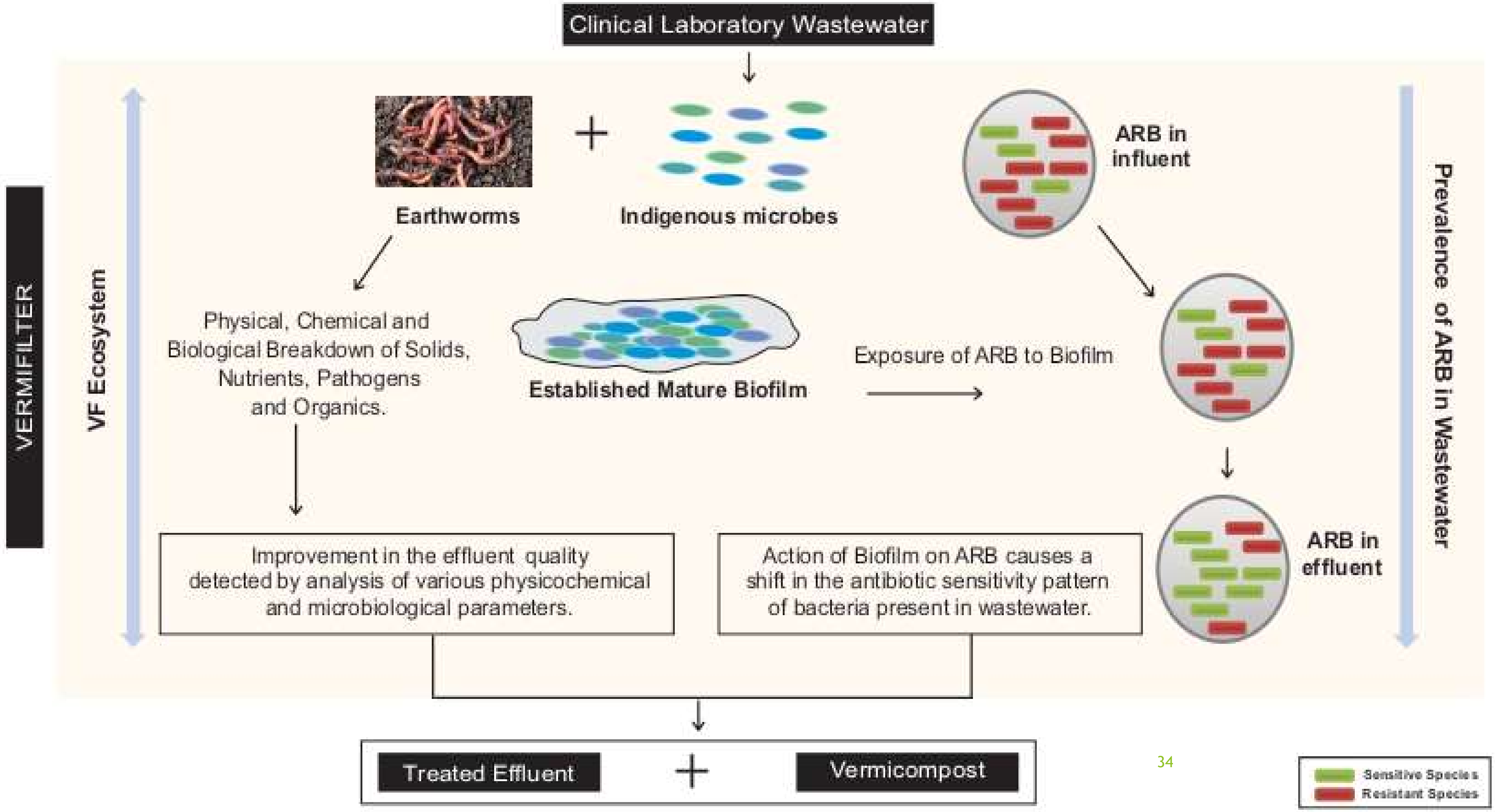



Effluent
Firmicutes
Bacillus sp.
Staphylococcus epidermidis
Gammaproteo-bacteria
Pseudomonas mallei
Escherichia coli
Proteus inconstans
Actinobacteria
Corynebacterium xerosis
Micrococcus varians
Influent
Gammaproteo-bacteria
Shigella sp.
Pseudomonas mallei
Proteus inconstans
Citrobacter intermedius
Actinobacteria
Micrococcus sp.
Corynebacterium xerosis
Firmicutes
Bacillus sp.
Staphylococcus sp.
Filter Media Layer
Actinobacteria
Micrococcus sp.
Corynebacterium xerosis
Gammaproteo-bacteria
Pseudomonas mallei
Proteus rettgeri
Escherichia coli
Klebsiella ozaenae
Firmicutes
Staphylococcus aureus
Earthworm
Gammaproteo-bacteria
Proteus rettgeri
Pseudomonas mallei
Providencia
Enterobacter
Citrobacter
Klebsiella ozaenae
Actinobacteria
Micrococcus sp.
Coelomic Fluid
Firmicutes
Bacillus cereus
Staphylococcus epidermidis
Influent
Effluent
Earthworm
Filter Media Layer
Coelomic Fluid

