GREENCAMPUS INITIATIVES

WATER AND WASTE MANAGEMENT

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The challenges addressed by the Karunya community to maintain and develop a Green campus include the following:

I. Scientific management of the natural resources of the campus namely water, land and bio-resources.
II. Developing a campus with zero waste-both solid and liquid by scientific waste management.
III. Conservation of the ecosystems and biodiversity of the campus and people by scientific conservation strategies.
IV. Energy conservation and resorting to sustainable energy sources-biogas and solar.
V. Reuse of waste materials-paper, food waste and solid and liquid waste.
VI. Restrictions on waste generation of all forms.
VII. Water conservation and recharge measures
VIII. Avoiding the use of hazardous materials.
IX. Awareness creation among students in the campus and hostels.
X. Introduction of courses and subjects on Environmental innovation and conservation in the curriculum.
XI. Establishment of NSS and different clubs to involve the student community.
XII. Complainer to the guideline of the international, national, state and local bodies.

1. Preamble

As an institution of higher educationand programmes in sciences, engineering, agriculture, management and media, Karunya is committed to teach and carry out research, extension and consultancy works in the areas of Sustainable Development Goals. The campus of Karunya with more than 300 acre of land serves as experimental and demonstration laboratories and to test field station and validate eco-friendly solutions to the problems in the areas of societal importance such as Water, Food, Healthcare and Sustainable energy. Most of the Technology missions of KITS also focus on research and demonstration projects in the areas related to a sound environment free from pollution sustaining the natural resources and ecosystems and conserving the biodiversity of the campus. These challenges have been recognized by the management and around 8000 students and faculty members residing in the Karunya campus right from the inception of the installation and all through the past three and half decades of existence.

2. Need of Green Initiatives in the campus

KITS campusis located in the foothills of Western Ghats known for its faunal and floral biodiversity. Karunya took up the challenge of conserving the natural resources, ecosystems and biodiversity of the campus restoring to the scientific and technological advancements andthe commitment of its student and faculty to build a green campus.

Further challenges and opportunities include:

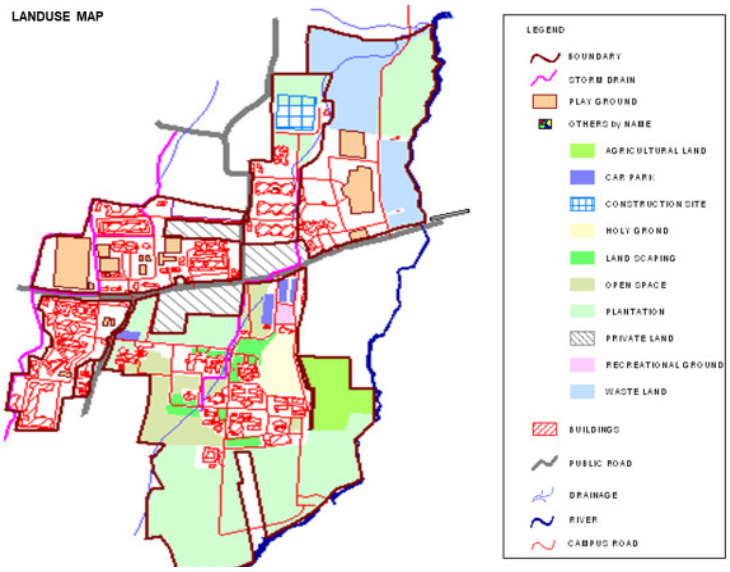

Figure 1 Land Use Map

- A fully residential with 13 residences for boys and girls (FDR, EGR, P.R. Garg Residence, HR, AR, JMR, SGBRR, JVR, BYR, EVR/Oprah, SRR, SPR, DMR).
- 15 Quarters (Zion, Alpha, Bethel, Elim, Canaan, Carmel, Kidron, Tabor, Sinai, Hebron, Frankincense, Pat Robertson, Antioch, Bethsaida, Beersheba) with around 500 residents.
- The agriculture land of 329 acres mainly utilized by the students and faculty of the

Department of Agriculture for their academic, research and community development activities.

3 Water Resource Management Strategies and Technologies

In KITS campus, there are 19 bore wells and 5 open wells which supply water to 166 storage tanks which meet the water demand of the students, faculty and staff residing in and around the campus. Thecampus area, residences, quarters and the agriculture land are given in the layout.

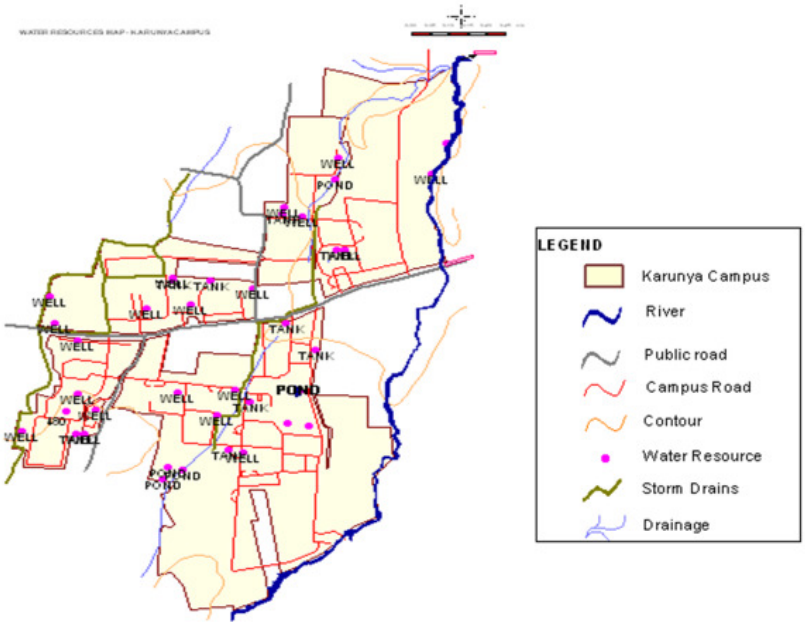

Figure 2Location of Water Resources

For efficient water management and effective handing of liquid, solidwaste and e-waste in the campus, green initiatives have been introduced. The major issues addressedin the integrated water management sector are: overflow of the storage tank and sump, grey and black water management, challenges of recycling and reuse of treated water and storm water management. The issues are tackled byinnovative, cost-effective and eco-friendly solutions and awareness creation on water conservation and management in the campus. The strategies, techniques and measures followed for sustainable development, management and conservation of water in the campus include:

1. Development of water harvesting structures (roof-top harvesting structures – ferrocement storage tank, and underground soak pits);
2. Water conservation measures (using IoT based automated water control structures);
3. Reuse of treated grey water (after bathing, laundry and dishwashing);
4. Recovery and utilization of biogas for cooking (in hostel kitchens) from black water (wastewater that includes human faeces, urine and other materials from toilets, urinals or bidets);

5. Water quality monitoring of treated water and suggesting remedial measures;
6. Capacity building and awareness creation programs, training workshops, extension and community development activities, innovative solutions, and pilot projects through various Karunya Technology Missions on 'Water and Desalination', 'Wetland Conservation', 'Rural Development', 'Smart Technology for Precision Farming Session' and 'Food Security'.
7. Development of innovative cost-effective and eco- friendly solutions to address the water related issues like availability of potable drinking water, grey and black water treatment, groundwater remediation through Interdisciplinary Research & Development activities in the campus and bringing out products and technology.
8. Water Auditing forms an integral part of the water management strategy in the campus; this is carried out by certified internal facultyand external agencies.

3.1 Water Demand

The water demand for meeting the water required of the residences, quarters, cooking and cleaning in the hostel residences, gardening and irrigation, laboratories and workshop in the campus is calculated based on the standards (135-150 lpcd).

3.2 Water Resources and Supply

3.2.1 Water supply from bore wells, Open wells and Siruvani water supply scheme

The water demand of the campus is met from available surface and groundwater resources (through 23 bore wells, 5 open wells, storm water storage in the campus and through the Siruvani water supply scheme).

3.2.2 Water distribution

Water is supplied from all the sources simultaneously to have 24 hours continuous supply of water in the whole campus. The main components of the water distribution system are:

- Main water supply sources
- Pump house
- Primary pipelines
- Overhead tanks
- Secondary pipelines
- End users

3.3 Rain Water Harvesting

3.3.1 Ferrocement Storage Tank (partially underground)

In the campus, a roof top rainwater structure (with capacity of 25,000 litres) made of ferrocement has been installed to collect the storm water from the roof of administrative block with an area of 1900 sq. m. The rain water that is being collected in the tank is supplied for washing purposes in the same block.

3.3.2 Soak Pit

Around 33 soak pits (3 m depth with 1.8 m diameter) are used to harvest roof top water, which are located in front and backside of all the academic departments and student residences. These structures improve the groundwater recharge in the campus and augment the groundwater potential. The details of locations of these structures and the area of catchment are furnished along with a photographs of (a) few of the structures.

Figure 3. Soak pits located at (a) Administrative block (b) Aero space lab (c) Civil lab (d) Guest house (e) Hepzibah hostel and (f) Father Duraisamy Residence hostel

3.4 IoT Enabled Water Conservation

The wastage of water due to the overflow in the storage tanks and sumps is controlled by using sensor-based pump operating system. The sumps in the campus and student residences are connected to ensure water supply at all times in the case of any reduction in groundwater level or mechanical failure of pumps in the borewells. Three IoT enabled automated water controllers have been installed in the overhead tanks and sumps by which 20% of water and energy are saved.

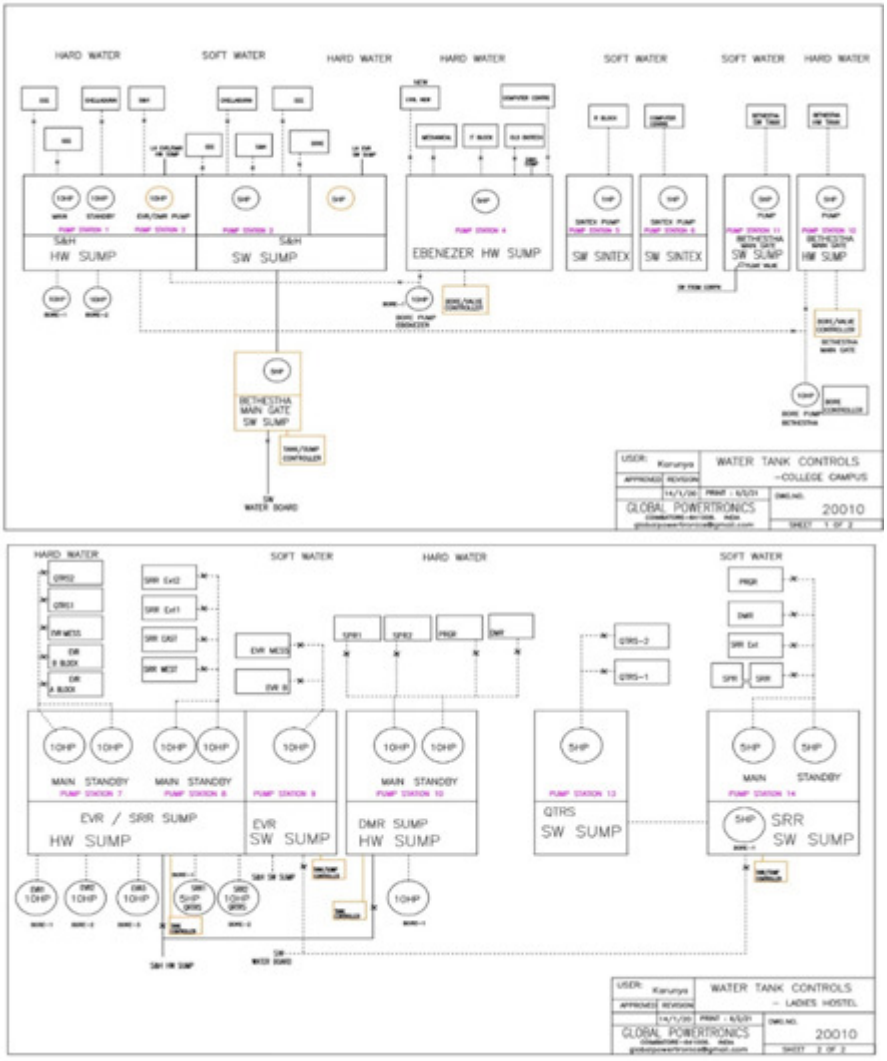

Figure 4.Layout of automatic waterflow control units

Figure 5Automatic Waterflow Control Units

4. Waste Management

4. 1 Liquid Waste Management

4.1.1. Recycling and reuse of greywater and blackwater treatment:

- Five Sewage Treatment Plants (STP) are available in the Student Residences to treat the grey water.
- Four Biogas Plants are available in the Students Residences to treat black water and recovery of biogas to substitute two to three commercial cylinders for cooking everyday.
- Treated or recycled wastewater is reused for gardening (from 118 STP treated water outlets)

Table showing the details on the capacity of each STP and the inflow rate with the quantity of treated effluent

Average Treated water Output from STP's in KITS Campuses				
Sl.No	Location	Capacity of STP	Wastewater Flow Rate in STP	Output (Treated Water)
1	JMR STP	1000 KLD	650 KLD	600 KLD
2	FDR STP	400 KLD	250 KLD	240 KLD
3	Ladies Hostel STP	450 KLD	250 KLD	220 KLD
4	PR GARG STP	600 KLD	350 KLD	320 KLD
5	Bethesda STP	8 KLD	4 KLD	4 KLD

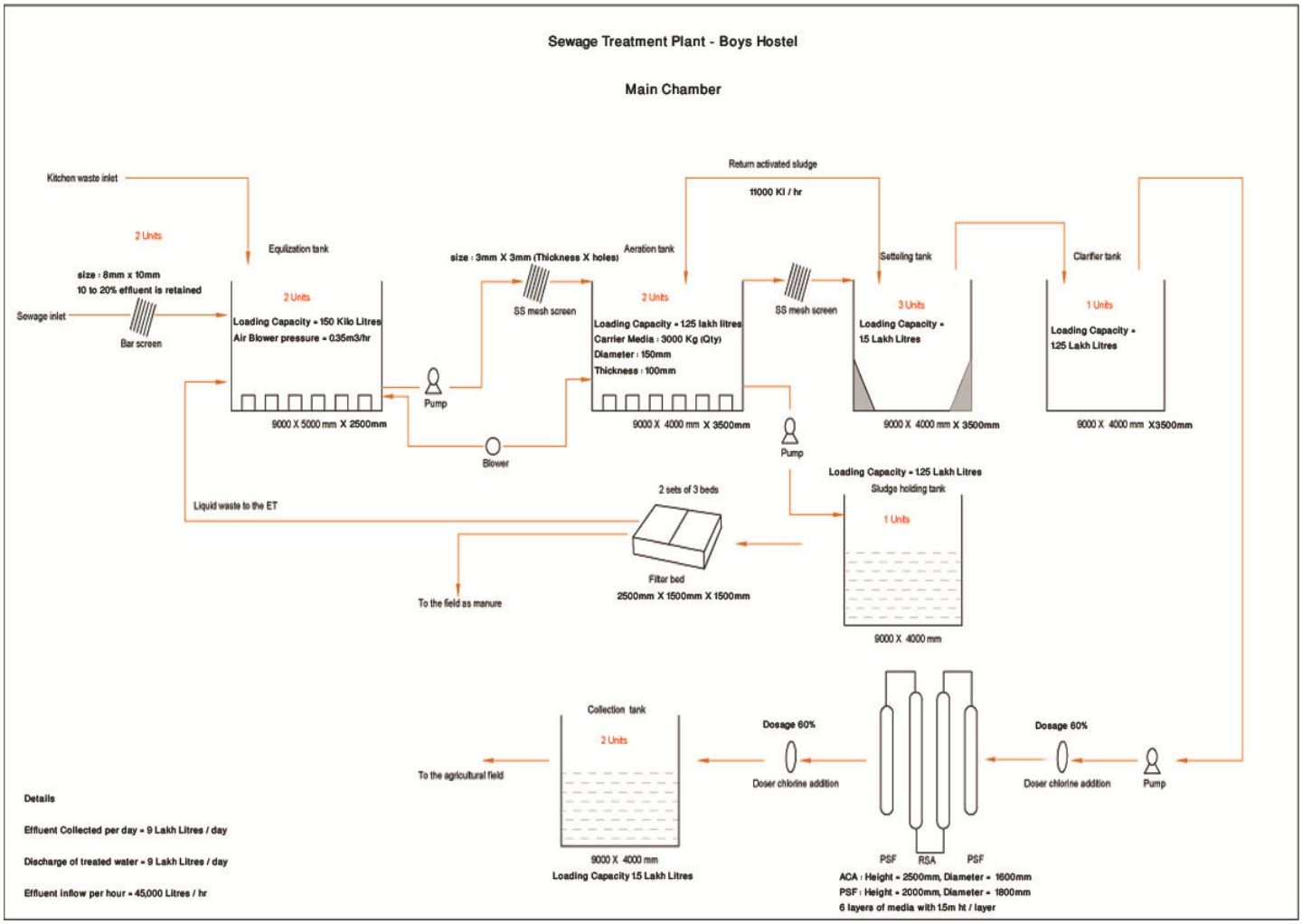

Figure 6. Layout of Sewage Treatment Plant

General Process and Functions:

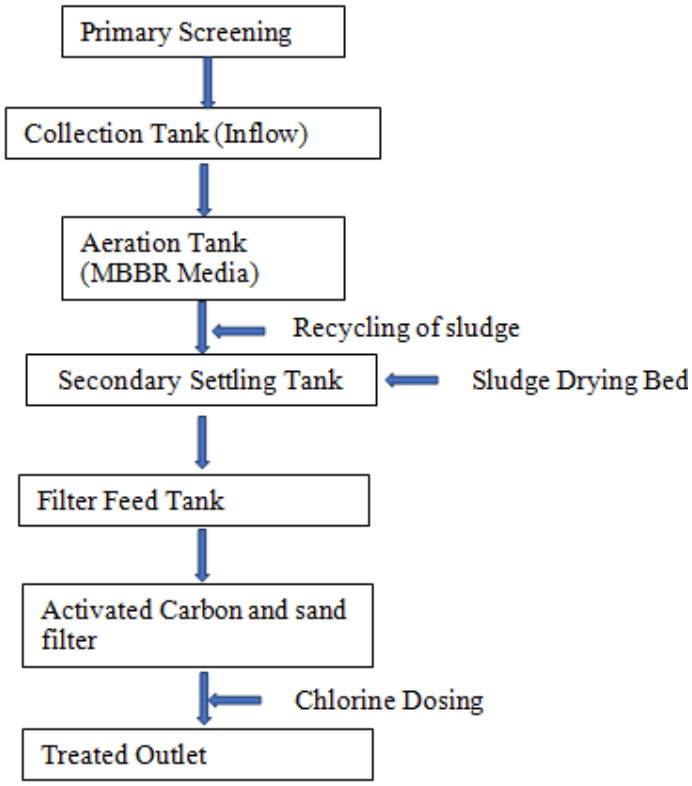

Figure 7.Steps involved in treatment process

Primary screening:

Screening essentially involves the removal of large non-biodegradable and floating solids that frequently enter the wastewater systems, these constitute of rags, paper, plastics, tins, containers, and wood.

Figure 8. Primary screening unit

*Fluidised bed bio-reactor:*The sewage is brought into a biological aeration basin where it is degraded by naturally occurring bacteria. After an "extended" period, typically 24 hours of detention time, the mixed liquor (ML) is sent to a clarifier, where it is allowed to settle. Secondary effluent (SE) is drawn off the clarifier and the settled biomass is returned to the head of the plant.

Figure 9.Fluidizedbed reactors

Settling tank:

In the settling tank, the sludge settles down in the hopper and is sent back to aeration tank to retain the biomass in the aeration tank.

Figure 10. Settling tank

Filtration system:

Thefiltration unit, that comprises of pressure sand filter and activated carbon filters, removes suspended matters such as flocs, micro-organisms, algae etc.

Figure 11. Filtration system

Sludge drying bed:

The generated sludge is allowed to dry by evaporation and excess water is drained over a period of several weeks depending on the climatic condition.

Figure 12. Sludge drying bed

Reuse of Treated Water: The treated water is reused for gardening and in the agricultural farm.

