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)

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It's most unfortunate but it's true.
There is an urgent need to develop Affordable, Easy, Natural, sustainable and decentralised solution for wastewater and sludge treatment.
OPPORTUNITY

- Novel, Innovative, Green, Natural, Sustainable, technology
- Efficient, Effective, Affordable & Robust
- Less Operation & Maintenance, Easy to manage, Potential for scale up, sludge co-digested with sewage

COST EFFECTIVE
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

Symbiotic & Synergistic interactions

Decomposition & degradation of organic matter by the enzymatic activity

Earthworms

Removal of pathogens by antimicrobial activity

Specialized Microorganisms

High nutrient rich end products for recycle & reuse

OFMSW

Treated effluent for Non-potable reuse

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.
OUR RESEARCH FINDINGS IN LAST 8 YEARS

2012 - 2015
Lab scale & pilot scale researches on optimizing the vermicomposting technology

2016
Field scale vermicomposting and investigations on microbial community dynamics inside the vermicomposter & earthworms for the treatment process

2017
Post-treatment option for vermicomposter 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 plant 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 media, Overall performance VFR > VFM > VFG > VFC

VFM = Mud balls

VFC = Coal

VFG = Glass balls

OPTIMUM Filter media = River bed material.
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

- **Run 1: Stocking density**
  - 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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent (Avg ± SD)</th>
<th>Effluent VF (Avg ± SD)</th>
<th>% Reduction (R)</th>
<th>Effluent GF (Avg ± SD)</th>
<th>% Reduction (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.97 ± 0.1</td>
<td>7.23 ± 0.1</td>
<td>-</td>
<td>7.89 ± 0.2</td>
<td>-</td>
</tr>
<tr>
<td>Temperature</td>
<td>27.4 ± 1.5</td>
<td>27.2 ± 0.5</td>
<td>-</td>
<td>27.3 ± 1.5</td>
<td>-</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>0.05 ± 0.01</td>
<td>5.4 ± 1.5</td>
<td>-</td>
<td>4.45 ± 1.9</td>
<td>-</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>235.7 ± 30</td>
<td>15 ± 9.9</td>
<td>93.6%</td>
<td>25.5 ± 8.8</td>
<td>89.2%</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>463 ± 28</td>
<td>125 ± 25</td>
<td>73.1%</td>
<td>137 ± 28</td>
<td>70.4%</td>
</tr>
</tbody>
</table>

Neutralizing pH, Buffering capability
Aerobic conditions, Maintains DO naturally
Significant BOD & COD Removal
## RESULTS

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

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)

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

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

- **Gram-Negative rods**: 49%
- **Gram-Positive rods**: 29%
- **Gram-Positive cocci**: 22%

**Percentage composition of bacterial isolates**

- Bacillus: 22%
- Enterobacter: 37%
- Enterococcus: 12%
- Pseudomonas: 5%
- Aeromonas: 2%
- Corynebacterium: 5%
- Clostridium: 5%
- Micrococcus: 5%
- Staphylococcus: 5%
- Unidentified: 2%
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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Influent</th>
<th>Final effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.0-7.8</td>
<td>7.0-7.5</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25-30</td>
<td>25-30</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>40-100</td>
<td>&lt;5</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>0-0.5</td>
<td>3-4</td>
</tr>
<tr>
<td>Color</td>
<td>Grey or Dark Grey</td>
<td>Slightly pale yellow</td>
</tr>
<tr>
<td>Odor</td>
<td>Strong</td>
<td>Odorless</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>100-200</td>
<td>&lt;5</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>200-400</td>
<td>&lt;30</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>1000-1500</td>
<td>50-100</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>250-300</td>
<td>20-30</td>
</tr>
<tr>
<td>NO₃⁻ (mg/L)</td>
<td>30-50</td>
<td>10 ± 2.7</td>
</tr>
<tr>
<td>PO₄³⁻ (mg/L)</td>
<td>1-5</td>
<td>&lt;3</td>
</tr>
<tr>
<td>SO₄²⁻ (mg/L)</td>
<td>100-200</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Fecal coliforms (MPN/100 mL)</td>
<td>3-4 X 10³</td>
<td>Below Detection Limit</td>
</tr>
</tbody>
</table>