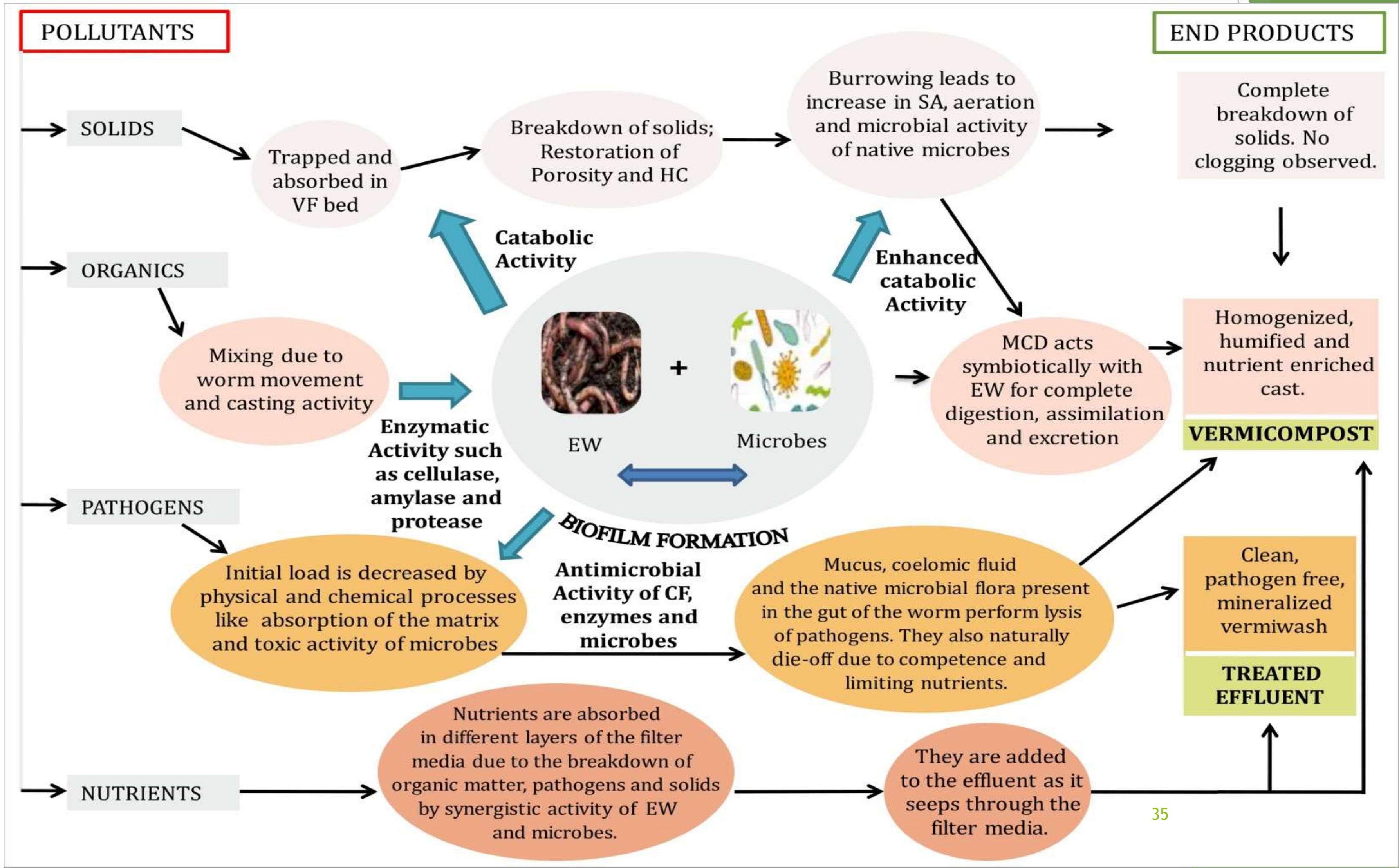
		Bacterial Diversity		Antimicrobial Acitivity Index	Enzymatic Activity Index	Dominant Strains	Classification
		Gram Positive %	Gram Negative %				
Influent		50	50	69%	44%	*Bacillus, Enterobacter, E.coli, Micrococcus, Psuedomonas, Staphylococcus, Proteus,Corynebacterium*	Firmicutes, Gammaproteobacteria, Actinobacteria
Earthworm	Control	50	50	50%	83%	*Bacillus, Proteus, E. coli, Klebsiella*	Firmicutes, Gammaproteobacteria
	180 days	12.5	87.5	100%	100%	*Micrococcus,Pseudomonas, Alcaligenes, Shigella, E. coli , Serratia*	Actinobacteria, Gammaproteobacteria, Betaproteobacteria
	360 days	33	67	100%	100%	*Lactobacillus, Citrobacter, unidentified sp.*	Firmicutes, Gammaproteobacteria
	Coelomic Fluid	100	0	33%	N/A	*Bacillus cereus Bacillus megaterium*	Firmicutes
Active Layer		100	0	60%	40%	*Micrococcus, Staphylococcus*	Firmicutes, Actinobacteria
Effluent		93.33	6.66	27%	40%	*Bacillus, Staphylococcus, Micrococcus, Proteus, Corynebacterium*	Firmicutes, Gammaproteobacteria, Actinobacteria