JMR Details:

Figure 13 JMR collection tank, settling tank (after filtration)

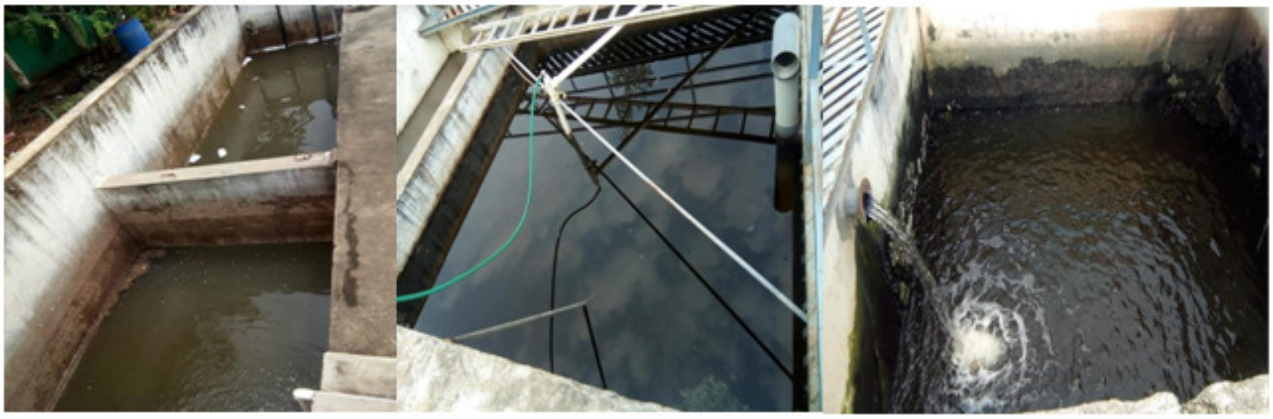

Figure 14 FDR collection tank, settling tank (after filtration)

Figure 15 LH collection tank (after filtration)

Figure 16. PRG collection tank, settling tank

Figure 17. Storage tanks for treated water (before reuse in thegarden)

Figure 18. JMR and LH STP – aeration tanks

Figure 19. Outlet for the recycled wastewater from STP

Cleaning of tank:

Figure 20. JMR STP cleaning – Collection tank, settling tank, filtered water tank and reuse water storage tank

Figure 21 FDR STP Cleaning – Collection tank, settling tank, filtered water tank and reuse water storage tank

Figure 22. LH STP Cleaning – Collection tank, settling tank, filtered water tank and recycled water storage tank

Figure 23. PRG STP CLEANING – Collection tank, settling tank, filtered water tank and reuse water storage tank

4.2. SolidWaste Management

Based on the study conducted by 'M/S Hand in Hand India Ltd.' in Karunya Campus, it is estimated that an average of 7,500 kg of waste is generated daily, the breakup is given below:

i) Food waste (37%)

ii) Recyclable waste (27%)

iii) Other organic waste (36%).

Collection and Segregation of bio-degradable andnon-degradable waste materials:

- For the purpose ofsegregation of waste (organic, recyclable, non-recyclable and e-waste) at source and collecting the same,Waste Bins (3,000 numbers) have been placed at designated locations in the Karunya Campuses, viz. KITS, EMHSS, KCS, Students Residences and Staff Quartersand Guest House.
- A Contractor, namely, M/s Metro Support Services, Coimbatore**,**has been engaged for the collection, segregation and shiftingof waste materials generated in the Campus.
- Organic compost is generated from biodegradable wastes in Karunya North Farm.

Details of the waste management practices in Karunya Institute of Technology and Sciences are highlighted below:

1) Bio-degradable waste handling
2) Paper recycling plant
3) Bio-gas plants (4 Nos.)
4) Disposal of e-Waste

4.2.1 Biodegradable waste handling

1. Food waste: A portion of food waste is being pulverized and used in the bio-gas digester

2. Organic waste like dry leaves, vegetable cuttings, etc are sent for bio-composting.

4.2.2 Paper recycling plant

Around 150 - 200 kg (45,000 – 60,000 kg for 300 days in a year) of wastepaper is segregated from waste generated in the Campus daily.To recycle the segregated wastepaper, an Eco-friendlyPaper Recycling Planthas been installed in the campus to produce 25 – 30 tons of paper board every year. Products like files, folders and decorative articles are made using the paper boards.

Processing of wastepaper to produce paper boards (grey boards)

1. Pulping

Figure 24. Hydro Pulper

Raw material (waste paper) is fed into the hydro pulper and wet grinding is done for 30 minutes.

2. Refining

Figure 25. Disc Refiner

The paper pulp is refined by the Disc Refiner and pumped into the pulp chest. Here, the agitating impellers agitate the pulp for constant consistency. Then the pulp is mixed with back water and pumped into the regulating tank of the flow mold wet-end paper making machine.

3. Board making (wet boards)

In this process, the pulp is molded as layers by the molding wire cloth, picked up by the couch roll and transferred to the press part through the conveyer. In the press part, the wet layer is squeezed between the press rubber roller and the heavy metal cutting drum. Wet paper boards are collected from the cutting drum and transferred to the drying shed.

Figure 26. Cutting drum

Figure 27. Rubber Roller

4. Drying

In the drying process, the wet boards are allowed to dry in the sunlight (sundry) for 8 hours.

Figure 28. Sun Drying

5. Calendaring and Cutting of Paper Boards

   The dried papers are polished by the Calendaring machine followed by edge trimming and cutting into desired size and packed.

Figure 29. Roller Mold

Figure 30. Edge Trimming

Figure 31. Final Board

4.2.3 Biogas project

Biogas – an overview

- A biogas plant is a decentralized energy system, which leads to self-sufficiency in heat and power needs, and at the same time reduces environmental pollution.
- Biogas is a gas mixture of carbon dioxide (CO_2) and methane (CH_4), which is generated when organic compounds are fermented in the absence of air (anaerobic fermentation).
- Organic matter such as manure (human or animal) is composed and used to feed the plant.

Biogas plants in Karunya Campus

Being a residential campus, thenight soil and food waste generated in the Student Residences of Karunya Campus are treated in the biogas plant installed in the following locations:

Sl. No.	Location	Capacity of the Bio-gas Plant	Year of Installation	Cost of the Plant (in Lakhs)	Savings in terms of LPG Cylinders (19Kg) /Day	Savings in terms of cost /Year (Rs.)
1	FDR Campus	$100m^3$	2017	32.0	2 Nos.	6.60 Lakhs
2	JMR Campus	$80m^3$ (Multifeed)	2010	26.0	2 Nos.	6.60 Lakhs
3	Ladies Hostel (PRG Campus)	$100m^3$	2017	32.0	2 Nos.	6.60 Lakhs
4	Ladies Hostel (EVR Campus)	$80m^3$	2017	26.0	1.5 Nos.	4.90 Lakhs

- The treated effluent from the biogas plant is diverted to the STP for storage and utilization for irrigation/gardening. Thisreduces the organic load coming to two STPs of a capacity of 6 and 4.5 lakh litres of sewage and their operational and maintenance cost.
- The biogas produced from the plant can be utilized for cooking, and

- The residual dung or the digested slurry left after generating biogas is used as manure for agricultural purposes.

4.2.4 Disposal of e-waste

- In compliance to the E-Waste Management Rules, 2016, Government of India,e-waste materials collected from Karunya Institutions are being segregated and besold to agencies which are authorized by the Polution Control Board (PCB) for handling e-waste.
- The details of sale of e-waste during the last 5 years given below:

Sl.No.	Year	Qty. Sold	Name of the agency with address
1.	2017	1,500 kg	M/s Green Recycling, 48, Vijay Building, Near Sakinaka Telephone Exchange, Kurla Andheri Road, Mumbai – 400 072.
2.	2019	4,630 kg	M/s Green Era Recyclers, SF No. 344/2, Sivanandha Industrial Complex, Dr.MS Udhayamurthy Nagar, Door No. 37, Thadagam Road, Edayarpalayam, Coimbatore – 641 025.
3.	2020	4971 kg	M/s Green Era Recyclers, SF No. 344/2, Sivanandha Industrial Complex, Dr.MS Udhayamurthy Nagar, Door No. 37, Thadagam Road, Edayarpalayam, Coimbatore – 641 025.

5.Annexure 1: Scientific Interventions

i. Evaluating the performance of the unit operations in the STPs, based on modeling and simulation studies – Model

ii. Periodic monitoring and characterization of the raw and treated effluents from STPs

iii. Biochar preparation using agricultural farm waste for purification of contaminated water

iv. Isolation of microbes from campus STPs for the bioremediation of waste water and treatment of effluents from small scale textile dyeing units in and around Coimbatore

v. Treatment of used cutting fluid from mechanical workshops on campus using the Petroleum Remediation Product (PRP®), a NASA product from UniRem Technology, Pittsburgh, USA

vi. Treatment of greywater using microbial fuel cells (Publication) Recycling and reuse of detergent water using electrocoagulation technology

vii. A conceptual model on decentralized wastewater treatment at household and community level

6. **Annexure 2: Related Documents**

(Purchase orders, Bills related to purchase, maintenance and operation).

Annexure 1
Scientific Interventions

Annexure 2

Documents

(Purchase orders, Bills related to purchase, maintenance and operation).

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ORIGINAL ARTICLE

Desalination and removal of organic pollutants using electrobiochemical reactor

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Abstract

Electrobiochemical reactor is one of the energy sustainable technologies for desalination which simultaneously treats waste water by microorganisms that are used as the catalyst for removal of salts and energy production. The microbial-induced electrochemical reaction occurring during the process and the energy produced in the reactor induce desalination. The reactor used in this study consists of 3 chambers: anode, middle and cathode which are separated using the ion exchange membranes. In this study, an air cathode with two different catholytes was used and comparative studies were carried out. The use of acidic water in the cathode chamber showed more efficiency than the phosphate-buffered solution. There were maximum removal of 60% electrical conductivity and 100% hardness during treatment. The maximum current of 1 mA and voltage of 850 mV were generated using this reactor. Apart from this, there is a reduction in 45% BOD removal in the anode chamber. The maximum power density and current density obtained were 251.8 mW/m^2 and 296.29 mA/m^2, respectively. The characterization of the deposited sludge on the membranes during the reaction was performed using scanning electron microscopy, X-ray diffraction, Fourier transform infrared spectroscopy.

Keywords Electrobiochemical reactor · Desalination · Membrane · SEM

Introduction

Water scarcity is the major problem faced by every continent. A few studies show that around 1.2 billion people live in water scarce areas and 500 million people are approaching this situation (United Nations Department of Economics and Social Affairs 2003). Countries like India, Kenya, Yemen, Crimea and others face severe water shortage (Glass 2010; Marshall 2011; Prakash et al. 2013; Tänzler et al. 2011). Only 3% of water is fresh and remaining 97% is found in ocean and sea as salt water. This uneven distribution and global water shortage have necessitated the requirement for desalination (Lattemann et al. 2010; Greenlee et al. 2009). Most of the current desalination technologies are energy-intensive (Carter 2013; IREA 2012; World Bank 2012; Garud et al. 2011; Elimelech and Phillip 2011; Semiat 2008). Though there are many studies carried out in minimizing the energy consumption, still it remains as a challenge (Zhang 2012).

It is estimated that in the next 20 years the average per capita supply of clean water will decrease by one-third. Desalination is one option for producing potable water from brackish water and seawater in many parts of the world, but most water desalination technologies are energy- and capital-intensive. The main desalination technologies currently used are reverse osmosis, electrodialysis and distillation. Continual improvements in desalination processes, particularly in the past decade, have made these systems more reliable and have reduced capital costs, but high energy requirements remain a concern in many parts of the world. Increasing attention is being placed on developing desalination processes powered by renewable energy, such as solar- and wind-driven electricity (Zhang 2012). New membrane systems are also being developed that reduce the need for high water pressure through the use of forward osmosis. All of these systems, however, require heat sources or electrical energy input. Reverse osmosis units require a minimum of 3–5 kWh/m^3 for water desalination (Figs. 1 and 2).

Bioelectrochemical systems (BESs) represent an array of processes capable of converting the chemical energy

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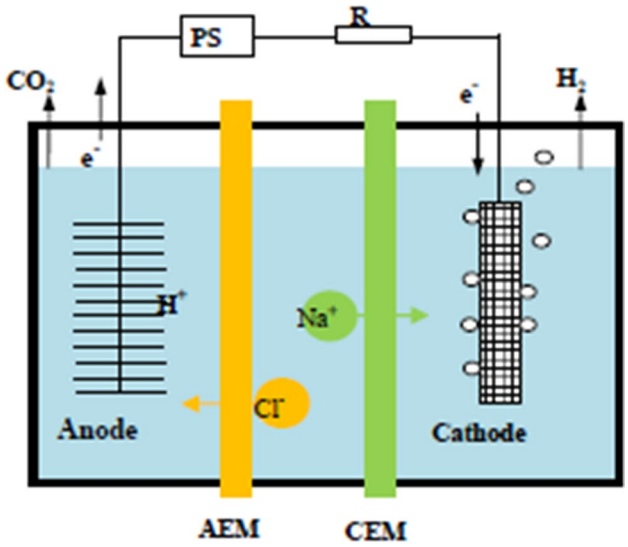

Fig. 1 Microbial desalination cell (Luo et al. 2010)

Fig. 2 Laboratory setup of microbial desalination cell

embedded in biodegradable materials including wastewater and sediments into direct electricity. BES is a new bioelectrochemical process that offers simultaneous water desalination, renewable energy production and wastewater treatment. A new technology for seawater desalination was recently developed on the basis of microbial fuel cell (MFC), called microbial desalination cell (MDC). The working principle of MDC is on the basis of a phenomenon that ion transport is needed to maintain charge balance in the anode and cathode of MFCs. The main difference of MDC compared with MFC is that MDC contains an additional desalination chamber between anode and cathode chambers separated, respectively, by AEM and CEM. In a typical three-chambered MDC system (Fig. 5), when electrochemically active bacteria oxidize organic matters and transfer electrons to the external circuit, protons are released into solution and accumulated in the anode, the AEM deposited in the anode side prevents positively charged species from leaving the anode, and thus, negatively charged species move from the middle chamber to the anode to maintain charge balance. In the cathode chamber, protons are consumed to form water with electrons, resulting in the movement of positively charged species from the middle chamber to the cathode chamber. In such way, the salt ionic species are removed from the middle chamber resulting in water desalination.

The desalination using electrobiochemical reactor (EBCR) is a new technology which treats the salt water in an energy-efficient way (Ren and Luo 2010; Kim and Logan 2013). It is designed by modifying microbial fuel cell by including an additional chamber in the middle (Mehanna et al. 2010). It usually consists of three chambers: anode, middle and cathode, separated using ion exchange membranes (Kim and Logan 2013; Mehanna et al. 2010; Cao et al. 2009; Luo et al. 2012a). In EBCR, exoelectrogeneous bacteria oxidize organic matter present in the wastewater in anode chamber and release electrons to the anode electrode and protons to the water (Mehanna et al. 2010). Anion exchange membrane which is provided between anode and middle chambers prevents the movement of protons from anode chamber. The charge carried by the protons is balanced by anions (Cl^-) separated from the desalination chamber into the anode chamber.

In the cathode chamber, protons released from catholyte combine with electrons and oxygen to form water. The electrical neutrality occurs with charge balanced by cations (Na^+) separated from the desalination chamber. The reactions occurring in the anode and cathode chamber are given below:

Anode Chamber

$$C_6H_{12}O_6 + 4H_2O \rightarrow 6HCO_3 + 3OH + 24e^-$$

Cathode chamber

$$4H^+ + 4e^- + O_2 \rightarrow 2H_2O \text{ (Sabat et al. 2013)}$$

Based on the factors such as stability, conductivity, biocompatibility, strength and resistivity, the selection of the electrodes is being done. The large surface area of the anode facilitates the effective growth of bacteria responsible for degradation. The electrode modified with catalysts in the cathode chamber increases the rate of oxygen reduction. Generally, the cathode and anode may of the same material. The commonly used materials are carbon brush, graphite rod, carbon paper, etc. In few studies, ferricyanide has been used as catholyte. In this study, graphite electrode was positioned as an anode and air cathode in cathode chamber. To reduce the internal resistance and increase the power generation, membranes are used. In addition, the solutions in the anodic and cathodic compartments are to be replaced several times over a cycle, resulting in less

efficiency (Mehanna and Logan 2010). Advanced studies using stacked model, series of stacked model, series of EBCR, upflow EBCR and algae-mediated microbial cell have been carried out by researchers (Kim and Logan 2011; Zhang and He 2012; Kokabian and Gude 2012; Qu et al. 2013). EBCRs are also used for softening the water and for removing the heavy metals also (Brastad and He 2013). EBCR proves to be more efficient than MFC in energy production and waste water treatment (Luo et al. 2012b). But the pH imbalance is the major problem faced by EBCR. The recirculation of anode and cathode is a favorable solution for this problem (Qu et al. 2012). Biofouling and scaling of membrane reduce the efficiency of EBCR (Brastad and He 2013; Ping et al. 2013). By applying external voltage, hydrogen gas can be released from the cathode chamber (Ren and Luo 2010).

Materials and methods

Reactor design

The EBCR used in this study is a three-chambered bioelectrochemical reactor. It has three chambers like anode chamber, middle chamber and cathode chamber. The anode and middle chambers were separated using an anion exchange membrane (AEM; AMI-7001, Membrane International, Inc., NJ, USA), and the cathode chamber and the middle chamber were separated using a cation exchange membrane (CEM; CMI-7000, Membrane International, Inc.). The membranes were preconditioned by emersion in 5% NaCl solution at 40 °C for 24 h for membrane expansion and hydration. Graphite rods of 5 cm diameter and 10 cm height were inserted into the anode and cathode chamber as the anode electrode and cathode electrode, respectively. Each chamber was designed with a volume of 3375 cm^3. The liquid volumes in all the chambers were kept approximately at 3000 ml. The electrodes were connected using electric wire using a closed circuit with a multimeter.

Operating conditions

In the anode chamber, sodium acetate was added to ensure an adequate electron supply to drive. About 3 g of NaCl, 0.5 g of $MgSO_4$, 0.015 g of $CaCl_2$, 0.02 g and 0.53 g of vitamins and glucose were added as nutrients for the active growth of microorganisms responsible for degradation of organic matter. About 50 mM phosphate-buffered solution (PBS) was added to maintain pH and to increase the alkalinity. The aerobic sludge from aeration tank of wastewater treatment plant was inoculated into the anode chamber as a seed for acclimatization process. The anode chamber was maintained in anaerobic condition to get more efficiency. The anolyte was replaced with fresh waste water after 24 h in the first cycle, whereas it was replaced after 12 h in the second cycle. In the first operation cycle, 50 mM phosphate-buffered solution (8.5 g of KH_2PO_4, 21.75 g of K_2HPO_4, 33.5 g of $Na_2HPO_4{\cdot}7H_2O$, 1.7 g of NH_4Cl) with conductivity 12 mS/cm and pH 7.2 was used as a terminal electron acceptor catholyte. In the second cycle, acidic water of pH 1 prepared by adding sulfuric acid to tap water was used. Air cathode was used in this reactor. The solution in the cathode chamber was continuously aerated to provide dissolved oxygen. The seawater sample from Nagapatinam, Tamil Nadu, and Calicut, Kerala, was kept in the middle chamber for treatment. The reactor was operated at room temperature (31 °C). All the solutions were replaced once the voltage generation was less than 40 mV.

Performance of EBCR based on analysis of parameters

The power generation is determined by the potential between the anode and cathode which is one of the important parameters responsible for movement of electrons. The anode and cathode potentials determine the generation of theoretical maximum voltage generated by EBCR. The overall calculations depend on the electrochemical reactions that occur in between the low potential anode (electron donor, i.e., substrate) and high potential cathode (electron acceptor). The parameters such as working voltage on the external resistance are monitored by multimeters, voltage meters and data acquisition systems. Based on the measured parameters, the current generated is calculated by Ohm's law and the energy efficiency (ε) is another important parameter for evaluating how efficient of MFC compared with more traditional techniques such as anaerobic digestion. The systematic energy efficiency is calculated as the ratio of power actually produced EBCR to the heat energy obtained by substrate combustion. Electrode potential (V) $E = \mathrm{Eo} - \mathrm{RT}/(\mathrm{nF})\ln(a_{\mathrm{red}}/a_{\mathrm{oxy}})^a$; current (A) $I = E/R$, E is voltage, R is external resistance (Ω); power (W) $P = E^2/R$ or $P = IE$; current density (A/m^2) $I_A = I/A$, A is projected electrode surface area (m^2); power density (surface area) (W/m^2) $P_A = E^2/R/A$; volumetric power density (W/m^3) $P_A = E^2/R/v$, v is the reactor volume (m^3).

Characterization studies using XRD, SEM and FTIR

XRD is a nondestructive technique to identify atomic arrangement in crystalline phases, phase composition, structural properties like lattice parameters (10^{-4} Å), grain size, thickness of thin films and multi-layers. By X-ray diffraction, the atomic planes of a crystal cause an incident beam

of X-rays to interfere with one another as they leave the crystal. Although Bragg's law ($n\lambda = 2d\sin\theta$; the variable 'd' is the distance between atomic layers in a crystal, and the variable lambda 'λ' is the wavelength of the incident X-ray beam; 'n' is an integer) was used to explain the interference pattern of X-rays scattered by crystals, diffraction has been developed to measure the average spacing between layers or rows of atoms, determine the orientation of a single crystal or grain, find the crystal structure of an unknown material and measure the size, shape and internal stress of small crystalline regions.

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample with resolution better than 1 nm, by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals, containing information about the sample's surface topography and composition. The number of secondary electrons emitted is a function of the angle between the surface and the beam. On a flat surface, the plume of secondary electrons is mostly contained by the sample, but on a tilted surface, the plume is partially exposed and more electrons are emitted. By scanning the sample and detecting the secondary electrons, an image displaying the tilt of the surface is created.