### Results of Performance Analysis for Clinical Laboratory wastewater treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Influent</th>
<th>Final effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6-8</td>
<td>7.0-7.5</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25-30</td>
<td>25-30</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>100-200</td>
<td>&lt;10</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>0-0.5</td>
<td>3-4</td>
</tr>
<tr>
<td>Color</td>
<td>Grey or Dark Grey</td>
<td>Slightly pale yellow</td>
</tr>
<tr>
<td>Odor</td>
<td>Strong</td>
<td>Odorless</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>200-250</td>
<td>&lt;15</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>350-400</td>
<td>&lt;30</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>1000-1500</td>
<td>50-100</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>250-300</td>
<td>20-30</td>
</tr>
<tr>
<td>NO₃⁻ (mg/L)</td>
<td>30-50</td>
<td>10 ± 2.7</td>
</tr>
<tr>
<td>PO₄³⁻ (mg/L)</td>
<td>1-5</td>
<td>&lt;3</td>
</tr>
<tr>
<td>SO₄²⁻ (mg/L)</td>
<td>100-200</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Fecal coliforms (MPN/100 mL)</td>
<td>3-4 X 10³</td>
<td>Below Detection Limit</td>
</tr>
<tr>
<td></td>
<td>Bacterial Diversity</td>
<td>Antimicrobial Activity Index</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td>Gram Positive %</td>
<td>Gram Negative %</td>
</tr>
<tr>
<td>Influent</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Earthworm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>180 days</td>
<td>12.5</td>
<td>87.5</td>
</tr>
<tr>
<td>360 days</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>Coelomic Fluid</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Active Layer</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Effluent</td>
<td>93.33</td>
<td>6.66</td>
</tr>
</tbody>
</table>
Results of Antimicrobial resistance during Vermifiltration

![Prevalence of Antibiotic Resistant Bacteria](image-url)

**Antibiotics**
- AMX10
- AMP10
- TCC75/10
- CAZ30
- CTX10
- CTR10
- S10/HLS300
- HLG120/GEN10
- E5
- TE10
- C10
- CIP10/5

**Legend**: ARB in influent □ ARB in effluent

- ARB: Antibiotic Resistant Bacteria
- AMX: Ampicillin
- AMP: Ampicillin
- TCC: Tetracycline
- CAZ: Carazidime
- CTX: Cefotaxime
- CTR: Cephradine
- HLS300: High Level Spectinomycin
- HLG120: High Level Gentamicin
- GEN10: Gentamicin
- E5: Erythromycin
- TE10: Tetracycline
- C10: Chloramphenicol
- CIP10/5: Ciprofloxacin

**Percentage Prevalence**
- 0
- 10
- 20
- 30
- 40
- 50
- 60
- 70
- 80
- 90
- 100
Role of Earthworm-Microorganisms Interaction in AMR Reduction

- **Influent**
  - 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.

- **Effluent**
  - 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  |
Mechanism of Earthworms- Microorganisms interactions

[Diagram of the mechanism of Earthworms- Microorganisms interactions showing the interactions between pollutants, organics, pathogens, nutrients, and end products. The diagram illustrates the process of biofilm formation, enzymatic activity, and nutrient absorption and enrichment.]
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
Vermifiltration

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

HOSPITALS/CLINICAL LABORATORIES

HOTELS

DOMESTIC

INDUSTRIAL WASTE WATER WITH HIGH ORGANIC CONTENT

RURAL & URBAN WATER BODIES
Cultivated in our specialized laboratory from the body and coelomic fluid of earthworms

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

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

Scalability
Work on various scales from 1 KLD to 1 MLD and can be expanded according to the need
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. Sandeep 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 (MNNIT), Jaipur

Mentor
Mr. Akhilesh Trivedi
- Global Mentor & Strategist
- Startup & Technology Business Incubation Development

Sanitation & Design Expert
Mr. Aditya Chaudhary
Ph. D (MNNIT, 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