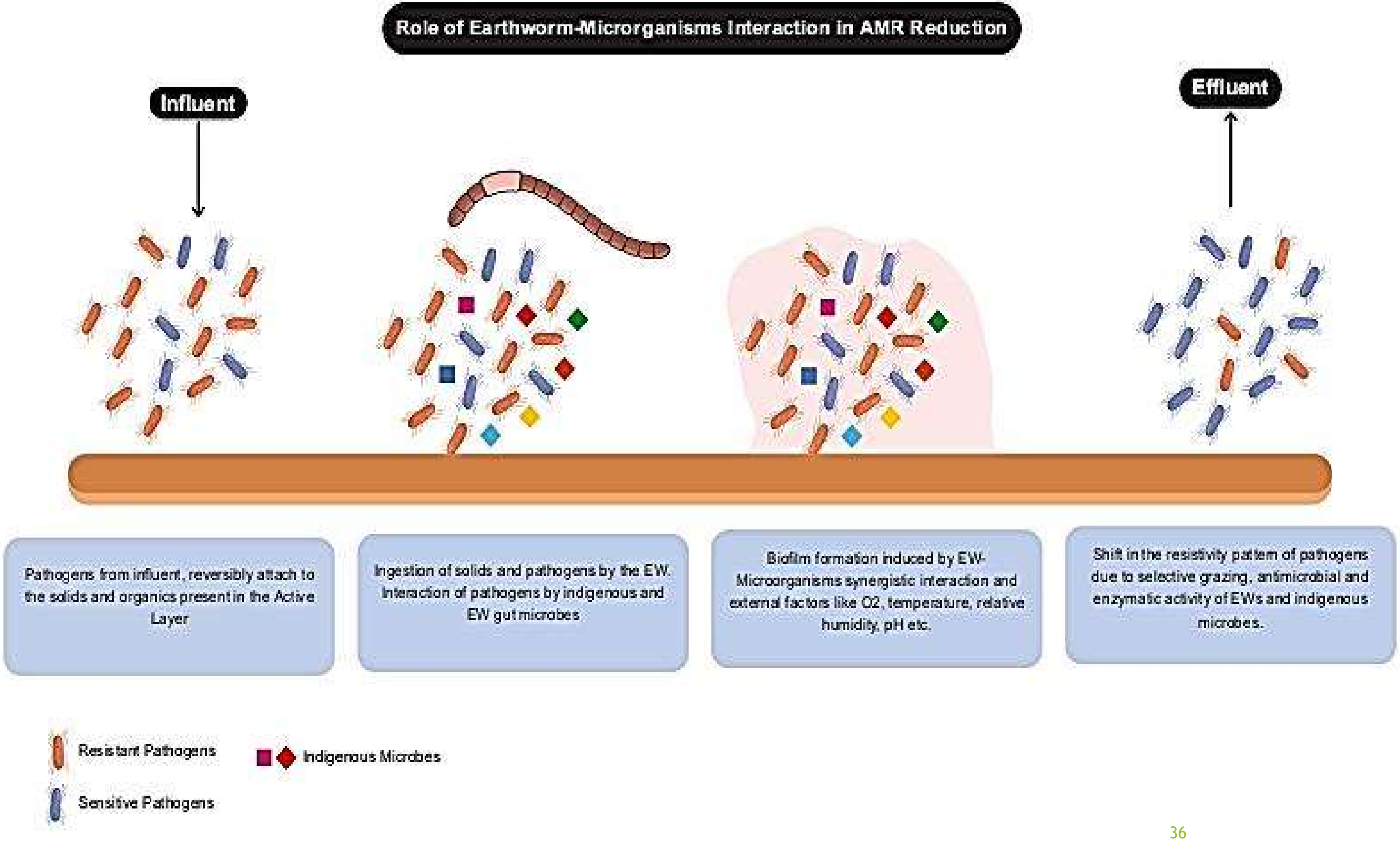
Results of Antimicrobial resistance during Vermifiltration