FTIR stands for Fourier transform infrared, in which IR radiation is passed through a sample resulting in a spectrum after partial absorbance of radiation by the sample and partial transmission of the radiation through the sample. No two unique molecular structures produce the same infrared spectrum that makes infrared spectroscopy useful for several types of analysis. It is a technique for quantitative analysis to identify the amount of components of unknown materials and determine the quality or consistency of a sample.

Measurements and analyses

Due to the variation in reactor design and operation conditions adopted by researchers, uniformity in data reporting is required to compare the results among different systems The performance of EBCR can be determined by the parameters such as voltage, power, power density (reactor), power density (anode/cathode), volumetric power density (reactor), volumetric power density (anode/cathode), current density (reactor), current density (anode/cathode), volumetric current density (anode/cathode), volumetric current density (reactor). The cell voltage, current and resistance were monitored every 1 h using a digital multimeter (Aplab VC97). The conductivity and total dissolved solids were measured using water quality analyzer kit (ELICO WQ Analyzer). The pH was measured with a benchtop pH meter (Hanna Instruments). Using the titrimetric method, the total hardness and chloride were determined (APHA standard methods). Current density (reactor), power density (reactor) and power density (anode/cathode) were calculated based on the observed values of current and voltage. The Biochemical Oxygen Demand (COD) of wastewater and anolyte (after and before changing) was measured using analytical method.

Table 1 Characteristics of waste water used in the study

Parameters	Values
BOD	160 mg/l
Conductivity	2 mS/cm
Alkakinity	200 mg/l
pH	7

Table 2 Characteristics of seawater samples

Parameter	Seawater from Nagapatinam coast (Sample I)	Calicut coast (Sample II)
Conductivity (mS/cm)	50	50.5
TDS (ppt)	31	32.32
Hardness (mg/l)	3000	3300
Chloride (mg/l)	18,744	20,022
pH	7.35	7.47

Results and discussion

Performance of EBCR

The wastewater from the influent to the aeration basin of Karunya Sewage Treatment Plant was used as a substrate in anode chambers of two identical EBCRs. The seawater samples taken from Nagapatinam coast, Tamil Nadu, and Calicut coast, Kerala, were filled in the middle chamber for treatment. The characteristics of wastewater and seawater samples are given in Tables 1 and 2. Both reactors were operated for almost the same lengths of time (140 h). PBS was used as catholyte in the first reactor while acidic water of pH 1 was used as catholyte in the second reactor.

In the first reactor with PBS as catholyte, there was a maximum current generation of 0.37 mA and voltage production of 460 mV. The maximum current density and power density obtained were 109.6 mA/m^3 and 50.37 mW/m^3, respectively. In the other reactor which was operated with acidic water as catholyte, there were better current generation and voltage generation. The maximum current and voltage obtained were 1 mA and 850 mV. The power

Table 3 Performance of EBCR during cycles

	Parameters	Cycle I	Cycle II
1	Current	0.37 mA	1 mA
2	Voltage	460 mV	850 mV
3	Power	0.17 mW	0.85 mW
4	Power density (reactor)	0.83 mW/m^2	4.19 mW/m^2
5	Power density (anode/cathode)	7.5 mW/m^2	37.7 mW/m^2
6	Volumetric power density (reactor)	1.86 mW/m^3	9.32 mW/m^3
7	Volumetric power density (anode/cathode)	50.37 mW/m^3	251.8 mW/m^3
8	Current density (reactor)	1.82 mA/m^2	4.9 mA/m^2
9	Current density (anode/cathode)	16.44 mA/m^2	44.4 mA/m^2
10	Volumetric current density (anode/cathode)	109.6 mA/m^3	296.29 mA/m^3
11	Volumetric current density (reactor)	4.06 mA/m^3	10.97 mA/m^3

Table 4 Performance of EBCR in removing salts from Sample I

S. no.	Parameters	Initial	Final	Percentage reduction
1	EC (mS/cm)	50.50	20	60.3
2	TDS (ppt)	32.32	12.8	60.3
3	Hardness (mg/l)	3300	0	100
4	Chloride (mg/l)	20,022	9230	53

Table 5 Performance of EBCR in removing salts from Sample II

S. no.	Parameters	Initial	Final	Percentage reduction
1	EC (mS/cm)	50.36	25.01	50.33
2	TDS (ppt)	32.23	16.00	50.35
3	Hardness (mg/l)	3000	0	100
4	Chloride (mg/l)	18,744	10,372	44.6

density and current density obtained were 251.8 mW/m^3 and 296.29 mA/m^3 (Tables 3, 4 and 5).

Desalination

There was 50% and 60% removal of EC in the first and second reactors, respectively. It was observed that there was 100% removal of hardness in both reactors. Graphs which correlate the current, voltage and percentage reduction in EC are given (Figs. 3, 4 and 5). It clearly shows that in the 100th hour there is a drop in current and voltage, whereas the percentage reduction in EC does not change after that hour. The graph correlating power density and current density show that there is a drop at 100th hour (Fig. 5).

BOD removal

The maximum reduction in BOD_5 was found to be 38% in the first reactor, and in the other one, there was 45% removal. It indicates that waste water is being treated and organic matter is getting degraded in the anode chamber (Table 6).

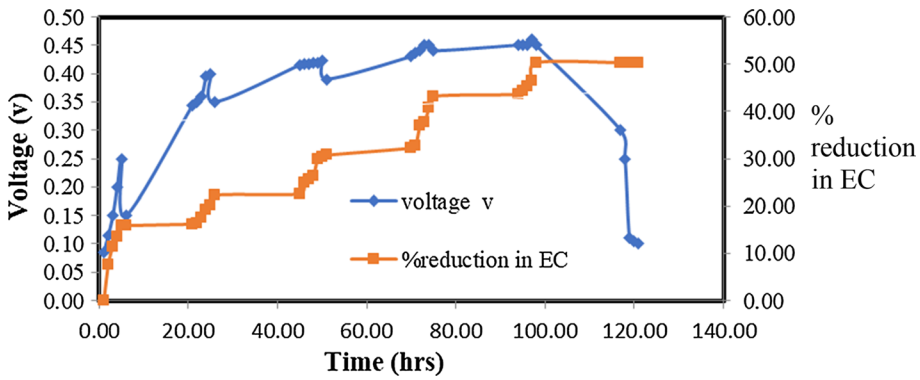

Fig. 3 Voltage versus reduction percentage in EC

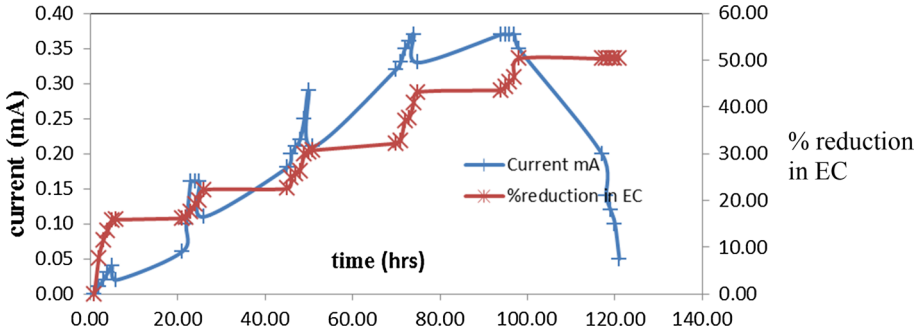

Fig. 4 Current versus percentage reduction in EC

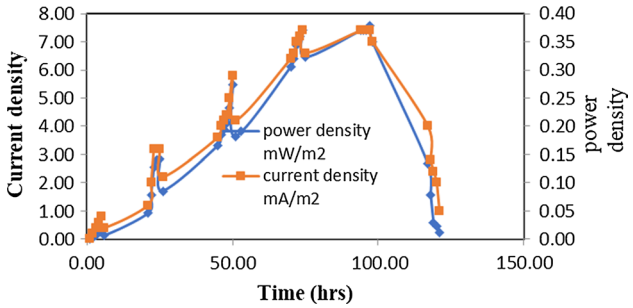

Fig. 5 Current density versus power density

Table 6 Performance of EBCR in removing BOD from Sample II

Sl. no.	Initial	After one day	% of removal
1	150	105	30
2	154	109	29
3	159	99	37
4	160	98	38
5	162	100	38

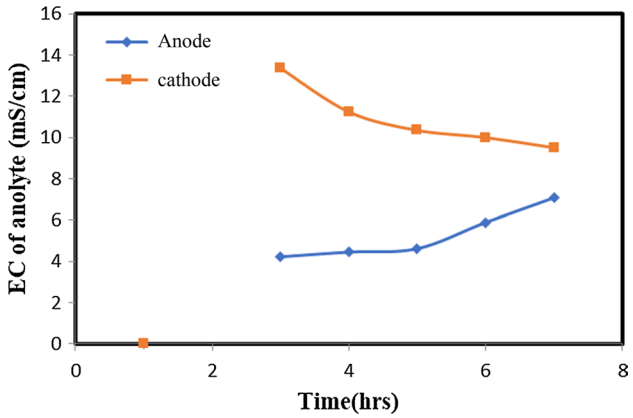

Fig. 6 Conductivity variation in anolyte and catholyte

Effects of desalination on EBCR performance

Effect on conductivity

The conductivity graph of anolyte and catholyte is shown in Fig. 6. The increase in conductivity in anode chamber is due to the addition of chloride ion into the anode chamber from middle chamber. The cathode conductivity gradually decreases due to the formation of pure water in the cathode chamber.

Effect on pH

The pH in the anode chamber gradually decreases while that in cathode chamber increases gradually. The pH of anode chamber goes below neutral range and becomes acidic, and that of cathode chamber goes above neutral range and becomes alkaline. The decrease in pH in anode chamber is due to the microbial activity of bacteria and due to the accumulation of protons. The increase in pH in the cathode chamber is due to the reduction in protons due to the formation of water. Neutral pH in anode and highly acidic pH in cathode are recommended. Using acidic water in the cathode chamber is preferable to maintain this condition.

Characterization studies of wastewater

FTIR spectral analysis was carried out to understand the unknown materials, quality and the amount of components of waste water. It also shows the structural changes, before and after the treatment process. Mostly the domestic waste water contains soap as a major contaminant. The IR spectrum of waste water contains the following frequency mainly due to organic pollutant from soap and detergents. The peak at 1647.21 cm^{-1} corresponds to alkene compounds –C=C– maybe from oleic acid or linoleic acid salts.

$$CH_3-(CH_2)_7-CH=CH-(CH_2)_7COOH \text{ or}$$
$$CH_3(CH_2)_3-(CH_2-CH=CH)_2-(CH_2)_7-COOH(\text{Linoleic acid})$$

The peak at 1550 cm^{-1} belongs to amino acid (N–O asymmetric stretching). The peak at 1419.61 cm^{-1} shows

antisymmetrical stretching due to COO– which is from soap. The peaks at 1022 cm^{-1} are due to aliphatic amines.

Membrane fouling

Biofouling

Biofouling of ion exchange membranes, especially in the AEM that was in contact with the anolyte, was expected. Through visual inspection, it was observed that the surface of the AEM turned black and was covered with a fungi-like deposit. The analysis of SEM images provided more detailed information about the conditions of the membrane surface and clearly showed the difference between a raw membrane (unused) and the fouled membranes. The raw membranes of both the AEM and the CEM exhibited a slightly cracked surface which may be due to mixture of bacteria and fungi. On the AEM facing the anode, both microorganisms and salt deposit were observed which confirmed the separation of Cl^- ions from middle chamber. A mud-shaped structure was observed on the membrane. Although microbial analysis was not performed, spherical-shaped microbial morphology was noted.

Inorganic scaling

The scaling on ion exchange membranes is mainly caused by the precipitation of various inorganic compounds such as sodium chloride, calcium carbonate and magnesium hydroxide. It was observed that a layer of whitish crystals and deposits is formed on the AEM and CEM facing middle chamber. Higher concentrations of sodium, calcium and magnesium ions in seawater are the major scaling ions. During the operation, both sodium ions migrated across the CEM into the cathode chamber as a result of the electricity generation. The scaling on the AEM (the side facing seawater) was less serious than that of the CEM. The SEM image of CEM facing cathode side shows the formation of sodium phosphate which indicates the movement of sodium ion. The salts were unevenly distributed on the membrane.

Crystal structure of membrane

AEM facing anode chamber

Figures 4, 5, 6, 7, 8, 9, 10, 11 and 12 show the crystalline structure of AEM facing anode chamber. The strongest peaks are at 29.3°, 31.70° and 32.30°. The crystallite size of the AEM is 34.5 nm.

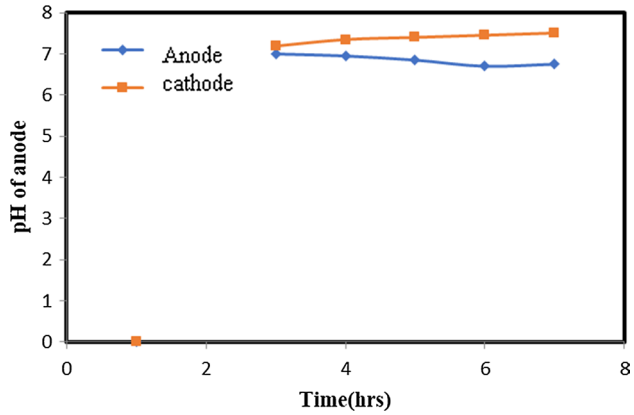

Fig. 7 pH Variation in anolyte and catholyte

AEM facing middle chamber

The above figure shows the crystalline structure of AEM facing middle chamber. The intense diffraction patterns were observed at 29.6° and 47.6° which correspond to NaCl crystalline (JCPDS 83-1728S). So it clearly indicated that sodium chloride was deposited on the AEM facing the middle chamber. It also infers that other impurities are not present in this. The crystallite size of sodium chloride is 39.0 nm.

CEM facing middle chamber

The above figure shows the crystalline structure of CEM facing middle chamber. The intense diffraction patterns were at 20.5° and 31.2° which correspond to $CaCl_2$ crystalline (JCPDS 49-1092). So it clearly indicated that calcium chloride is deposited on the CEM facing the cathode side and it is of good crystalline nature. It also infers that other impurities are not present in this. The crystallite size of calcium corresponds to 40.28 nm.

CEM facing cathode chamber

The above figure shows the crystalline structure of CEM facing cathode chamber. The intense diffraction patterns are at 30.9° and 25.2° which correspond to $NaPO_3$ crystalline (JCPDS 11-0650). So it clearly indicates that sodium phosphate is formed on the CEM facing the cathode side and it is of good crystalline nature. It is also inferred that the other impurities are not present in this. It suggests that the scaling has occurred when the sodium migrated across the CEM and bonded to the phosphate. The crystal structure which is formed is monoclinic, and crystallite size is 10.6 nm.

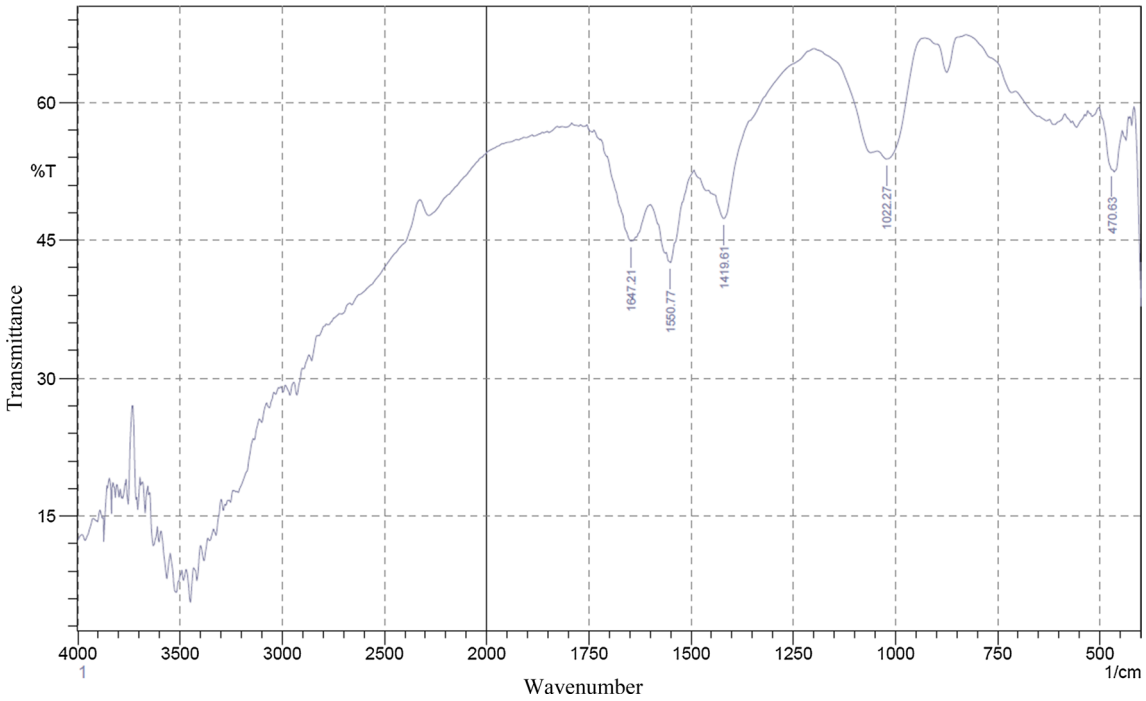

Fig. 8 FTIR of domestic waste water

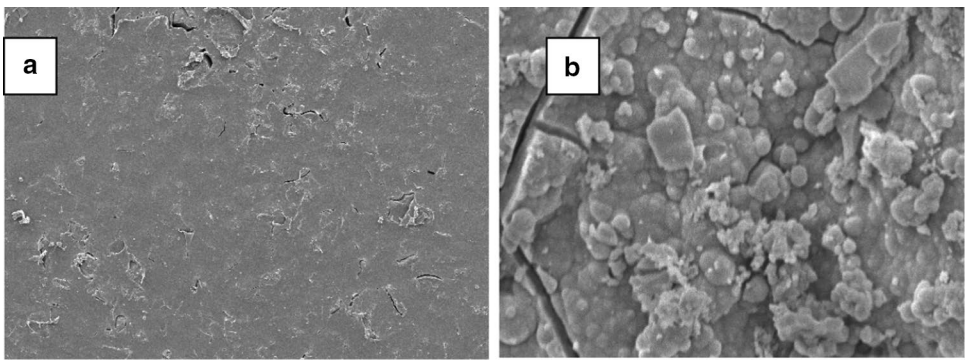

Fig. 9 SEM image of ion exchange membrane: **a** raw AEM membrane and **b** AEM facing anode chamber after treatment

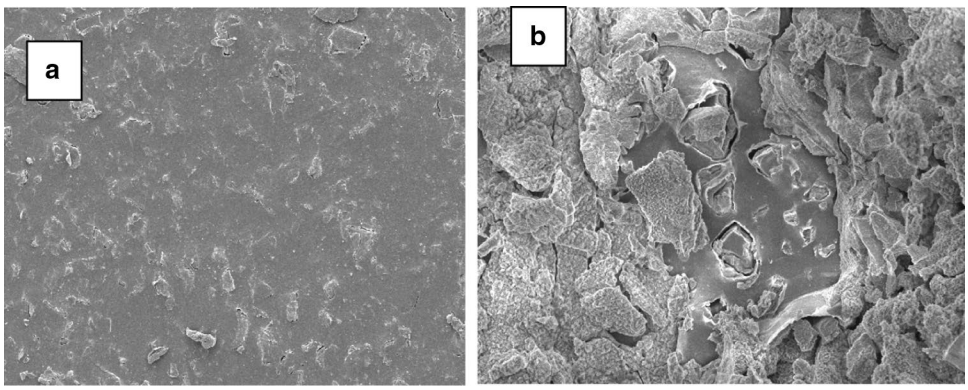

Fig. 10 **a** SEM image of raw CEM and **b** used CEM facing cathode chamber

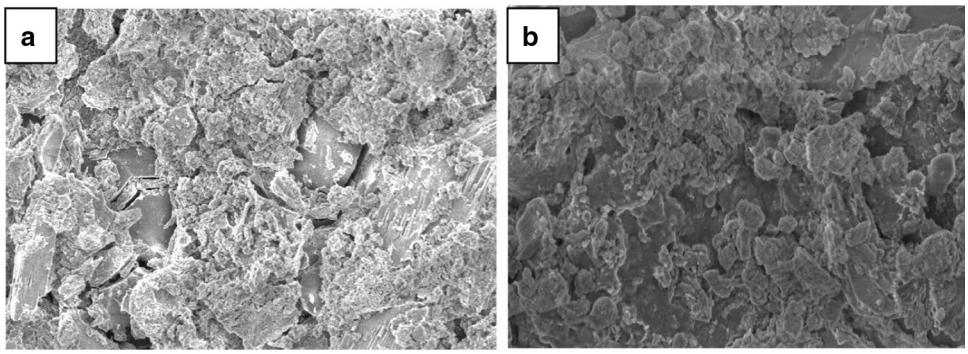

Fig. 11 **a** and **b** SEM images of used AEM facing seawater

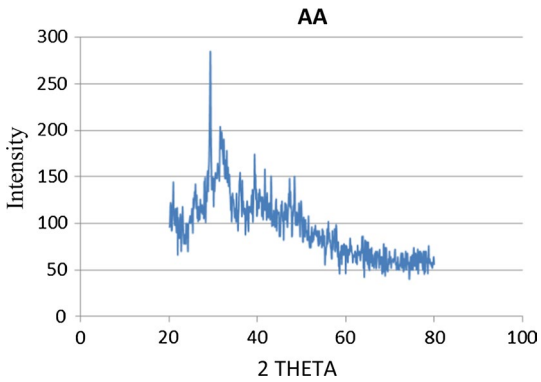

Fig. 12 XRD pattern of AEM facing anode chamber

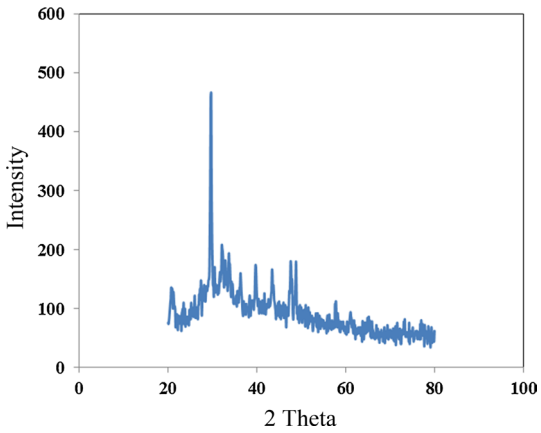

Fig. 13 XRD pattern of AEM facing middle chamber

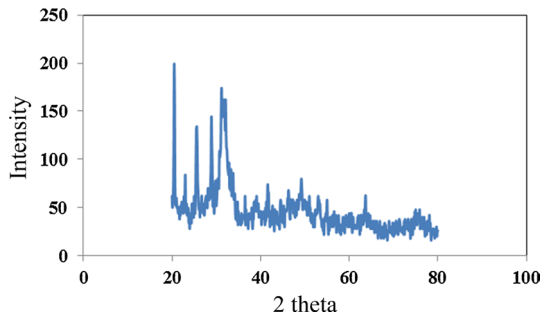

Fig. 14 XRD pattern of CEM facing middle chamber

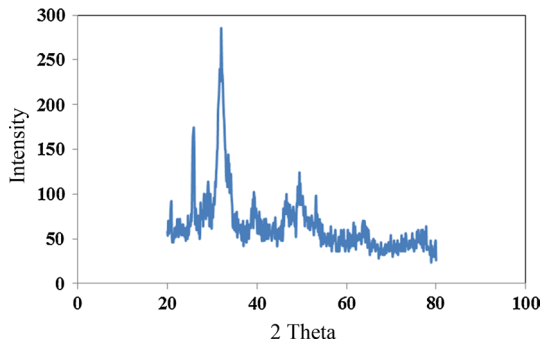

Fig. 15 XRD pattern of CEM facing cathode chamber

Conclusion

This study demonstrated that EBCR can be used for desalination along with other advantages like energy production and waste water treatment. The second reactor with acidic water as catholyte proved to be more efficient. It was noted that there was 100% removal of hardness during the treatment using EBCR. There was current generation of 1 mA with voltage of 850 mV. About 60% of EC and 40% of BOD were removed. A power density of 251.8 mW/m^2 and current density of 296.29 mA/m^2 were also obtained. From this, it can be concluded that the EBCR is the most energy-efficient alternative for desalination as well as for the waste water treatment (Figs. 13, 14 and 15).

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RESEARCH PAPER

In Situ Bioremediation of Textile Dye Effluent-Contaminated Soils Using Mixed Microbial Culture

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Abstract
In situ bioremediation is proposed as a low cost and environmental friendly technology for clean-up of land discharge sites of textile industries. Owing to the large range of dye molecules in the environment, a treatment system using mixed microbial culture is proposed. The mixed microbial population possesses higher degree of biodegradation due to synergistic metabolic activities of the microbial community. In the present study, mixed microbial cultures were cultured from four different sources. The dyes used in the experiments were Direct Red 28, Direct Blue 53 and Azur Blue. Batch studies for industrial application were also attempted to treat sludge landfills for chemical coagulation units of dye effluent treatment plants. Finally, a downward flow continuous flow soil reactor was set up to study the robustness of the technology over long duration of operation. Rate of recharge of mixed dye during the trials was 2 mg per hour per kg of soil. Colour removal of 85% (measured at 595 nm) was observed in the first day and 90% in the second day. A steady removal rate of 98% was observed from the 13th day of operation. Fate of the dye molecules was studied using scanning electron microscope (SEM) and Fourier transform infraRed (FTIR) analysis to establish colour removal due to biodegradation.

Keywords In situ bioremediation · Biodegradation · Contaminated soil · Textile dyes · Mixed microbial culture · Infrared spectroscopy

1 Introduction

The Indian textile industry is an important, if not, the most important economic activity in the country, providing the basic necessity of clothing and contributing to industrial output, employment generation and export earnings of the country. Its growth is closely linked to the phenomenal growth of the dyestuff sector of the chemical industry consuming almost 70% of all the dyestuffs produced in the country. But, all the dyes consumed by the textile industry do not bind to the fabric, and are returned as wastewaters contaminating water and soils in the vicinity of textile dyeing industries [1].

The use of various dyes is widespread and very common in the modern day. These dyes adhere on surfaces by physical adsorption, mechanical retention, formation of covalent bond, or complexes with salts or metals, or by solution [2]. The ability of certain chemicals to alter the colour of surfaces is what makes them useful as dyes. The specific colour of the dye is determined by its ability to absorb light within the visible spectrum. Dyes are used in many industries such as textile dyeing, paper printing and photography, foods, cosmetics, and others. In the textile industry, due to inefficiency of the dyeing process, upto 2,00,000 tonnes of these dyes are lost as effluent during dyeing and finishing operations [1]. In earlier times, dyes would come from natural sources such as from plants or animals. And, microbes, which are nature's tools, convert back these organic materials, such as dyes, to inorganic form through decomposition and mineralization [3]. Since many synthetic dyes have been developed and are now in use the natural microbial populations are unable to decompose them in due course of time. Also, the variety of dyes with unique chemical properties has made it difficult to estimate the effects of these on the local environment and on humans. The combined effect of high volumes of textile

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dye wastewater generated and the increased use of synthetic dyes, makes textile dyeing industry a substantial source of pollution in the current times.

Known toxicities of dyes are often low yet there are other environmental and health impacts of dyes even at low levels. Dyes can be visible in water at concentrations as low as 1 ppm [3–5]. Dyes cause decreased light penetration, decreased dissolved oxygen, and increased BOD of water [3, 4, 6]. The environmental impact and public health concerns surrounding textile dye contamination show a need for better treatment of textile dye effluent as well as remediation of sites contaminated with these effluents. Increased knowledge of the negative environmental and public health effects in recent years has led to more regulations concerning dye use and disposal. This increased awareness has led to increased interest in dye effluent treatment.

The physicochemical methods such as chemical coagulation, oxidation process, ion exchange and membrane technology that are largely employed for dye water treatment are too expensive, impractical and inefficient, as a result of which toxic dyes escape the treatment process and enter the environment [1, 2]. Their complex chemical structure causes the dyes to persist in the environment [3]. Bacterial degradation of these dyes has been suggested as an economically viable and efficient method for treatment. Decolorization is often used as an important marker for dye degradation. Decolorization occurs when the chromophoric center of the dye is cleaved. This decolorization process often leads to problematic intermediate compounds as many dyes contain carcinogenic aromatic amines. It has been shown that aerobic oxidation aids in metabolising these aromatic amines [7]. It has been observed that anaerobic decolorization followed by aerobic oxidation can lead to mineralization of dyes in some cases.

Manivannan et al. [8] in concluding their research highlight that textile industries contribute to much water and soil pollution. The water pollution by textile industries has been addressed both at research and industry levels. The problem of land discharge, though now a part of history, it has taken a new avatar in the form of landfilling of sludge from chemical coagulation of textile effluent. The clean-up of soil cannot be considered in isolation, but as a combined treatment of the soil-aquifer system. Stroo [9] observes that in situ bioremediation of groundwater has become a widely used technology for contaminated site treatment because of its relatively low cost, adaptability to site-specific conditions, and efficacy when properly implemented.

Contaminated soil remediation technologies broadly fall under two approaches: mobilising the contaminant and immobilising the contaminant. Mobilising the contaminant involves leaching out or flushing out the contaminants by flooding, or by use of suitable chemicals. This is done to extract the contaminant, without having to excavate the soil and clean it. Though the extraction of the contaminant happens ex-situ, the technology is in situ, since the soil remains in place. The second approach is to immobilise the pollutant within the soil matrix or any suitable barrier by sorption, or chemically or biologically transforming the pollutant into less toxic or non-toxic compounds. The available technologies that have been tried for clean-up of chromium-contaminated soils include geochemical fixation, permeable reactive barriers, reactive zones, natural attenuation, phytoremediation, electrokinetic remediation and vitrification [10].

Biodegradation of several dyes among various bacteria, microbes, and fungi has been researched. Decolorization is often used as an important marker for dye degradation. Decolorization occurs when the chromophoric center of the dye is cleaved [3]. The ability of various organisms to degrade dye is variable depending on the dye, organism, and several other conditions such as temperature, nitrogen source, carbon source, pH, dye concentration, and others. Due to this variability, it is expected that a mixed microbial culture may be ideal for effluent treatment and remediation as this allows a variety of microbes to work simultaneously to degrade the dye. These different bacteria would likely use different pathways and mixed microbial culture would thus, produce a versatile treatment method that could be used for a variety of scenarios [3]. The biodegradation of textile dye effluent has received enough attention in the literature [11–15]. These studies, to cite a few, do not address remediation of textile dye effluent-contaminated sites. The clean-up of soil cannot be considered in isolation, but as a combined treatment of the soil-aquifer system.

In situ bioremediation is a promising technology that aims at augmenting the existing microbial population by the addition of supplementary nutrients and introduction of more effective microbes into the environment for waste site clean-up [16]. Due to the complex nature of dye molecules, a single isolate of microorganism may not be effective in complete mineralization. The individual strains of a mixed microbial culture, can on the other hand, attack the dye molecule from different positions thus hastening the degradation process. This synergistic metabolic activity in mixed microbial community, found effective in achieving higher degree of mineralization, can be used positively as a treatment technology for biodegradation of mixed dye effluents as well. The proposed pathway of biodegradation of direct blue and congo red is first, the cleavage of the azo bond to form benzidine as an intermediate metabolite. FTIR spectrum has studied to see for benzidine and amino-phenyls. The author wishes to make this hypothesis, based on the biodegradation pathway of another diazo compound (Direct Black 38) referred by Hazrat Ali [4] in his review.

The aim of this paper is to study the efficacy of applying in situ biodegradation as a technology for remediation of textile effluent-contaminated soils using mixed microbial

cultures. The specific objectives that proceed form this aim include: first, to identify a viable source of mixed microbial culture for in situ bioremediation of textile dye effluent-contaminated soil and second, to study the performance of the mixed microbial culture in batch studies and in a continuous flow soil reactor.

2 Materials and Methods

2.1 Sources of Microorganisms

Four different sources for mixed microbial culture were identified. These were sludge (SE) from the sewage treatment plant in Karunya University Boys hostel (Daystar campus), soil (SS) from drying bed of the same sewage treatment plant, treated effluent from a textile dyeing unit (DE) and soil from mat formation inside the textile dying unit (SS). The samples were collected in a plastic container and brought to the laboratory as early as possible. The samples were stored in a refrigerator before being used in the microbiological trials.

2.2 Dyes

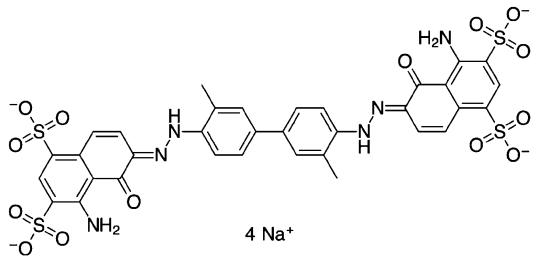
The dyes used in the biodegradation studies were Direct Blue (an imino dye) (Evans Blue; DB53), Direct Red (a diazo dye) (Congo red; DR28) and Azure A (Di-methyl thionine chloride) (AZA). These dyes were chosen for their complex chemical structure (Fig. 1). Hazrat Ali [3] suggests that the need for using real dye wastewaters in biodegradation studies for the results to be realistic. This is because actual dye effluents have variable composition of dyes, salts and other abiotic conditions. To simulate the field conditions, a synthetic mixed dye solution was prepared with DB53, DR28 and AZA in ratio 2:2:1.

2.3 Culture Media

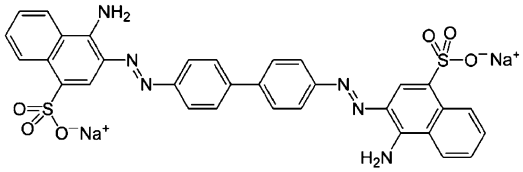
Three types of culture media were employed during the course of the study. Nutrient medium (NM) for microbial growth consisted of peptone 10 g, beef extract 2 g, yeast extract 1 g, and sodium chloride 5 g in 1 L of distilled water [16]. The mineral salts media (MSM) [13] contained (in g/L) NaCl (1.0), $CaCl_2$ $2H_2O$ (0.1), $MgSO_4$ $7H_2O$ (0.5), KH_2PO_4 (1.0) and Na_2HPO_4 (1.0). Nutrient agar medium used for making agar slants consisted of (in g/L) peptone (5.0), yeast extract (2.0), beef extract (3.0), NaCl (5.0), agar (16.0).

2.4 Soil

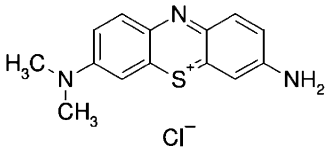
Soil used in the batch studies and in the downward flow soil reactor was sandy loam as per Textural Classification Chart



(a) Evans Blue



(b) Congo Red



(c) Azur A

Fig. 1 Chemical structure of dyes. **a** Evans blue, **b** congo red, **c** azur A

[17]. River sand was sieved using mechanical sieve shaker and fraction retained on (in millimetres) 4.25, 2, 1, 0.600, 0.425 and 0.150 sieves were stored separately. Fraction passing 0.150 mm sieve was also kept separate. The fraction finer than 0.150 mm was considered as silt in the textural classification. The sand was then mixed (Table 3.1) to achieve well-graded sand based on Indian Standard Soil Classification criteria for coarse-grained soils (IS1498:1970). The uniformity coefficient achieved was 6.67 and coefficient of curvature was 1.2. This well-graded sand was mixed with clay soil from the Karunya University campus to achieve a final clay content of 20%, silt content of 8% and sand content of 72% (see Table 1).

Table 1 Sieve fractions of river sand in synthetic soil

Sieve size (mm)	% Finer
4.25	100
2.00	90
1.00	60
0.60	45
0.425	30
0.150	10
Pan	0

2.5 Analytical Procedure

2.5.1 Concentration of Dye

Dyes produce colour because they selectively absorb electromagnetic radiation in the visible range. Absorbance is usually maximum at a single wavelength (λ_{max}). A regression line can be plotted between various dilutions of a stock dye solution and the absorbance at its λ_{max}. This standard plot can then be used to related absorbance with concentration of dye. Absorbance was measured using UV–Vis Spectrophotometer (JASCO).

2.5.2 Decolorization Measurement

Colour removal or decolorization is expressed as percentage reduction in the absorbance value measured at λ_{max} and is given by the formula

$$\%\ \text{of Decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100\%.$$

2.5.3 Characterization of Biodegradation Metabolites

Fourier Transform Infrared Spectroscopy (FTIR) serves atleast three functions—it indicates whether there is a backbone to the structure, whether the structure consists of linear or branched chains, it helps determine if there is unsaturation or aromatic rings in the structure and helps deduce specific functional groups present [18]. FTIR analysis helps to characterise the treated metabolites, change in position of FTIR peaks indicating biochemical cleaving of the chromophore center of the dye. FTIR analysis of the control mixed dye and the treated mixed dye from the continuous flow soil reactors was done using IR Prestige-21 (Shimadzu).

2.6 Experimental Methods

2.6.1 Enrichment of Viable Mixed Microbial Cultures

The biodegradation abilities of microorganisms can be enhanced by gradually introducing them to higher concentrations of the pollutant to be treated. This process is called acclimatisation. Adaptation of the microbial community to the toxic compound is useful in improving the decolorization process [6]. Microbial procedures adopted were as per standard methods [19]. 2 mL of sludge sample (SE) and dye effluent sample (DE) are transferred to separate 250 mL sterile flasks containing 100 mL of autoclaved nutrient media and incubated in static conditions at 34 °C for 24 h. Similarly, 2 g of soil sample from dyeing unit (DS) and soil from STP (SS) are transferred into separate 250 mL sterile flasks containing 100 mL of autoclaved nutrient media and incubated in static conditions at 34 °C for 24 h. These solutions are the stock microbial solutions for use in acclimatisation.

Sterile mineral salts medium (MSM) was amended separately with each of the textile dyes (100 mg/L) and subsequently inoculated aseptically with 2 mL of stock solution. Four stock solutions, and three different dyes, will yield 12 flasks with samples. Three controls were maintained, one each for each dye, with no inoculum added. Initial absorbance readings were taken for each dye and further readings were taken at 22 and 42 h after inoculation. After 42 h flasks containing solutions of the same dye were combined aseptically into a single flask. From this enriched stock, 1 mL of solution was aseptically transferred to sterile nutrient broth and incubated to be used in the biodegradation studies. The sub-cultures (stock) in nutrient broth were transferred to the refrigerator to be preserved to upto a week. In all experiments, SE, SS and DS were used as sources of mixed microbial culture.

2.6.2 Soil Column Trials for Bioremediation

The purpose of this trial was to investigate the ability of bacterial injections in remediating soil with dye contamination under static conditions. Two soil columns were studied each with 750 g of the synthetically mixed soil. First the soil was put into a beaker along with a volume of 10 ppm dye mixture that was roughly the total pore volume of the soil. This mixture was left to sit for 2 days so that the dye could saturate the soil and adsorb onto the soil particles. A UV spec. reading of leachate was taken at this point to determine initial dye reading from eluent. The soil was then transferred is as prepared condition, into separate plastic columns. Into one soil column (treatment), 150 mL of sewage effluent bacteria cultured in MSM was added along with 0.5 g yeast extract. In the other column (control) only 150 mL of MSM (without SE cultures) was added. After 2 days UV readings were taken from the eluent to determine the effect of microbial cultures on colour. After this period 50 mL of 20 ppm mixed dye solution was added in both soil columns and left to sit over 2 days. Samples (leachate) were taken and tested for absorbance and other chemical properties. 20 mL of 40 ppm solution was then added to both columns and tested for absorbance once column was saturated with this solution. 20 ml of SE was added to the treatment column and left overnight. Properties were measured next day. Absorbance was measured on third day and sixth day as well (see Fig. 2).

Fig. 2 Bacterial culture

2.6.3 Industrial Application—Batch Trial 1

Once promising bacterial sources for dye remediation were found experiments were conducted to test for the ability of bacteria from these sources in remediation real-world dye contaminants and by-products. To test the ability of bacteria to remediate important water-quality benchmarks in real-world dye removal by-products, a batch study was conducted. The batch study used leachate from sludge form chemical coagulation unit of dye effluent treatment plant. The sludge was collected from a nearby dye industry. To obtain sludge leachate, approximately 500 cc of the chemically coagulated sludge was placed into a column. 1500 mL of water was passed through the column three times. Effluent water produced was coloured light brown, indicating the presence of sludge material in the water. To test the ability of a microbial culture to remediate the compounds present in sludge this batch trial tested the ability of the most promising bacterial source which was determined to be sewage effluent (SE).

Four 2 L beakers were filled with 200 mL sludge effluent. Each received a 10 mL bacterial injection with varying bacterial densities. The stock SE bacteria solution was cultured and suspended in nutrient medium and was of high optical density (greater than 1). The four 10 ml inoculums contained different dilutions – 100%, 75%, 50%, and 25% of SE bacteria cultures. For example, for the 25% dilution, 2.5 mL of stock culture was added with 7.5 mL of distilled water to make 10 mL inoculums. After inoculation beakers were placed into a SERVO flocculator apparatus and were continuously mixed in between sampling. Samples were stirred for all sampling times with the only exception being between the 26 h and 48 h samples with 48 h being the overall sampling period. Parameters tested included: iron, nitrates, sulphates, and chlorides.