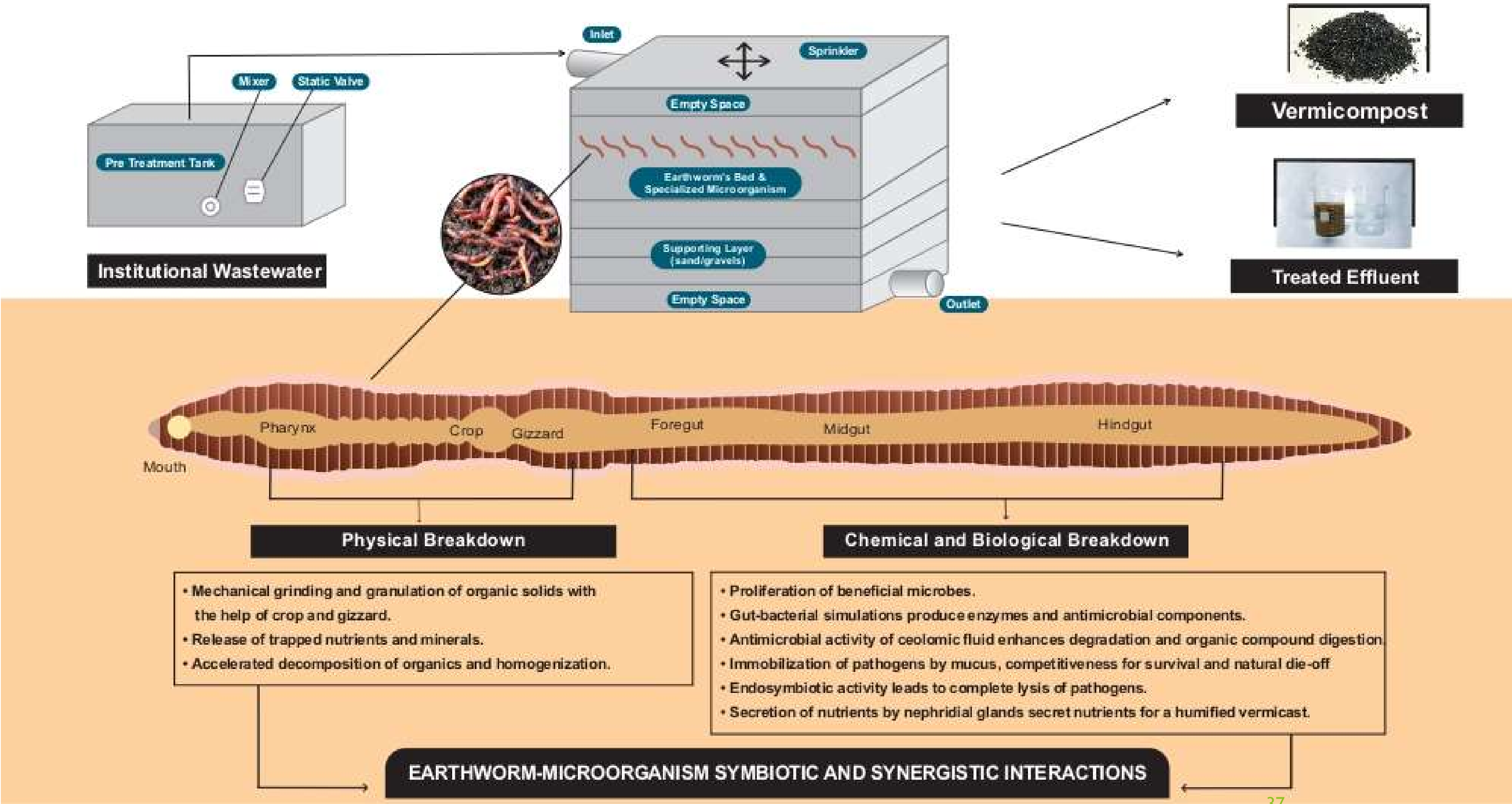


Clinical Laboratory Wastewater
VERMIFILTER
VF Ecosystem
Earthworms
Indigenous microbes
ARB in influent
Physical, Chemical and Biological Breakdown of Solids, Nutrients, Pathogens and Organics.
Established Mature Biofilm
Exposure of ARB to Biofilm
Prevalence of ARB in Wastewater
Improvement in the effluent quality detected by analysis of various physicochemical and microbiological parameters.
Action of Biofilm on ARB causes a shift in the antibiotic sensitivity pattern of bacteria present in wastewater.
ARB in effluent
Treated Effluent
Vermicompost
Sensitive Species
Resistant Species

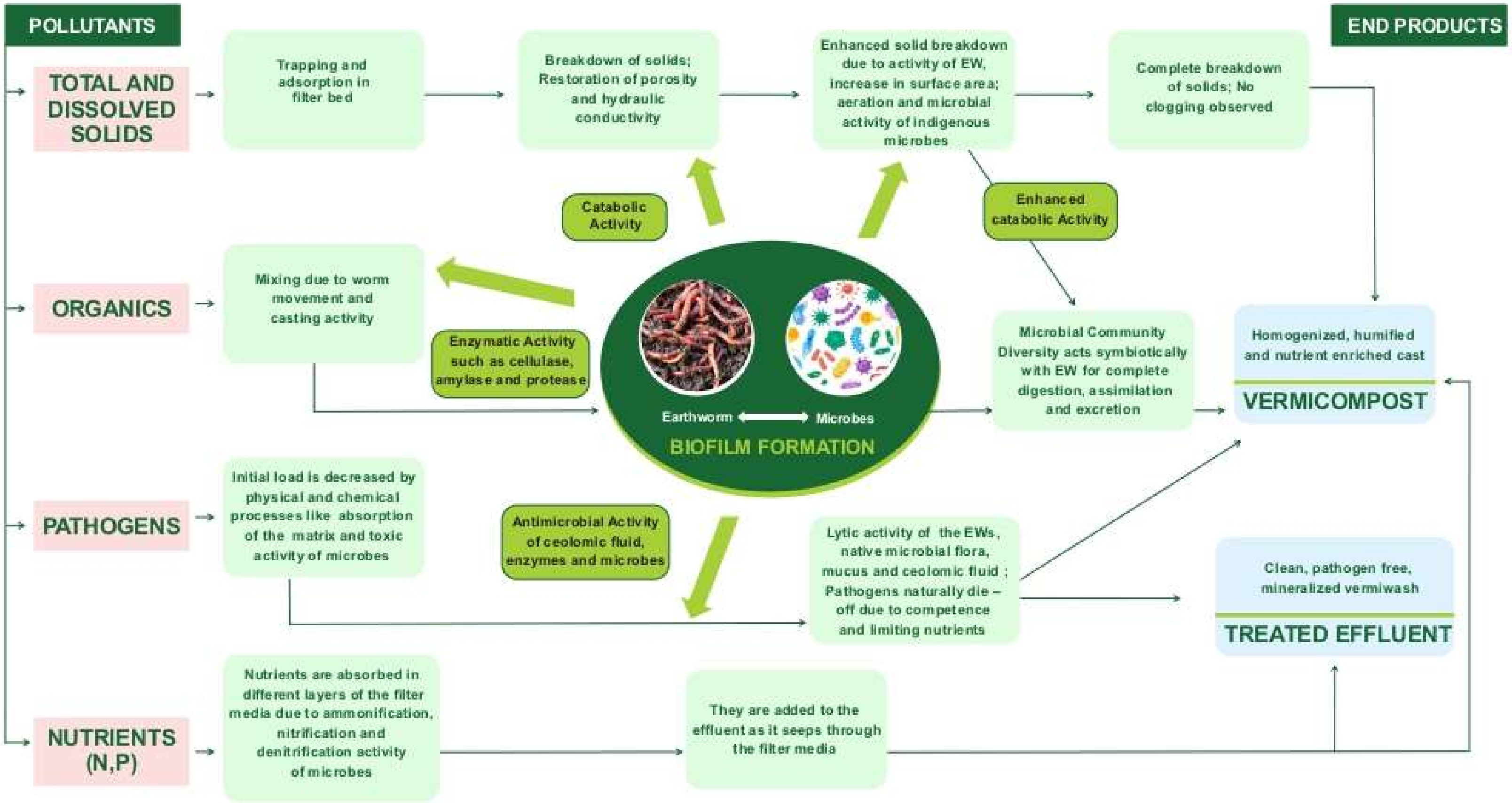

POLLUTANTS
END PRODUCTS
SOLIDS
Trapped and absorbed in VF bed
Breakdown of solids; Restoration of Porosity and HC
Burrowing leads to increase in SA, aeration and microbial activity of native microbes
Complete breakdown of solids. No clogging observed.
Catabolic Activity
ORGANICS
Enhanced catabolic Activity
Mixing due to worm movement and casting activity
EW
+
Microbes
MCD acts symbiotically with EW for complete digestion, assimilation and excretion
Homogenized, humified and nutrient enriched cast.
VERMICOMPOST
Enzymatic Activity such as cellulase, amylase and protease
PATHOGENS
BIOFILM FORMATION
Initial load is decreased by physical and chemical processes like absorption of the matrix and toxic activity of microbes
Antimicrobial Activity of CF, enzymes and microbes
Mucus, coelomic fluid and the native microbial flora present in the gut of the worm perform lysis of pathogens. They also naturally die-off due to competence and limiting nutrients.
Clean, pathogen free, mineralized vermiwash
TREATED EFFLUENT
NUTRIENTS
Nutrients are absorbed in different layers of the filter media due to the breakdown of organic matter, pathogens and solids by synergistic activity of EW and microbes.
They are added to the effluent as it seeps through the filter media.



Role of Earthworm-Microrganisms Interaction in AMR Reduction
Influent
Effluent
Pathogens from influent, reversibly attach to the solids and organics present in the Active Layer
Ingestion of solids and pathogens by the EW. Interaction of pathogens by indigenous and EW gut microbes
Biofilm formation induced by EW-Microorganisms synergistic interaction and external factors like O2, temperature, relative humidity, pH etc.
Shift in the resistivity pattern of pathogens due to selective grazing, antimicrobial and enzymatic activity of EWs and indigenous microbes.
Resistant Pathogens
Indigenous Microbes
Sensitive Pathogens