2.6.4 Industrial Application—Batch Trial 2

To test remediation capabilities of the bacterial sources, in real-world landfills, a column experiment was conducted with field samples. An 8.5 cm diameter, 60-cm tall acrylic column was filled with a mixture of sludge from the chemical coagulation unit of the effluent treatment plant and previously dye-contaminated soil from a nearby dye industry. The prepared soil column was filled with water and left to sit for several days so that full saturation and chemical leaching could occur. One trial was conducted for each of the three bacterial sources (SE, SS and DS) and properties of the column effluent were tested for remediation impact of bacteria. Stock solution of cultures was applied at the top of the columns [20], and sampling done through sampling ports along the vertical length of the column.

2.6.5 Performance Evaluation of Continuous Flow Soil Reactor

An experiment was conducted to test the ability of mixed microbial cultures to effect in situ biodegradation under continuous flow conditions. Two reactors were used and each were filled with a sandy loam soil synthetically mixed in the laboratory. Mixture was made in the same manner as in the previous trial. Columns were not of equal sizes, the larger column was used as the control column (no bacteria added) with the smaller one being used with microbial addition to the soil. For the control column 180 mL of water and 1 kg of the soil mixture were mixed and added into the column. In the other column 445 g of soil, 80 mL of SE bacteria in NM made up to 100 mL with distilled water was added and left to sit for 2 days to foster growth and attachment of microbial communities and then put into the column (see Fig. 3) (see Table 2).

A 40 ppm concentration of the mixed dye (2:2:1) was pumped through each column continuously over time. Bacteria column was operated with a peristaltic pump (Fig. 4) while the control column was operated with a standard impeller water pump. Experiments were often run during working hours and switched off overnight for ease of sampling. Effluent samples were tested for UV absorption. 0.5 g yeast extract was added on day 2 and day 8 to enhance bacterial growth and survival. UV readings of eluent from each column were taken at regular intervals to see effectiveness of columns in dye filtration and bioremediation. The columns were run for 42 days (6 weeks).

Fig. 3 Soil column study

Table 2 Continuous flow reactors experimental parameters

	Flow reactors	
	Treatment	Control
Area (cm^2)	19.6	44.2
Height (cm	15.5	15.5
Volume (cm^3)	305	685
Soil used (kg)	0.445	1
Initial water (ml)	20	180
Initial inoculum (ml)	80	0
Flow rate (mL/min)	0.4	Variable

3 Results and Discussion

3.1 Screening of Viable Source for Mixed Microbial Cultures

Mixed microbial cultures from four different sources were enriched for biodegradation capability by exposing them to high concentration of dyes (100 mg/L). Trial for dye effluent (DE) was not continued after 22 h as it was observed to have the lowest decolorization potential. The effluent was collected from the open drain near the boiler units of the dyeing plant and not from the direct effluent. High temperatures and fresh dripping of water from the boiler would have rendered the sample aseptic. DE was thus discarded as a viable source.

Among the other three sources, an average decolorization of 67% in DR28, 87% in DB53 and 18% in AZA indicate growth of microbial colonies in these media. Overall results show high decolorization for DB53 among the three promising sources. Low decolorization was observed for AZA among all sources. This could be due to higher initial concentration of AZA (170 mg/L in the control) as evidenced in the absorbance readings taken at 22 h. The decolorization dramatically increased for some samples between 22 and 42 h measurements. This increase could be due to differences in initial bacterial density as no process was implemented to ensure uniform cell density of inoculum. Other factors could include variation in bacterial properties, degree of acclimatisation to dye, or bacteria beginning to use dye as an energy source only after an initial growth phase in the yeast during the first 22 h.

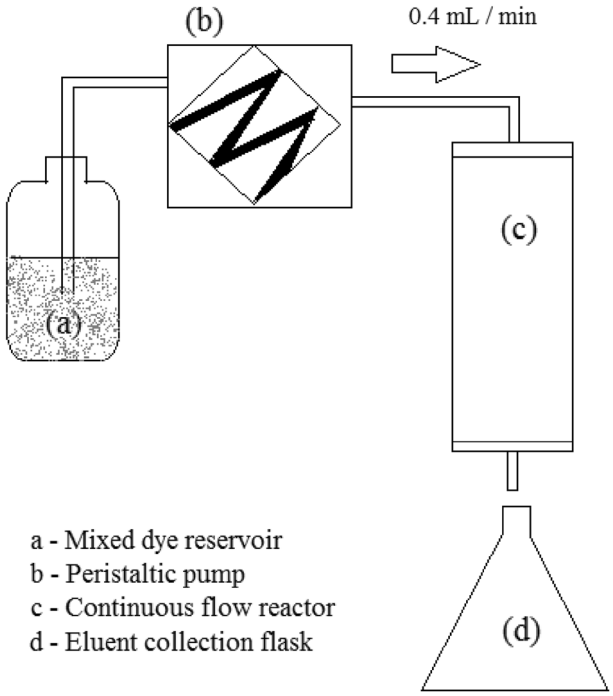


Fig. 4 Schematic of continuous flow soil reactor

3.2 Soil Column Trials

Maximum wavelength of the mixed dye observed at 606 nm. Another peak observed at 525 nm. Concentration of mixed dye in the leachate is reported by converting absorbance into concentration using calibration curve for mixed dye prepared separately. It is observed for small concentration of dye, the colour removal is same in both control and treatment columns. With higher concentration of dye, treatment columns show better colour removal after 3 days. The initial apparent high colour removal is due to sorption on the soil. But with time, the microbial treatment effects more colour removal compared to control column. The similar colour removal after 6 days in both treatment and control columns justifies the initial thesis that natural microbial communities, given sufficient time can mineralize the organic pollutant.

Table 3 Results from soil column trials

Initial concentration of dye ppm	Treatment duration days	Concentration of dye in eluent ppm	
		Treatment	Control
10	0	5	5
10	2	1	1
20	2	2	1
40	0	10	4
40	0.75	32	25
40	3	12	36
40	6	7	5

Bioremediation only augments the natural microbial ecosystems capacity to mineralize the pollutant, and hence speeds up the process, which is necessary in the case of continuous discharge of pollutants (see Table 3).

3.3 Industrial Application—Effect of Inoculum Size

Sampling from the beakers was done for a total of six times. Four water-quality parameters were monitored in each sample. Results show a decrease in nitrates and sulphates across samples. The decrease in nitrates and sulphates is greater for beaker with higher initial concentration of microbial cultures. The effect of inoculum size is illustrated (Fig. 5) comparing change in water-quality parameter for samples with 25% inoculum and 100% inoculum size.

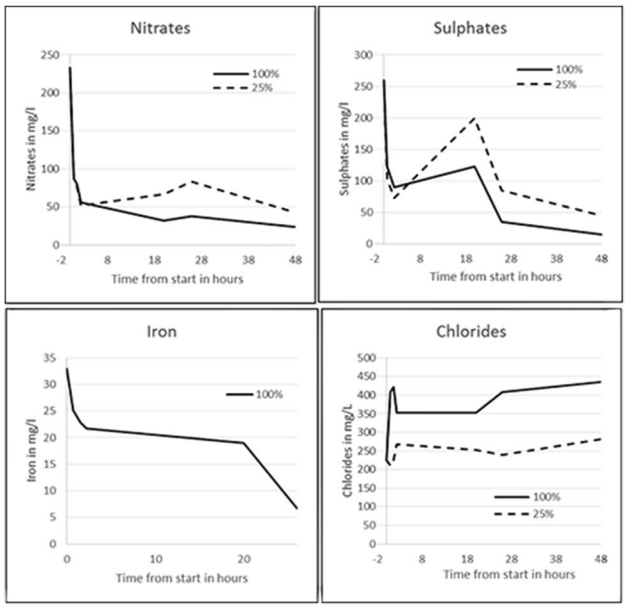


Fig. 5 Effect of inoculum size on remediation of textile dye sludge

Chlorides have increased slightly in all samples. It can also be observed that the decrease in iron is accompanied by an increase in chlorides. This is expected as the sludge from the industry is rich is $FeCl_3$ used as a coagulant. Iron was measured only for the beaker with 100% inoculum size. Iron content in the effluent decreased with time. It can be observed that the effect of initial inoculum size is insignificant in the first few hours of testing.

Similar results were obtained by Katherine Banks et al. [21] in their bacterial adsorption and transport studies through saturated soil column studies have applied microbes at the top of the soil column followed by flushing with water.

3.4 Industrial Application—Efficacy of Different Microbial Cultures

Results (Table 4) indicate an initial drop in important water-quality parameters with an increase of these values observed later on. This is expected to be due to limitation in microbial ability or survivability. The unmarked readings were reported above the upper range for measurement using HACH colorimeter.

3.5 Performance Evaluation of Continuous Flow Reactor

The continuous flow soil reactor was operated for a total duration of 42 days (6 weeks). Colour removal of 85% (measured at 595 nm) was observed in the first day and 90% in the second day in the treatment column. A steady removal rate of 98% was observed from the 13th day of operation. Observance of a new peak (480 nm) on the 5th day is indicative of intermediate metabolites being formed and accumulated in the soil matrix. Colour removal at 480 nm reached maximum of 95% in 40 days of operation. It can be observed from Fig. 6 that decolorization observed at wavelength of 480 nm runs almost parallel to the decolorization for DR28 dye measured at 498 nm. The new peak is then suspected to be due to recalcitrance of Congo red, a diazo dye, while the other dyes approach almost 100% decolorization.

3.6 FTIR Characterization of Biodegraded Product

The FTIR spectrum of the control mixed dye and the treated mixed dye (Fig. 7) indicated weakening of stretching and bending peaks in the treated solution indicative of reduction in concentration of the functional groups and skeletal structure of the parent dyes. At stretching frequency of 1634 cm^{-1} the treated mixed dye has weakened with a single peak, three peaks indicative of persistence of azo bond, but replacement of amino group with ketones. Table 4 summarises the functional groups and structural bonds from the FTIR analysis.

Table 4 Industrial application—batch trials comparing efficacy of different microbial sources

SE trial					
	Initial	18 h	42 h	48 h	70 h
Iron (mg/L)	3	2	–	–	–
Nitrates (mg/L)	18	18	11	20	25
Hardness (mg/L)	8600	5440	6600	6900	5100
Chloride (mg/L)	14,643	5914	7322	8026	6956

DS trial			SS trial		
	Initial	72 h		Initial	72 h
Iron (mg/L)	44	51	Iron	34	41
Nitrates (mg/L)	33	–	Nitrates	90	86
Hardness (mg/L)	5160	4900	Hardness	4360	3840
Chloride (mg/L)	7383	6805	Chloride	6266	5961

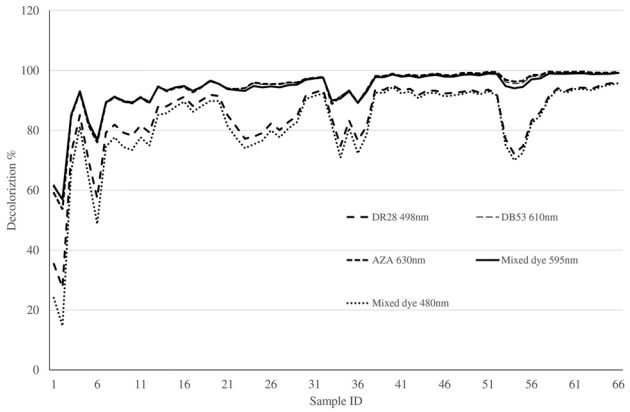


Fig. 6 Decolourization at different wavelengths in the continuous flow soil reactor

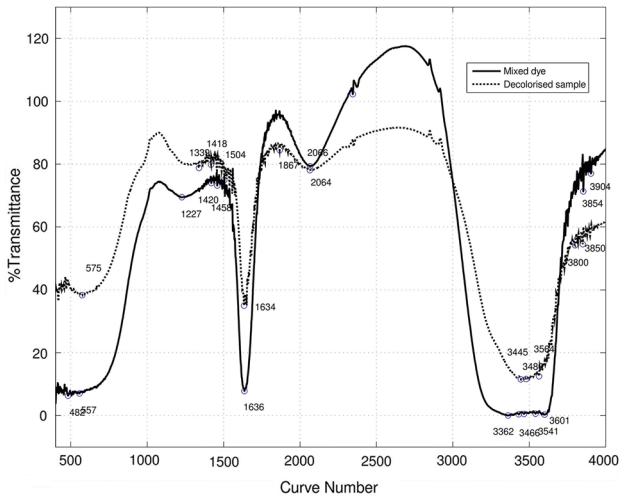


Fig. 7 FTIR spectrum of control mixed dye and treated mixed dye

When comparing FTIR (Fourier transform-infrared spectroscopy) spectrum of both control volumes and the treated sample revealed one different peak, similar results were obtained during dye removal through biodegradation, either the major visible light absorbance peaks were completely disappeared or got weakened and a new peak appeared [4, 13, 22, 23] (see Table 5).

3.6.1 SEM and EDAX Analysis

The elemental composition and surface morphology of the control column, dye-contaminated column and bacterial column were carried out using scanning electron microscopy-energy dispersive x-ray spectroscopy analysis (SEM-EDX) system. As shown in Fig. 8, the concentration of the elements C, O, S, Cl, Na, Mg, Al, Si, P, K, Ca and Fe in the three columns and it was observed that there is reduction in the concentration in the bacterial column due to degradation and demineralization.

4 Summary and Conclusions

Microbial biodegradation has been demonstrated as a viable treatment of textile effluent based on the batch studies. The domestic sewage treatment plant in Karunya Institute of Technology and Sciences itself is a viable source of mixed microbial culture for biodegradation of textile effluent. The batch trial on chemical coagulant sludge from the textile industry also yielded promising results. Microbial activity-mediated fixation of nitrates and sulphates was observed resulting in decrease of their concentration in the leachate.

Table 5 Summary of FTIR analysis

Dye peak	Description	Effluent peak	Description
557	Strong peaks indicating Aromatic C–H	575	Weaker peak indicating reduction in concentration of aromatic compounds and in the present study, reduction in concentration of dye
1227	Aromatic primary amine CN stretching	1339	Aromatic primary amine CN stretching
1420	Primary and secondary amines NH bending	1418	Primary and secondary amines NH bending
1458		1504	
1636	Strong peak. This band is characteristic of double-bonded nitrogen groups such as azo (N=N) and imino(C=N)	1634	Two strong peaks; but much weaker than initial dye indicating reduction in concentration of dye in effluent
2066	Alkyne stretching –C≡C–	2064	Alkyne stretching –C≡C–
3362 3466	NH stretching. This is expected since nitrogen is present in all the dyes used	3445 3480	NH stretching; peak less broader and weaker compared to initial dye
3541 3601	Intermolecular hydrogen bonding; in Evans blue keto–enol totomerism results in formation of phenol and stretching peak observed in this range	3564	Weakening of hydroxy peak observed

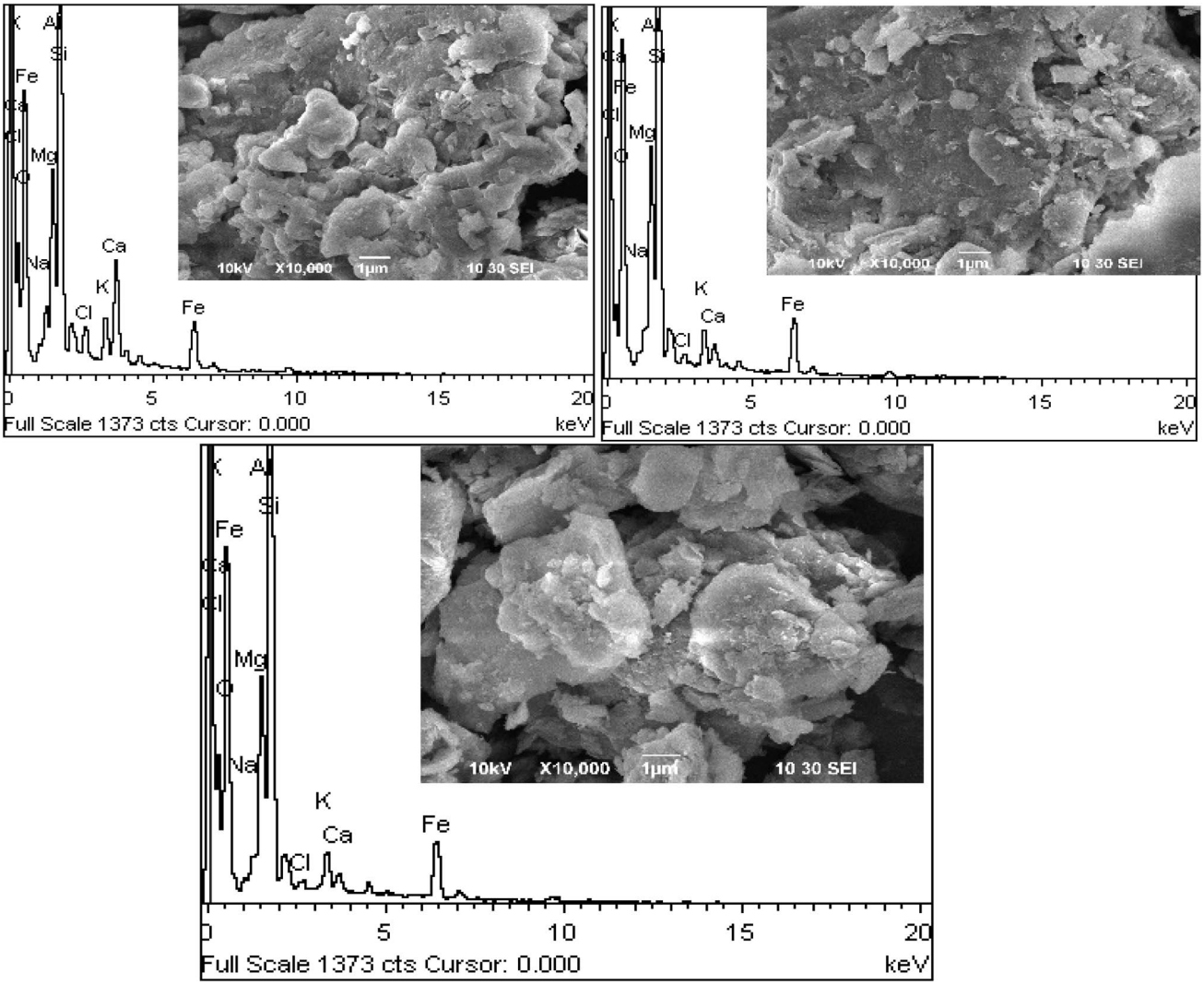


Fig. 8 The elemental composition the control column, dye-contaminated column and bacterial column

The fate of the sulphates and nitrates though are yet to be investigated. Lowering in concentration of iron in the leachate is also a promising result with wide-reaching implications for real world application.

The continuous flow soil reactor can be extended to reactive barrier technology in in situ application. The robustness of the system was demonstrated by 6 weeks of treatment without any external inputs. The application of these learnings in lab scale reactive zone technology prototype will enlighten the researcher on the feasibility of scaling up this technology. Field demonstration will have to be designed considering site specific conditions. Also, the toxicity of the biodegradation metabolites has to be investigated before field applications.

Clean-up of polluted sites is a much ignored area of research, and these series of trials have elicited much interest in the researcher to explore the topic in more detail through a further series of calibrated trials. Mixed microbial culture-mediated in situ bioremediation is a feasible solution to clean-up of textile dye waste-contaminated soil-aquifer systems.

Waste water inoculum or mixed microbial culture is a promising technology for cost-effective clean-up of dye waste-contaminated soils.

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Performance and Characterisation of Agriculture Residue Based Biochar in Removal of Textile Dyeing Effluent

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Abstract

Due to urbanisation and industrialisation, the demand for water and the discharge of pollutants are increasing exponentially. The textile industry is using more than 8000 chemicals in various processes of textile manufacturing processes including dyeing and printing. Many of these chemicals are toxic and lead to direct or indirect health hazards in human. Though there are many physico-chemical, biological methods and membrane technologies are available for the treatment of textile dyeing effluents, most of them are energy and cost intensive. More works have been reported to use the economically efficient and eco-friendly adsorbents to treat the textile dyeing effluent. Though adsorption using activated carbon is most common and efficient method of removal of dye, the process of preparation of activation carbon is energy intensive. The biochar which has been prepared from agricultural residue acts as both adsorbent and ion exchanger. It has got higher surface area and has been considered as a low-cost option for soil remediation and water treatment adsorbent. In this work, biochar has been prepared from different waste sources such as corn, sugarcane bagasse, coconut shell, bagasse + coir and moringa powder. The characterization techniques such as water holding capacity, moisture content, ash content, volatile matter was carried out. Among these, bagasse + coir shows high water holding capacity and higher water retention capacity. The prepared biochar materials were characterized by Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) and Fourier Transform Infra-Red Spectroscopy (FTIR). The prepared biochar where then incorporated into beads using sodium alginate and also Polyvinyl alcohol (PVA) and it was tested for its efficiency to remove textile blue dye. Alginate beads showed better removal than PVA beads. The maximum removal of blue dye was 99.92% for Corn biochar, 99.04% for Moringa biochar, 83.09% for sugarcane bagasse biochar and 77.96% for coconut shell biochar.