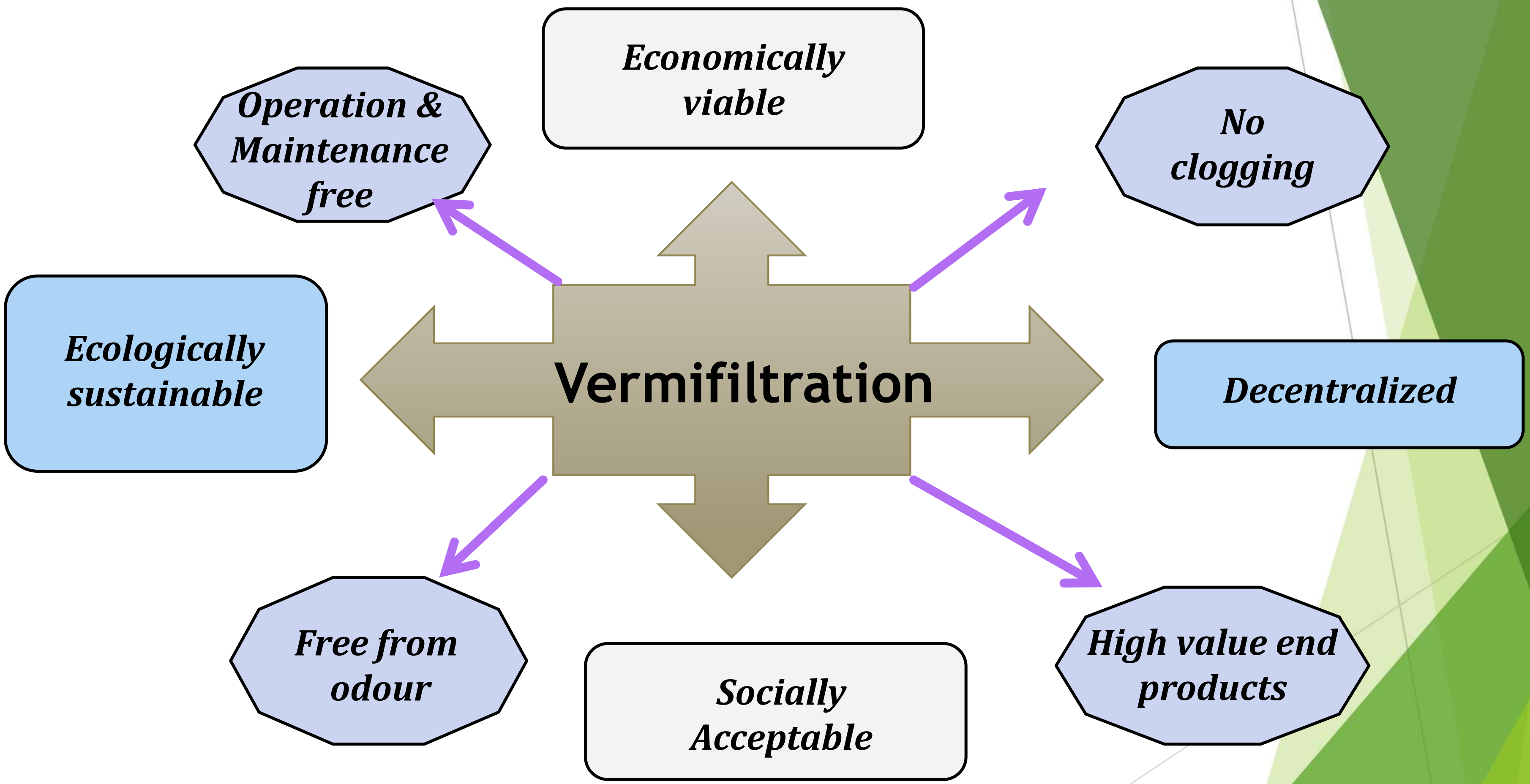

Inlet
Sprinkler
Mixer
Static Valve
Pre Treatment Tank
Institutional Wastewater
Empty Space
Earthworm's Bed & Specialized Microorganism
Supporting Layer (sand/gravels)
Empty Space
Outlet
Vermicompost
Treated Effluent
Mouth
Pharynx
Crop
Gizzard
Foregut
Midgut
Hindgut
Physical Breakdown
Chemical and Biological Breakdown
• Mechanical grinding and granulation of organic solids with the help of crop and gizzard.
• Release of trapped nutrients and minerals.
• Accelerated decomposition of organics and homogenization.
• Proliferation of beneficial microbes.
• Gut-bacterial simulations produce enzymes and antimicrobial components.
• Antimicrobial activity of ceolomic fluid enhances degradation and organic compound digestion
• Immobilization of pathogens by mucus, competitiveness for survival and natural die-off
• Endosymbiotic activity leads to complete lysis of pathogens.
• Secretion of nutrients by nephridial glands secret nutrients for a humified vermicast.
EARTHWORM-MICROORGANISM SYMBIOTIC AND SYNERGISTIC INTERACTIONS

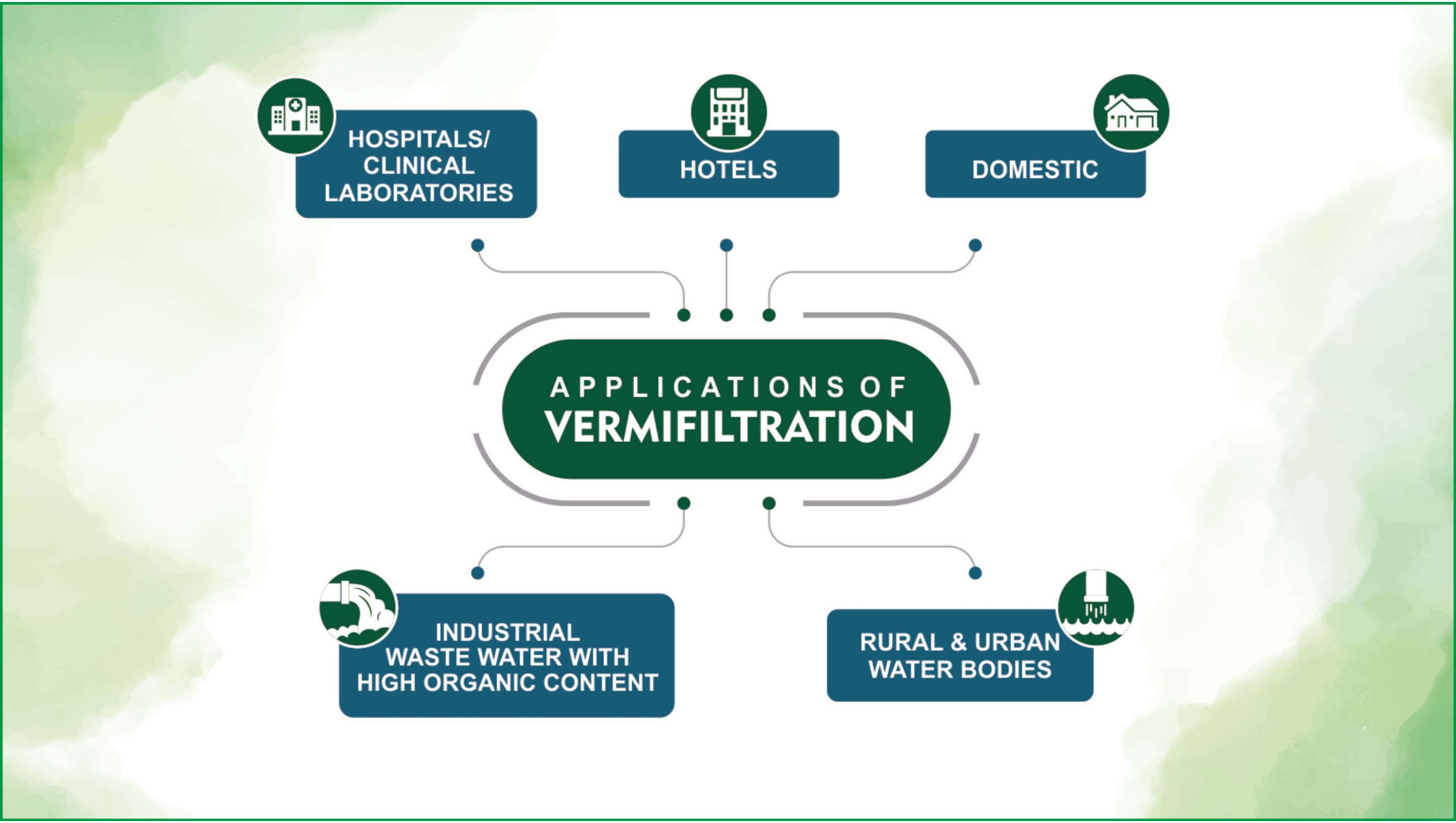
Mechanism of Earthworms- Microorganisms interactions