1. Introduction

The textile industry is using more than 8000 chemicals in various processes of textile manufacturing process e including dyeing and printing. Many of these chemicals are poisonous and damaging to human health directly or indirectly. Large quantities of water are required for textile processing, dyeing and printing. The daily water consumption an average sized textile mill having a production of about 8000 kg of fabric per day is about 1.6 million litters. 16% of this is consumed in dyeing and 8% in printing. Specific water consumption for dyeing varies from 30 - 50 litres per kg of cloth depending on the type of dye used. Dyeing section contributes to 15% - 20% of the total waste water flow. Water is also required for washing the dyed and printed fabric and yarn to achieve washing fastness and bright backgrounds. Washing agents like caustic soda-based soaps and enzymes etc. are used for the purpose.

The colloidal matter present along with colours and oily scum increases the turbidity and gives the water a bad appearance and foul smell. It prevents the penetration of sunlight necessary for the process of photosynthesis. The resulting effluent is usually high in colour. A complimentary treatment process is needed to remove colour and if possible residual impurities. The textile industry has been condemned to be the world's worst environment polluters. It requires large amounts of chemicals and water at every step of the textile manufacturing and finishing process. Water is needed to convey the chemicals into the fabric and to wash it at the beginning and end of every step. It becomes full of chemical additives and is then expelled as wastewater; which in turn pollutes the environment.

For all of these reasons, it is really important to treat the water from these types of industries due to that the quantity of waste water is high.

Effluent treatment methods can be classified into physical, chemical and biological methods. Exclusive treatment by one of these three methods has proved to be insufficient in removing colour and other effluent from textile industry wastewater

Biochar was a solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment and also it is a highly porous material produced from plant waste. It is mostly used in agriculture as a soil conditioner, in livestock farming as a feed supplement, in metalworking as a reducing agent, it can also be used for cleaning "grey water", as an absorber in sports clothing and as well as having excellent insulating properties, improving air quality, being able to soak up moisture and protect from radiation. Biochar has a lot of uses in different fields of study. In this case, the use is orientated to the adsorption. Biochar has great potential to reduce the greenhouse gas effects by sequestering carbonic soils. Because the time of residence of carbon in biochar is in the range of hundreds to thousands of years. Biochar was an ingredient that is eco-friendly. The structure from bio-char is also having greater positive influence in Environmental issue. We can use bio-char as replacement for fine materials in building construction. It will also, reduce the carbon footprint of concrete because the biochar consumes the higher carbon content in concrete, Improve the mechanical properties and durability of concrete. Reduce the release of CO_2 back in the atmosphere when the carbon is stored in the soil. Biochar used slow pyrolysis to permit various wood sizes, moistures and nature's anomalies to be slowly processed into consistent, high quality biochar.

2.Experimental:

2.1. Production of biochar

The preparation of biochar was done from five types of materials: sugarcane bagasse, coconut shell biochar, corn bagasse, bagasse + coir and raw moringa powder. Biochar was prepared using two methods. One of them is via pyrolysis and the other one is via muffle furnace. The products of these procedures have different properties

First, the mass of the container is taken, then the container is filled packed with the biomass and measured in the balance. Later, the container is placed inside the muffle, at 500°C for an

hour. Once the experiment is started, we noticed the presence of intense smoke from the muffle, but disappeared in ten minutes. After one hour the sample was taken out from muffle and then made into fine powder by using mortar and pestle and used for further analysis.
Second, the biochar from sugarcane bagasse was produced using the pyrolysis reactor in our lab. The conditions of this reactor were the same as the muffle furnace. But the final properties of the biochar were different.

Muffle furnace

Pyrolysis reactor

Biochar from moringa biochar

Sugarcane bagasse

Biochar from plantation leaves

Biochar from corn bagasse

Biochar from sugarcane via pyrolysis

2.2. Preparation of Biochar beads:

Beads were produced from two different solutions, one of them was PVA and the another one was sodium alginate. Sodium alginate, a hydrophilic biopolymer obtained from brown seaweeds has been found to be highly promising with respect to drug delivery because of its high biological safety. Alginate was typically used in the form of a hydrogel in biomedicine, including wound healing, drug delivery and tissue engineering applications. Hydrogels were three-dimensionally cross-linked networks composed of hydrophilic polymers with high water content.

For bead formation, 100 mL of a 2-2.5% w/v aqueous solution of sodium alginate was introduced dropwise from a glass syringe into 100 mL of an aqueous calcium chloride solution being stirred at 400 rpm. The concentration of $CaCl_2$ in the solution ranged from 1% w/v to 3% w/v. The stirring was continued for one hour and the calcium alginate beads were harvested by filtration, washed with distilled water, and air dried overnight.

Beads were one of the particulate delivery systems used to achieve protection and/or controlled delivery of different active ingredients or microorganisms. Polyvinyl alcohol is a non-toxic and biodegradable polymer and possesses extensive applications as a biomaterial. Poly (vinyl alcohol) (PVA) hydrogel beads crosslinked with boric acid have been widely utilized for microorganism immobilization. These beads were obtained by extrusion dripping of a boric acid–polyvinyl alcohol aqueous solution into a basic aqueous gelling bath. However, a drawback of this method for preparing conventional PVA–boric acid beads is that microorganisms enclosed in the PVA matrix are drastically damaged by boric acid during the bead preparation process. In this work, we utilized sodium alginate as an inducer for cross-linkage of PVA for fabricating microorganism-enclosing PVA hydrogel beads to avoid the drastic decrease in cell viability caused by saturated boric acid solution. The shape of immature and mature beads was found to be dependent on the viscosity of the dripping solution for the former and the maturation time for the latter.

3.Characterization Methods:

The characterization test was carried out for each type of biochar and the procedure for each type of test was as follows:

3.1. Moisture content

The amount of water contained in the wood, was expressed as a percentage of the mass of the oven-dry wood. The moisture content of wood or other wood-based materials can be expressed on either as a percentage of oven-dry mass of the sample (oven-dry basis) or as a percentage of initial mass.

To do this test, standard ASTM test Designation: D 4442 – 92 Standard Test Methods for Direct Moisture Content Measurement of Wood and
Wood-Base Materials was followed

Oven—A forced-convection oven that can be maintained at a temperature of 103 +/- 2°C

throughout the drying chamber for the time required to dry the specimen to the endpoint should be used. Ovens should be vented to allow the evaporated moisture to escape.

Sample

Test Material—Any conveniently sized wood or wood -based material could be used.

Procedure

2g of test specimen was taken in the petri dish and the weight of petri dish along with specimen was determined and it was placed in the drying oven at 100 to 105°C with the lid semi open. After 1 hour, the lid was replaced on the petri dish and cooled in a desiccator and then weighed.

The drying was carried out for periods of every 1hr and weighed until the weight becomes constant to within 0.1 mg and the weight was noted periodically. It was assumed that the endpoint has been reached when the mass loss in a 3h interval was equal to or less than twice the selected balance sensitivity. Dried samples shall be stored in a desiccator with fresh desiccant until it has reached room temperature.

3.2. Ash content

The ash content was an approximate measure of the mineral content and other inorganic matter in wood. The ASTM Standard for ash in wood Designation: D 1102 – 84 protocolled was followed. This test method included the determination of ash, expressed as the percentage of residue remaining after dry oxidation (oxidation at 580 to 600°C), of wood or wood products.

Materials

Crucibles, with tightly fitting lids, having a capacity of 30 mL or more, should be used. Platinum crucibles were preferred, but silica or porcelain crucibles may be used.

Muffle Furnace—An electric furnace was recommended for igniting the wood sample. A furnace was fitted with an indicating pyrometer, so that the desired temperature could be maintained was preferable.

Analytical Balance, sensitive to 0.1 mg.

Drying Oven, with temperature controlled between 100 and 105°C.

Desiccator

Sample:

2 g of wood has to been grounded to pass through No. 40 (425-µm) sieve. Care should be taken to ensure that it was representative of the entire lot of material being tested.

Procedure

The empty crucible was ignited and covered over a burner or in the muffle at 600°C and cooled in a desiccator and weight was found to be about 0.1 mg. 2g of test specimen was placed in the crucible and the weight of crucible along with specimen was determined and then placed in the drying oven at 100 to 105°C with the crucible cover removed. After 1 hour, crucible cover was replaced and cooled in a desiccator and then weighed. The drying and weighing was repeated

until the weight becomes constant to within 0.1 mg. The crucible cover was removed and it was placed inside the muffle furnace and then ignited and it started to heat slowly to avoid flaming and protect the crucible from strong drafts at all times to avoid mechanical loss of test specimen. The recommended temperature of final ignition was 580 to 600°C. Heating was avoided above this maximum temperature and the crucible with its contents was removed and then the cover was replaced and cooled and weighed accurately. Repeat The heating for 30mins periods was repeated until the weight after cooling becomes constant to within 0.2 mg.

Calculations

The percentage of ash was calculated based on the weight of the moisture-free wood, as follows:

$$\textbf{Ash, \% = } (W_1 / W_2) \textbf{ x 100}$$

where:

W_1 = weight of ash, and

W_2 = weight of oven-dry sample

3.3. Volatile matter

This test method was used to determine the percentage of gaseous products and moisture vapour present in the analysis sample of particulate wood fuel that was released under the specific conditions of the test. The ASTM Standard Test Method for Volatile Matter in the Analysis of Particulate Wood Fuels Designation: E 872 – 82 method was followed. Volatile matter was determined by establishing the loss in weight resulting from heating wood under rigidly controlled conditions. The weight loss was measured and corrected for moisture as determined in Method E 871 and the volatile matter content was established.

Materials

Crucible: with closely fitting cover, pre-fired to oxidize and stabilize the weight. The crucible should be of not less than 10 or more than 20-mL capacity, not less than 25 or more than 35 mm in diameter, and not less than 30 or more than 35 mm in height.
Muffle furnace: It should be regulated to maintain a temperature of 950 +/- 20°C in the crucible, as measured by a thermocouple positioned in the furnace.

Procedure

The sample size was reduced to a smaller particle size by using cutting or shearing type laboratory mill. The final product should pass through a 1-mm or smaller screen. The crucible along with cover was measured and it was found to be about 0.01 g and crucible weight was expressed as W_c. 1 g of sample was placed in the crucible and then the crucible along with cover was measured and it was recorded as initial weight (W_i). The covered crucible along with

sample was placed on platinum or nickel-chromium wire supports and inserted directly into the furnace chamber and temperature should be maintained at 950 +/- 20°C. Regulating the temperature to be within the prescribed limits was found to be critical. There will be disappearance of the luminous flame after the discharge of volatile matter and after this the crucible has to be checked to verify whether the lid was still properly seated. After heating for a total of exactly 7 min, the crucible was removed from the furnace and, without disturbing the cover, it was allowed to cool in a desiccator. The covered crucible along with sample was weighed as soon as cold to the nearest 0.1 mg and the final weight was recorded as $W_{f.}$

Calculations

The volatile matter percentage in the analysis sample was calculated as follows:

The weight loss percentage was calculated as follows:

Weight loss, % = 100 x $(W_i - W_f)/ (W_i - W_c)$

W_C = weight of crucible and cover, g,

W_I = initial weight, g, and

W_f = final weight, g.

Volatile matter in analysis sample, % = A - B where:

A = weight loss %, and B = moisture, %, as determined using Method E 871

3.4. Water holding capacity

This test was performed to prove the ability of the biochar to be applied to some soils. One of the main functions of soil is to store moisture and supply it to plants between rainfalls or irrigations. Evaporation from the soil surface, transportation by plants and deep percolation combine to reduce soil moisture status between water applications. If the water content becomes too low, plants become stressed. The plant available moisture storage capacity of the soil provides a buffer which determines a plant's capacity to withstand dry spells. Soil water holding capacity can defined the amount of water that a given soil can hold for crop use. Soil water holding capacity is a term that all farms should know to optimize crop production.

3.5.SEM

Biochar was also characterized using Scanning Electron Microscope (SEM). This analysis is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the intensity of the detected signal to produce an image.

3.6. EDAX

Energy-dispersive X-ray spectroscopy (EDS, EDX, EDXS or XEDS), Energy Dispersive X- Ray Analysis (EDX), referred to as EDS or EDAX, is an x-ray technique used to identify the elemental composition of materials, present in the sample. It relies on an interaction of some source of X-ray excitation and a sample.

The data generated by EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analysed. Elemental mapping of a sample and image analysis are also possible.

3.7. XRD

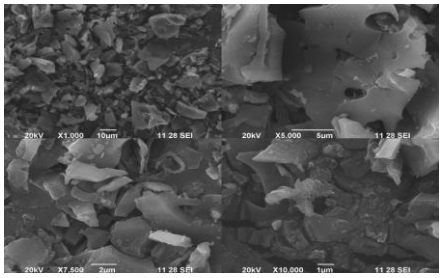
X-Ray Diffraction, (XRD) was a non-destructive test method used to analyse the structure of crystalline materials. XRD analysis, is used to study the crystal structure, and also to identify the crystalline phases present in a material and thereby reveal chemical composition information. X-ray diffraction is useful for evaluating minerals, polymers, corrosion products, and unknown materials. In most cases, the samples were analysed at Element are analysed by powder diffraction using samples prepared as finely ground powders. This test method was performed by directing an x-ray beam at a sample and measuring the scattered intensity as a function of the outgoing direction. Once the beam was separated, the scatter, also called a diffraction pattern, indicates the sample's crystalline structure.

3.8. FTIR

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method used infrared light to scan test samples and observe chemical properties. FTIR analysis services can identify compounds and the general type of material being analysed when there are unknowns. This technique is used to assess the purity of some inorganic samples and is highly reliable for identifying polymer composition.

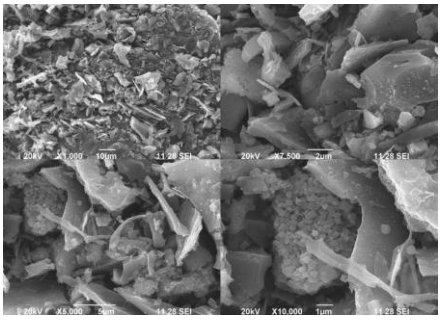
4. Results and Discussion:

4.1. SEM:

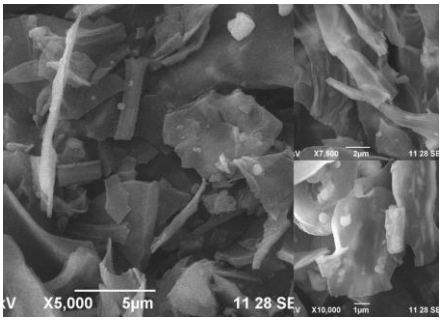


Coconut Shell Biochar

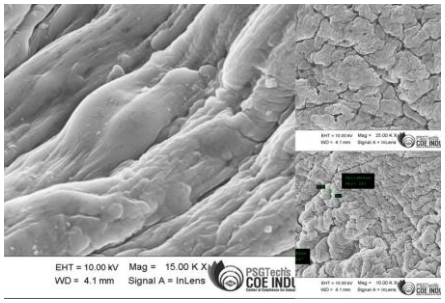
The obtained SEM result of coconut biochar was similar to the result of Swapan et.al. From this we could conclude the presence of formation of coconut shell biochar. The biochar showed flakes like structure.



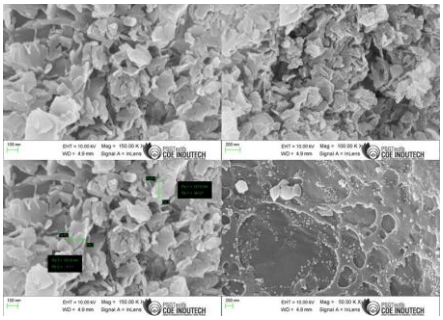
Plantation Leaves Biochar



Sugarcane Bagasse + Coir Biochar



Sugarcane Bagasse Biochar

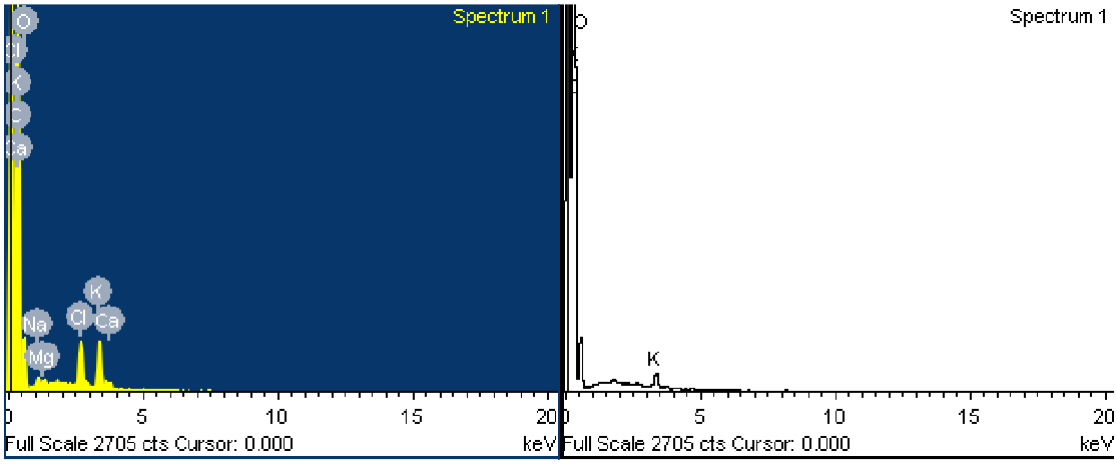


Corn Biochar

The SEM result of as prepared Corn biochar was consistent with results of Yuting et.al. The particle size of corn biochar was found to be 127 nm. The atoms were distributed uniformly on the surface.

4.2. EDAX:

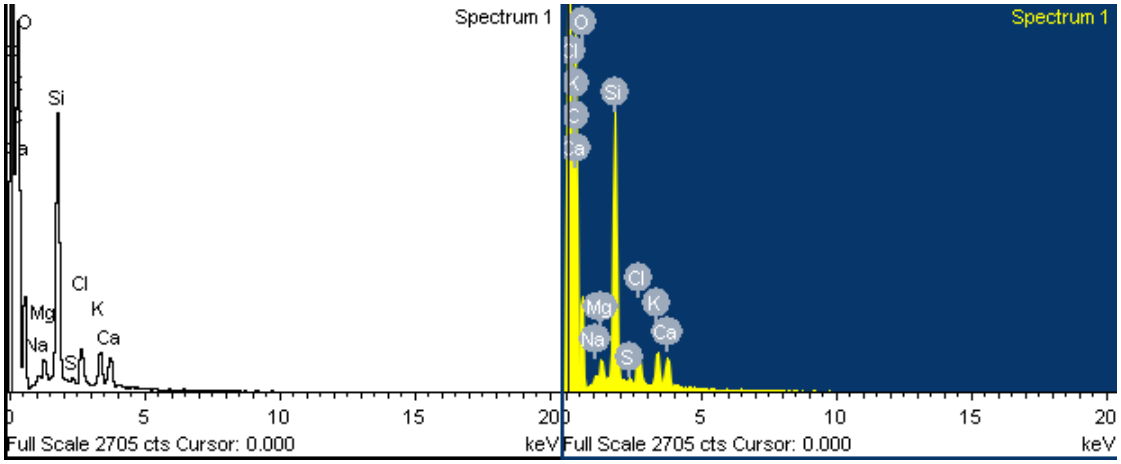
Coconut Shell Biochar



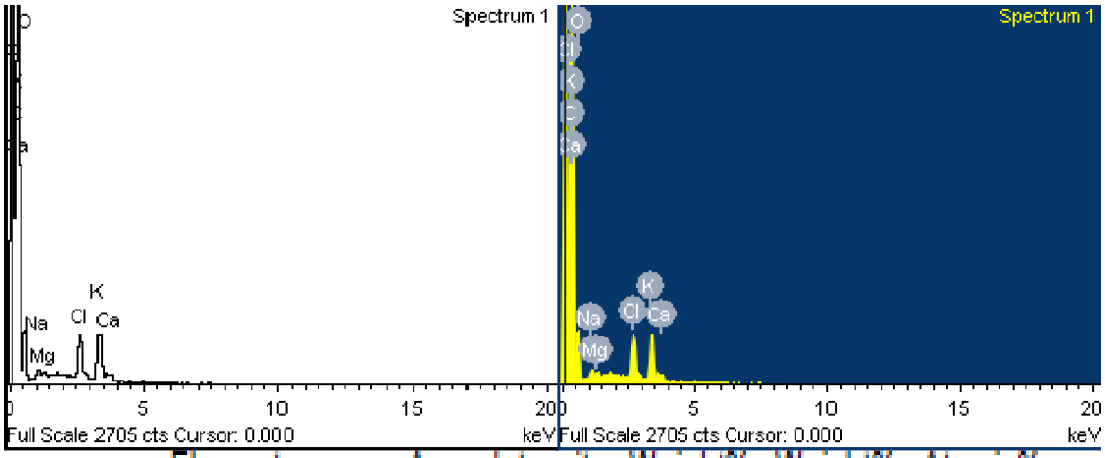
Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	158.61	1.6646	79.58	0.80	84.12
O K	8.16	0.3454	19.73	0.80	15.66
K K	0.87	1.0435	0.70	0.06	0.23
Totals			100.00		