Significance

- Cost –effective treatment technology as it does not require external aeration, 80% decrease in operational cost because VF does not need aerating oxygen pumps and heavy maintenance.
- Does not produce odor as the entire system is aerobic due to burrowing activity of earthworms.
- No problem of clogging
- No problem of excess sludge production


Operation & Maintenance free
Economically viable
No clogging
Ecologically sustainable
Vermifiltration
Decentralized
Free from odour
Socially Acceptable
High value end products


HOSPITALS/ CLINICAL LABORATORIES
HOTELS
DOMESTIC
APPLICATIONS OF
VERMIFILTRATION
INDUSTRIAL WASTE WATER WITH HIGH ORGANIC CONTENT
RURAL & URBAN WATER BODIES

NOVELTY

Bioengineered & Customizable

Fully Customized & Unique design based on user centric requirements

Unique and Specialized Design

Specially developed bio-media by mixing organic fraction of **solid waste, cow dung, and vermicasts, and optimized operating conditions**

Specialized Micro Flora

Cultivated in our specialized laboratory from the body and coelomic fluid of earthworms

Scalability

Work on various scales from 1 KLD to 1 MLD and can be expanded according to the need

Applicability in various segments

Wide applicability in municipal sectors, individuals farm house, rural segments, Industries targets like hospitals, hotels, pharmaceuticals, etc.

OUR TEAM

PI/ Expert of Field

Dr. Sudipti Arora

- Research Scientist, Dr. B. Lal Institute of Biotechnology, Jaipur
- Ph. D (Environmental Engineering)
- Indian Institute of Technology (IIT), Roorkee
- More Than 8 Years Research Experience In Waste Management And Sanitation through Vermifiltration Technology

---

Co-PI/ Environmental Expert

Dr. Sonika Saxena

- Vice Principal, Dr. B. Lal Institute of Biotechnology
- Developmental/ Environmental Biotechnology

Microbiologist & AMR Specialist

Dr. Sandeeep K. Srivastava

- Associate Professor, Dr. B. Lal Institute of Biotechnology
- Molecular Medical Microbiology
- Transcriptomics & Imagery
- Allergy & Infectious diseases

Key Advisor/ Domain Expert

Dr. A. A. Kazmi

Professor,
Indian Institute of Technology
(IIT), Roorkee

Scientific Advisor

Dr. A. B. Gupta

Professor,
Malaviya National Institute of
Technology (MNIT),
Jaipur

Mentor

Mr. Akhilesh Trivedi

- Global Mentors & Strategist
- Startup & Technology Business Incubation Development

Sanitation & Design Expert

Mr. Aditya Chaudhary

Ph. D (MNIT, Jaipur)

Marketing

Mr. Rajat Sharma

ASST. General Manager
Marketing & Communication
Dr. B. Lal Group

Financial & Legal Advisor

Mr. Sankalp Gupta

Dr. B. Lal Group

Fulfilling the Sustainable Development
Agenda 3 and Agenda 6

Thank you for your attention!

For more details, Please contact: sudiptiarora@gmail.com