Plantation Leaf

Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	96.28	0.8261	68.88	1.47	77.61
O K	13.19	0.3672	21.23	1.10	17.96
Na K	0.42	0.9129	0.27	0.06	0.16
Mg K	0.80	0.8279	0.57	0.06	0.32
Si K	9.38	0.9625	5.76	0.27	2.78
S K	0.28	0.9345	0.18	0.04	0.08
Cl K	1.33	0.8100	0.97	0.07	0.37
K K	1.91	1.0293	1.10	0.08	0.38
Ca K	1.69	0.9600	1.04	0.08	0.35

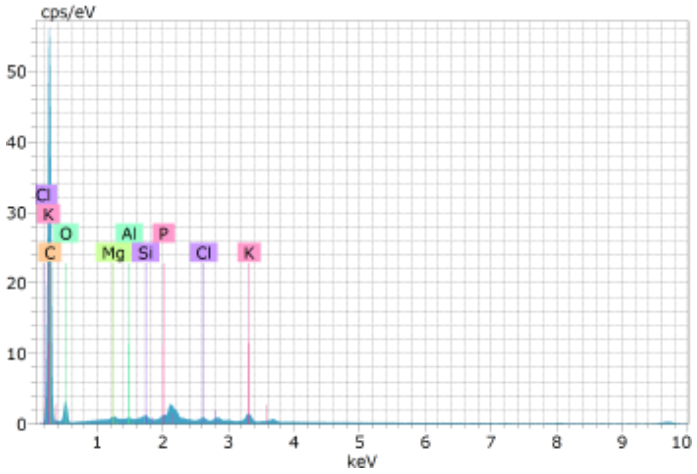


Sugarcane bagasse + Coir Biochar



Element	App Conc.	Intensity Corrn.	Weight%	Weight% Sigma	Atomic%
C K	134.97	1.1650	74.47	1.38	81.42
O K	10.92	0.3474	20.20	1.20	16.58
Na K	0.66	0.8962	0.47	0.08	0.27
Mg K	0.22	0.8120	0.17	0.06	0.09
Cl K	2.58	0.8425	1.97	0.13	0.73
K K	3.99	1.0392	2.47	0.15	0.83
Ca K	0.36	0.9595	0.24	0.06	0.08

Corn Biochar



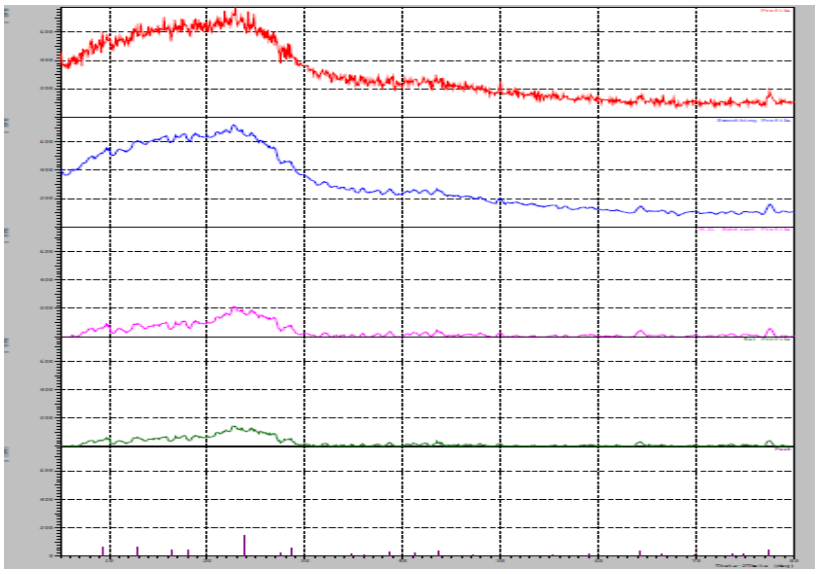
Spectrum: CORN BIOCHAR

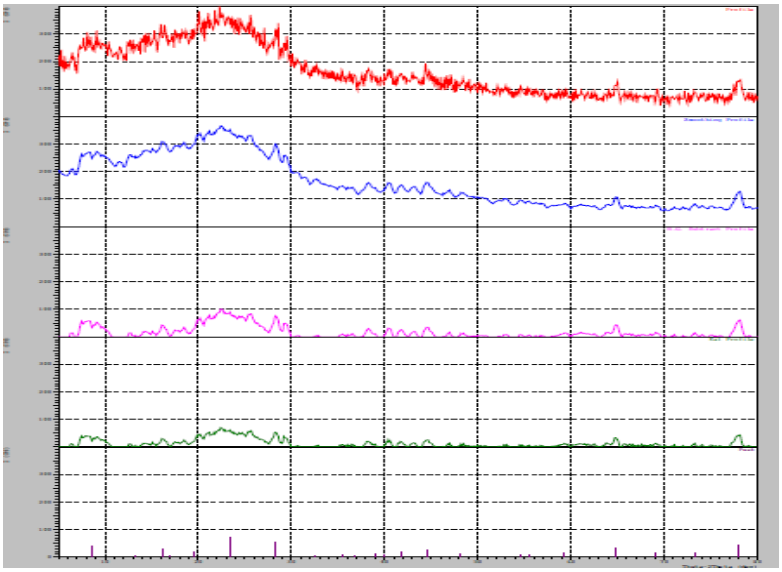
Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	81.42	81.42	85.95	27.51
Oxygen	K-series	16.95	16.95	13.43	7.60
Potassium	K-series	0.79	0.79	0.26	0.16
Silicon	K-series	0.15	0.15	0.07	0.10
Magnesium	K-series	0.16	0.16	0.08	0.11
Aluminium	K-series	0.10	0.10	0.05	0.10
Chlorine	K-series	0.16	0.16	0.06	0.10
Phosphorus	K-series	0.27	0.27	0.11	0.12
	Total:	100.00	100.00	100.00	

Corn Biochar	Sugarcane Bagasse Biochar	Sugarcane Bagasse + Coir pith Biochar	Plantation leaf Biochar	Coconut shell Biochar
C [85.95]	C [85.79]	C [74.47]	C [68.88]	C [79.58]
O [13.43]	O [13.30]	O [20.20]	O [21.23]	O [19.73]
		Na [0.47]	Na [0.27]	
Mg [0.08]	Mg [0.44]	Mg [0.17]	Mg [0.57]	
Cl [0.06]	Cl [0.02]	Cl [1.97]	Cl [0.97]	
K [0.26]	K [0.23]	K [2.47]	K [1.10]	K [0.70]
		Ca [0.24]	Ca [1.04]	
Si [0.07]	Si [0.01]		Si [5.76]	
	S [0.08]		S [0.18]	
Al [0.05]	Al [0.03]			
P [0.11]	P [0.05]			
	Cu [0.04]			

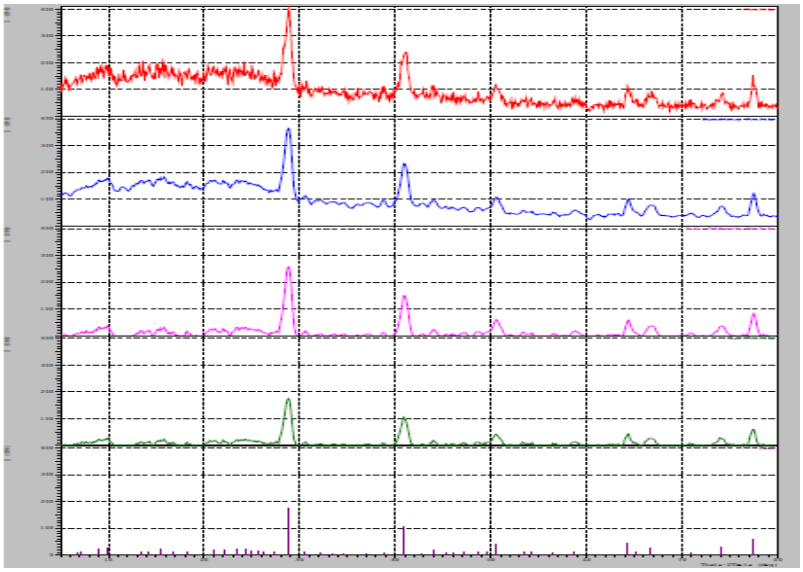
4.3. XRD:

Coconut Shell Biochar

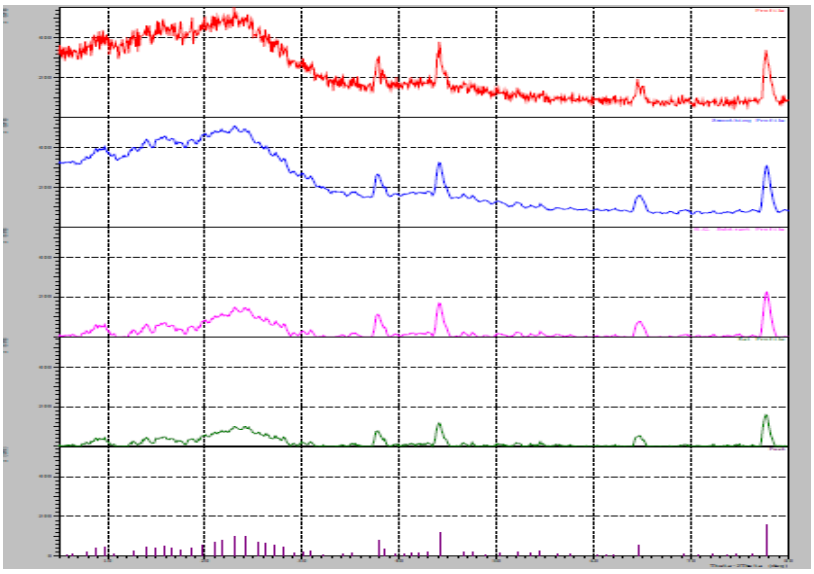




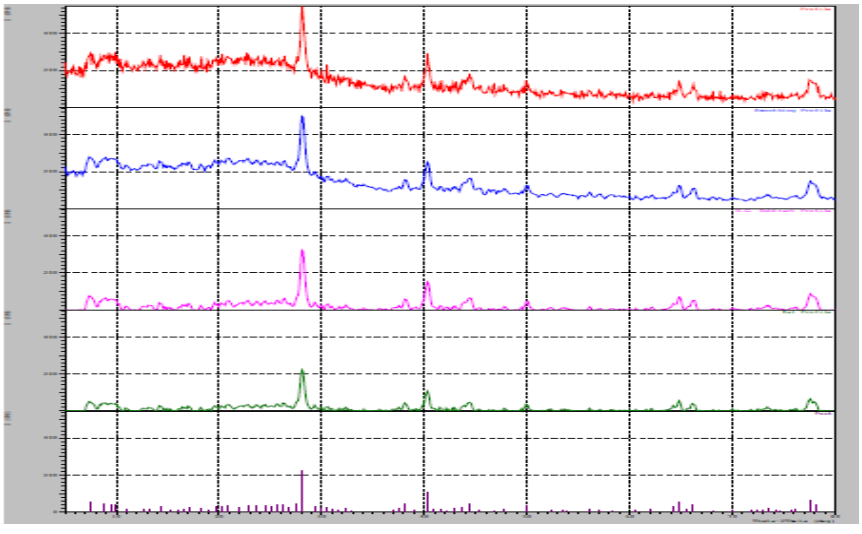
Sugarcane Bagasse+ Coir Biochar



Sugarcane Bagasse Biochar



Corn Biochar



Coconut Shell

The XRD pattern of the sugarcane biochar was shown in the figure. XRD pattern exhibited a strong peak at 2Θ = 24° and particle size with an interlayer distance of about 3.721 Å . These peaks could be assigned to diffraction of graphitic framework. The greater prevalence of these peaks was consistent with the higher content of SiO_2, CaO, and MgO. The sharp and the strongest peak at 2Θ=26° is originated from crystalline SiO. The X-Ray diffraction peak confirmed that biochar possessed a heterogeneous surface.

Plantain leaf

The XRD pattern of the sugarcane biochar was shown in the figure. XRD pattern exhibited a strong peak at 2Θ = 24° and particle size with an interlayer distance of about 5.433 Å . This confirmed the presence of graphitic carbon present in the biochar.

Corn biochar

The XRD pattern showed strong peak at 2Θ = 27°, 39°and 72° which were probably indexed as an orthorhombic unit cell having three lattice parameters a = 4.12 ˚A, b = 4.54 ˚A, and c = 4.14 ˚A. The results revealed that chemical activation has no significant influence on the crystal form of the as-prepared biochar from corn. The present data suggested that most of the crystalline regions were present in the biochar. It exhibited a strong peak at 2Θ = 27° and particle size with a interlayer distance of about 3.171Å

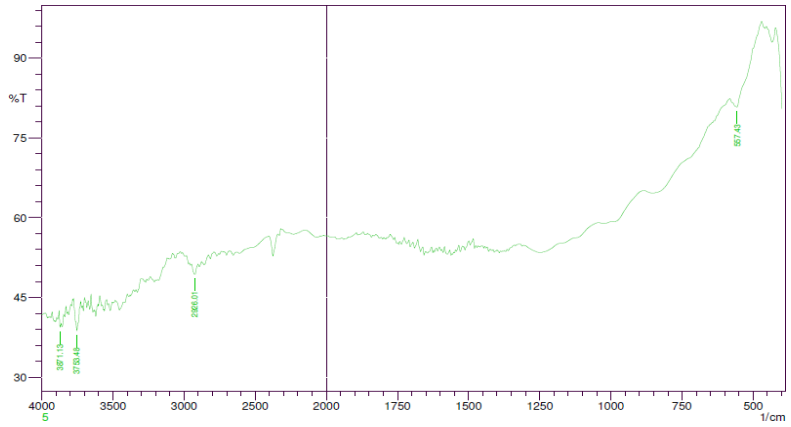
Sugarcane bagasse + Coir

The XRD pattern of the sugarcane biochar was shown in the figure. Sharp peaks in the SB indicated the presence of various inorganic compounds. The XRD pattern showed strong peak at 2Θ = 28°, 40° which attributes to the graphitic carbon and confirmed the existence of micropores in the structure of biochar. It exhibited a strong peak at 2Θ = 28° and particle size with an interlayer distance of about 3.177 Å.

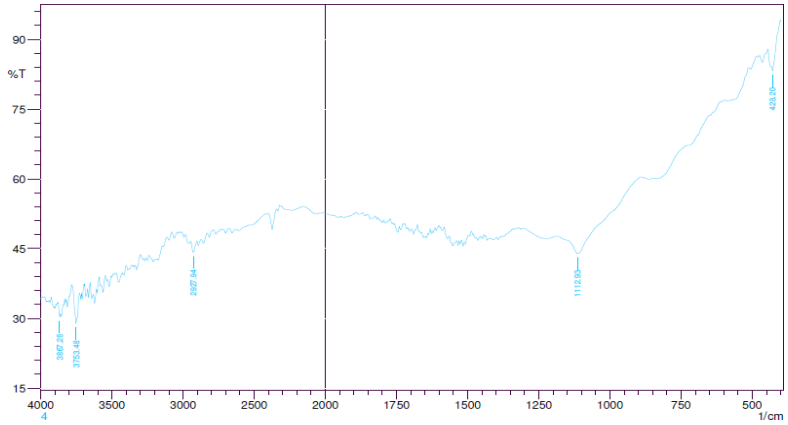
Sugarcane bagasse

The XRD pattern of the sugarcane biochar was shown in the figure. Sharp peaks in the SB indicated the presence of various inorganic components, which were related to the crystalline forms of SiO_2 and CaO. The present data suggested that most of the crystalline regions present in the biochar came from the cellulose crystallinity and turbostratic crystallites was very limited in the structure. It exhibited a strong peak at 2Θ = 56° and particle size with a interlayer distance of about 1.227 Å.

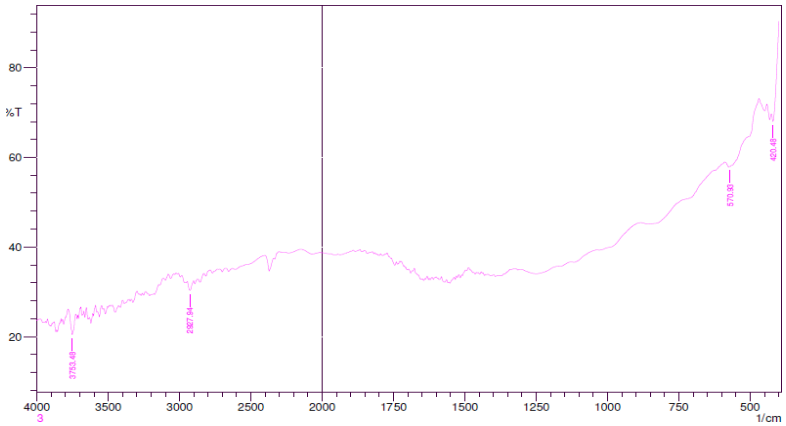
4.4. FTIR:



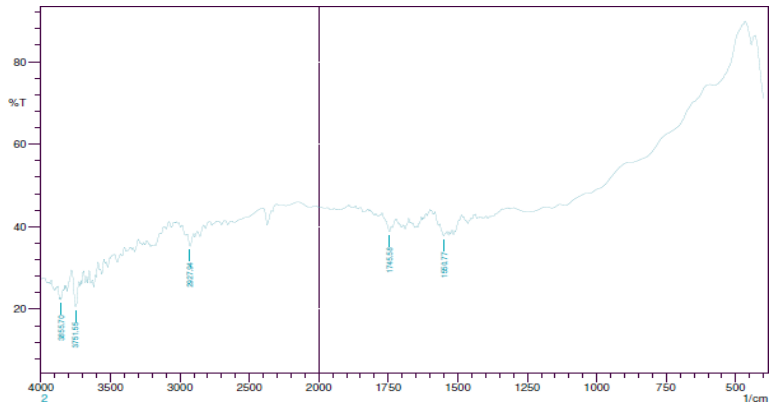
Coconut Shell Biochar



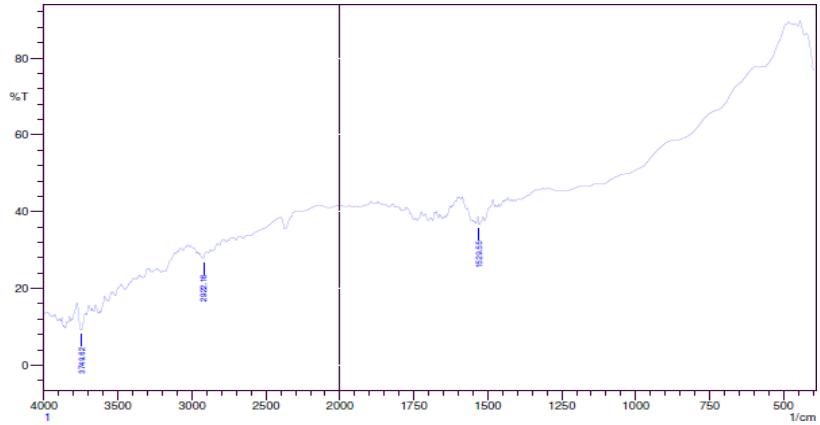
Plantation Leaf Biochar



Sugarcane Bagasse +Coir Biochar



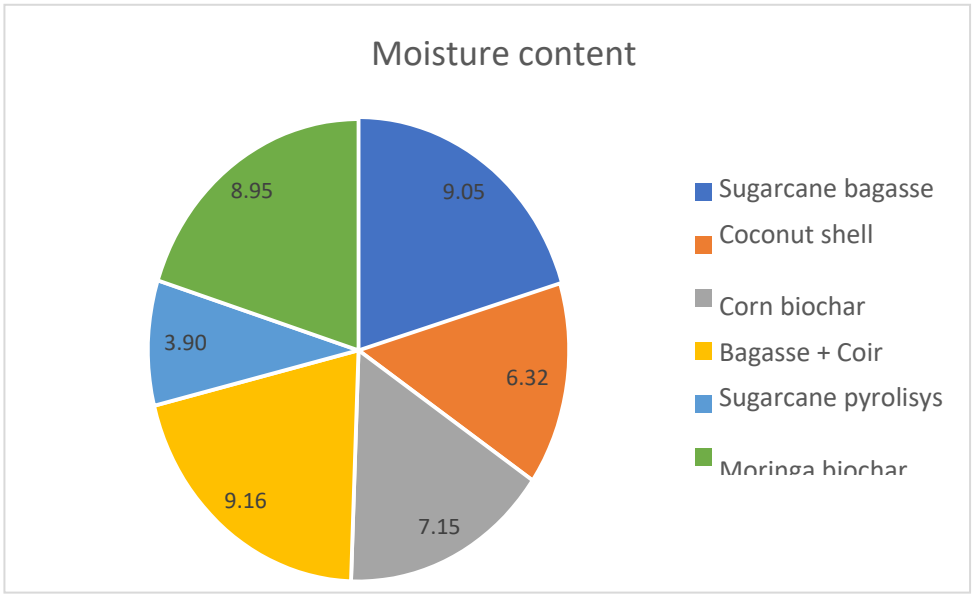
Sugarcane Bagasse +Coir Biochar



Corn Biochar

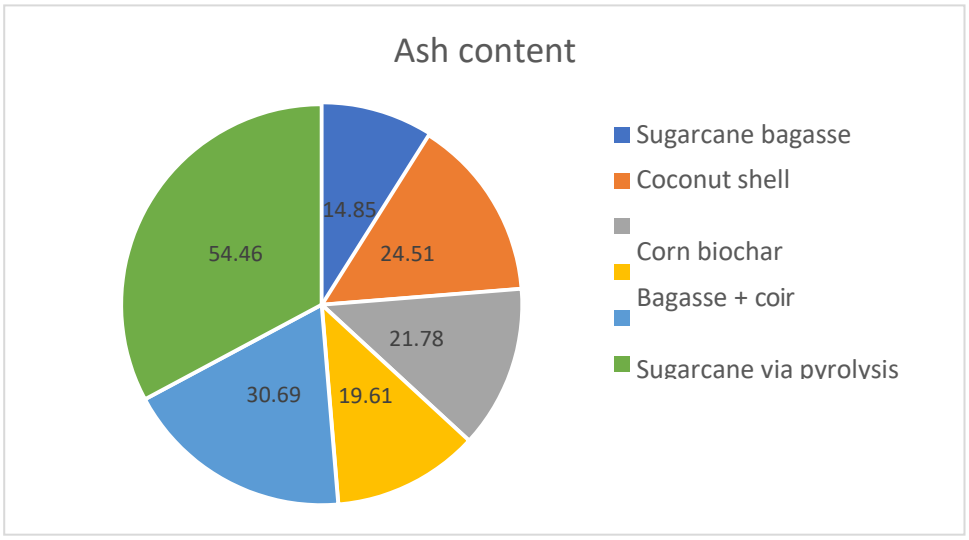
3749.62cm^{-1} (OH Stretching Vibrations)	3751.55cm^{-1} (OH Stretching Vibrations)	3753.48cm^{-1} (OH Stretching Vibrations)	3753.48 cm^{-1} (OH Stretching Vibrations)	3753.48 cm^{-1} (OH Stretching Vibrations)
2922.16cm^{-1} (Asymmetric C-H Stretching vibrations)	2927.94cm^{-1}(Asymmetric C-H Stretching vibrations)	2927.94cm^{-1}(Asymmetric C-H Stretching vibrations)	2927.94cm^{-1}(Asymmetric C-H Stretching vibrations)	2926.01cm^{-1}(Asymmetric C-H Stretching vibrations)
	1745.58 cm^{-1}(Carboxyl C=O Stretching mode)			
1529.55 cm^{-1}(C=C bond due to lignin)	1550.77 cm^{-1} (C=C bond due to lignin)			
			1112.93 cm^{-1} (OH bending vibrations)	
		570.93cm^{-1} (Presence of alkyl halides)		557.43 cm^{-1}(Presence of alkyl halides)
		420.48 cm^{-1}(C-C Stretching)	428.2 cm^{-1}(C-C Stretching)	

4.4. Moisture Content:



With these results, we could observe the difference in final values, the highest was the biochar from bagasse+coir, in correspondence with the results of water holding capacity.

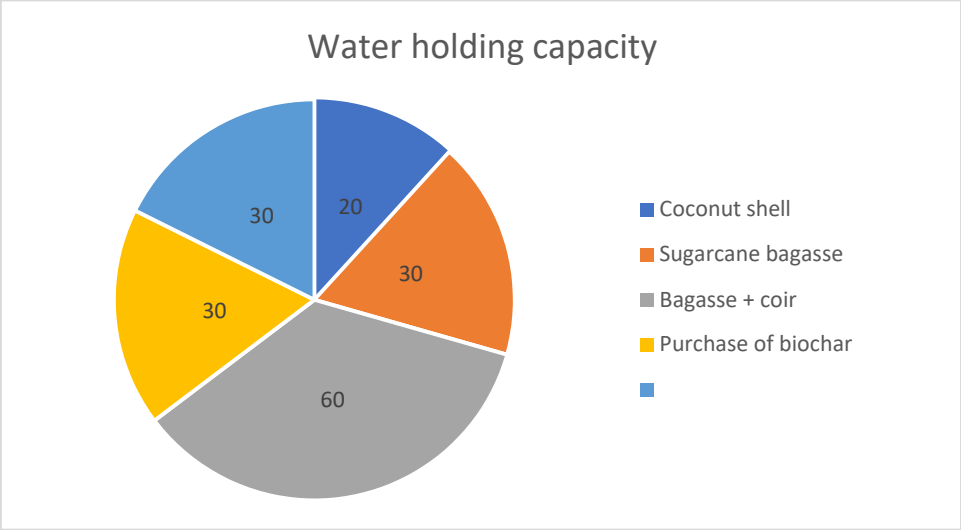
4.5.Ash Content:



We could conclude in these results, that the material with most quantity of ash was sugarcane via pyrolysis and the material with most quantity of ash content in the muffle furnace was moringa biochar. The meaning of this, was that the container of moringa powder was opened inside of the muffle and some part of the powder burnt. In the case of sugarcane biochar

obtained by pyrolysis, the reactor didn't close properly, so some quantity of air went to inside the reactor and a part of this material burnt too.

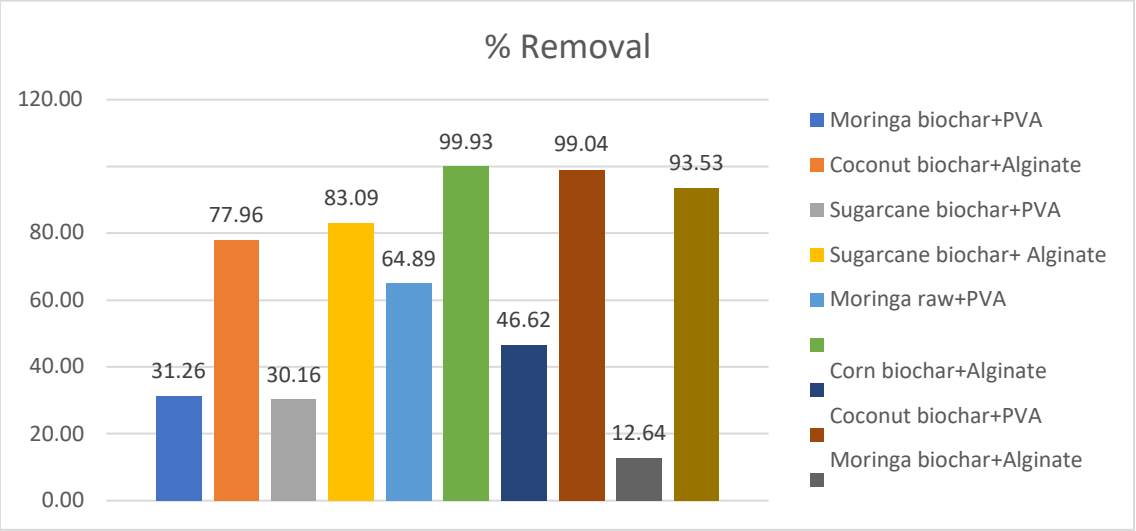
4.6. Water Holding Capacity:



In this table we could see the differences between quantities absorbed of water and conclude that the best material to apply for the agriculture fields was bagasse + coir, because it presents a higher capacity of retention.

5. Adsorption Study:

Different type of beads were chosen for adsorption study of blue dye. Batch adsorption equilibrium experiments were performed by taking 40mL of 0.01% blue dye water and 10g of each adsorbent. Sample was taken at the time interval of every hour and reading was noted. Parameters like pH, TDS, EC, Abs were measured. This procedure was repeated for different periods of time for two days. Among these 10 different types of beads Moringa biochar+ alginate, Corn biochar + alginate and Moringa powder raw+ alginate shows higher removal efficiency. For this test, 10 flasks, blue dye water and a shaker were required and in each flask 10g of each beads was taken, and 40ml of blue dye water was added in each flask. Then It was kept in a shaker for one hour. After that, the parameters, such as pH, electrical conductivity and total solids dissolved for each sample was measured. Then, placed in the shaker again. This procedure was repeated in different periods of time for two days. The best adsorbent was the beads made of corn biochar + alginate, the second one was moringa biochar alginate and the third one was moringa raw + alginate. With these results, we can conclude that the best agent to produce beads was alginate. It shows good properties to do beads with a higher removal efficiency Also, the higher efficiency was shown at the second measure, and then the values were almost constant.



6.Conclusion

Biochar was made from different sources. The characterization results confirmed the presence of biochar in the prepared samples. It could be concluded that corn biochar shows highest removal efficiency. Beads made from alginate shows better removal than PVA beads. Corn biochar shows highest removal of 99.94% of textile blue dye.

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Comparison of Mixed culture, *E. Coli, Aspergillus Niger and Bacillus sp.* in Bioremediation of Effluent from Small Scale Textile Dyeing Industry

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Abstract

Dyes are the visible contaminants that are released from the textile industries. Bioremediation of textile dye effluents using microbes would be the best suited greener method owing to their degradation potential of varied range of pollutants. Bacteria, fungi, yeast and algae have synergistic metabolic activities that alter the chromogen and degrade the absorbed dye colour. This work was aimed at investigating the dye declorization potential of a mixed microbial culture (MMC) obtained from different soil and sludge samples. . A single dye (Direct Blue 53) was used for comparison studies. The mixed microbial cultures were incubated for 9 d in mineral salt medium (MSM) with dye and the absorbance of its filtrate at 647nm (Blue dye) and 308nm (Industrial dye) was noted down for every 22hrsThe colour removal efficiency (CRE) by MMC were 47.04% , 46.77% , 45.21% and 35.02% for soil of textile dyeing unit (DS), sludge from STP (SE) soil (SS) from drying bed of STP and sludge from membrane reactor of dyeing unit (DE)respectively. Further, the maximum CRE of 98.35% was recorded by microbial culture from drying bed soil of STP (SS), followed by microbial culture from STP sludge (SE) was 97.96%, textile dyeing unit soil (DS) of 96.99%, and sludge form membrane reactor of dyeing unit (DE) was 96%. *Bacillus sp.* isolated from eco-bio block was tested against the blue dye and gave colour removal of about 89.25%. From this study, it is observed that the microbe present in soil obtained from the dyeing unit is naturally acclimatised to the dye waste and hence shows highest removal.

Keywords: Biodegradation, decolourization, textile dyes, mixed culture

I. Introduction

Textile industry is one of the largest industries in India providing employment for more than 35 million people in the entire country. Around 14% of the world's production of textile fibres and yarns were contributed from India. Textile industries accounts for 30% of the total exports and 14% of the total industrial production, thus playing an important role in deciding the national economy. The textile industry is interdependent to the dyestuff sector. Nearly 70% of the dyestuff produced is consumed by the textile industry (Chequer et al., 2013).

Different types of intermediate dyes were used in textile industries for fabric coloring such as; azo dyes, direct dyes, disperse dyes, vat dyes and reactive dyes. . The colour of the dye is due to the presence of the chromophore group. Wherein, the auxochrome group helps the dye in imparting the colour on the fabric. In the process of dyeing the dyestuff will attached with the cloth in different ways such as i) it will creates a covalent bond, ii) form a complexes with

metals or salts, iii)adhere onto the surface of the fabric by physical adsorption or by mechanical retention ((1)).

The dyestuff used in the dyeing process is not completely taken-up by the material, about 10-15% of the total dye is lost or discharged as waste during the process. Every year 28000 tons of dye waste were discharged from the dyeing industry. The waste discharge from textile dyeing industry contain toxic organic and inorganic compounds. The frequent use of artificial dye may cause environmental pollution and related health effects. Some dyes and their N-substituted aromatic biotransformation products are toxic and/or carcinogenic. Release of dye contaminated water into fresh water bodies tend to reduce the penetration of light into it and thereby affecting the photosynthetic activities of aquatic flora. Further, it decreases the dissolved oxygen and increases the biological oxygen demand of the contaminated water bodies (Ali H20, 2010).

Conventional effluent treatment methods consist of physiochemical processes such as chemical oxidation, precipitation, adsorption, electrolysis, coagulation, etc. The chemical stability of dyestuffs in the effluent makes the conventional treatment methods ineffective. Moreover, difficulties in handling of the waste generated and high operational cost are the major disadvantages of existing physiochemical methods. Thus, there is a need for the development of newer technologies that are efficient and environment friendly to reduce the dye contaminants to an acceptable level. Biological methods are known to be environment friendly to treat the dye waste water. It also operates at low cost and the end products formed are non-toxic in nature. These make the biological method the most effective method for the remediation process of dye effluent. The use of microbes ensures declorization of very complex synthetic dyes also. However, the activity of microbes and their decolorization ability depends on the dye, other conditions such as temperature, nitrogen source, carbon source, and pH.

Bioremediation of textile dyes using microbes is drawing the attention of researchers in the recent past due to their declorization potential. Microorganisms degrade dyes either by adsorption on microbial biomass or biodegradation by the cells or enzymes. Microbial adsorbents include bacteria, fungi and microalgae. In bio-adsorption process, the original dye structure is disrupted and often entirely decomposed (5). Microbial dye degradation takes place in two steps: i) reductive cleavage of azo linkages resulting in colorless but potentially hazardous aromatic amines ii) degradation of aromatic amines (Ali H, 2010).

Isolation of pure cultures from textile wastewater can be time-consuming and laborious. In addition, it is difficult to obtain complete decolorization by a pure bacterial culture. Mixed bacterial cultures, due to their cooperation for an enhanced effect, provide better results in decolorization and mineralization. They can efficiently degrade toxic aromatic amines. Nonetheless, the fact that these results are not readily reproduced and that mixed cultures do not provide the exact view of the dye metabolism makes the process and the results hard to interpret [7]

Biodegradation of several dyes among various bacteria, microbes, and fungi has been researched. Ecolorization is often used as an important marker for dye degradation. Decolorization occurs when the chromophoric center of the dye is cleaved [3]. The ability of

various organisms to degrade dye is variable depending on the dye, organism, and several other conditions such as temperature, nitrogen source, carbon source, pH, dye concentration, and others. Due to this variability, it is expected that a mixed microbial culture may be ideal for effluent treatment and remediation as this allows a variety of microbes to work simultaneously to degrade the dye. These different bacteria would likely use different pathways and mixed microbial culture would thus, produce a versatile treatment method that could be used for a variety of scenarios [3]. The biodegradation of textile dye effluent has received enough attention in the literature [11–15]. These studies, to cite a few, do not address remediation of textile dye effluent- contaminated sites. The clean-up of soil cannot be considered in isolation, but as a combined treatment of the soil-aquifer system

The textile dyeing industry is one of the complex and oldest of all industries. Ajmal (2016), in his studies stated that, India contributes the maximum textile waste water in South Asia. He also stated that, Because of the presence of more raw materials and industries in the regions of Gujarat and Maharashtra resulted in 90% of dye production in these areas.

Characteristics of textile organic dyes:

Discharge of textile organic dye wastes into water bodies causes pollution that is visible through naked eye. These dyestuffs are non-biodegradable in nature and they are accumulated as sediments in aquatic forms. The harmful dyes such as benzidine and other aromatic compounds. These dyes are generally carcinogenic. Similarly, Azo dyes are highly carcinogenic as they tend to damage the DNA leading to malignant tumors (Saini, 2017).

Physiochemical methods of dye degradation:

Adsorption is one of the most effective methods used for dye removal. An adsorbent is used to remove the dyestuff from the effluent. Activated carbon is commonly used adsorbent with pore volume of 0.3-1cm^3/g; bulk density of 300-550 g/L and specific surface area of 500-1500 m^2/g (Ajmal, 2016).

Irradiation uses radiations from monochromatic UV lamp that works under 253.7nm. This method requires constant supply of oxygen to breakdown the organic compounds in the dye.

Coagulation process utilizes chemical coagulants to remove the pollutants from the effluents. Lime, aluminium sulphate, ferric chloride, aluminium chloride, ferrous sulphate, etc are commonly used coagulants. These coagulants are reported to destabilize the minute suspended particles. After that these particles will colloides to form clusters (Coagulate) that settles as sediments which can be removed by filtration (Zaharia, 2007).

Biological methods:

Industries uses synthetic dyes for dyeing. Since, they are recalcitrant if they discharged into wastewaters it will cause severe environmental hazards. Certain microorganisms such as fungi and bacterial species have the capability to disintegrate different synthetic dyes (Fu and Viraraghavan, 2001; Pearce et al., 2003): Some enzymes of fungi are capable of dye removal have been isolated (Michel et al., 1991). In azo dyes the decolorization is started by degradation of the azo bond, and after that some aerobic microorganisms mineralize the colourless aromatic

amines (Zimmermann et al., 1982). In the first step, the dye reduction is the rate limiting step in the dye removal process. The NAD (P)H-dependent cytoplasmic 'azoreductases' have been described (Chen, 2006), but the azoreductase enzymes were not effective intracellularly (Blümel et al., 2002), because their enzyme activity will only reacts while using cellular extracts (Russ et al., 2000). Since, many dyes are very polar in nature the molecules will be permeable through the cell membrane (Pearce et al., 2003). The Bacterial dye reduction is considered as an extra cellular process (Keck et al., 1997; Kudlich et al., 1997). Through electron transferring proteins, the bacteria form a link between their electron transport systems inside the cell and the extracellular dye (Pearce et al., 2003). Under anaerobic conditions the dye removal rates of Shewanella strains J18 143 (Pearce et al., 2006) and decolourations were reported. Roy (2018) examined the effect of *Enterobacter* sp. on crystal violet dye. Different isolates of this species showed 81.25% removal efficiency against the crystal violet dye.

Mojsov in 2016 studied that the enzymes isolated from both anaerobic and aerobic bacteria have been identified which is very effective in the decolonization of the dyes. The enzymes were extracted from white rot fungi *Phanaerochaete* and *Trametes* during the limitation of nutrients levels such as carbon, nitrogen or sulphur, some other enzymes such as lactose, manganese peroxide and lignin peroxidase are synthesized by whiterot-fungi. They will oxidize different dye compounds, and therefore they will be examined in the textile dye effluent treatment.

The bacterial species, *Lactobacillus delbruckii* have a good dye removal efficiency. The decolourization was based on the dye concentration, pH and temperature and the optimization conditions for the decolourization were studied. *Lactobacillus delbruckii* is the best microorganism for the treatment of dye effluent.

II. Materials and Methods

a. Sources:

Different sources were identified for the microorganisms isolation. It include sludge (SE) from sewage treatment plant in Karunya Ladies Hostel, soil (SS) from sewage treatment plant in Karunya Ladies Hostel, effluent (DE) from textile dyeing industry and soil (DS) from the textile dyeing industry near Coimbatore. The soil sample was collected in zip-lock bags and the sludge samples were collected in plastic containers. Onions with fungal molds are selected for the study to isolate *Aspergillus niger*. The samples are freshly collected before use and if it is to be stored it is kept in refrigerator before being used.

b. Isolation – Mixed culture, *E. coli, Aspergillus niger, Bacillus sp.*

Nutrient Medium:

Weigh the needed quantities of beef extract, peptone, yeast extract & NaCl. Dissolve the ingredients in distilled water and make up the volume to 1L.Adjust pH of the solution to 7 ± 0.2.Take the solution in a clean conical flask and cotton plug the opening with a non-absorbent cotton. Sterilize the medium at 121°C/ 15psi pressure for 15 minutes.

Nutrient Agar Medium:

Weight the needed quantities of beef extract, peptone, yeast extract & NaCl. To the ingredients add 16g of Agar and mix gently. Dissolve the ingredients in distilled water and make up the volume to 1L. Care should be taken to dissolve the agar completely. Adjust pH of the solution to 7 ± 0.2.Take the solution in a clean conical flask and cotton plug the opening with a non-absorbent cotton. Sterilize the medium for 121°C/ 15psi pressure for 15 minutes. After sterilizing the solution, pour the medium onto a sterile petri-plate (approx. 15ml) and allow the medium to solidify before use.

Mineral Salts Medium:

Weigh the needed quantities of the ingredients using a sterile paper. Dissolve the ingredients in distilled water and makeup the volume to 1L. Adjust pH to 7 ± 0.2.Cotton plug the opening the conical flask firmly. Sterilize the medium by autoclaving. Add the ingredients one after the other and make sure to completely dissolve an ingredient before adding the next one.

Potato Dextrose Agar:

Weigh 19.5g of commercially available PDA.Dissolve it in distilled water and makeup the volume to 1 L.Adjust pH of the medium to 7 ± 0.2.Cotton plug the mouth of the conical flask and sterilize the medium by autoclaving.

Eosin-Methylene Blue Agar:

Weigh 35.9g of EMBA and dissolve it in distilled water. Adjust pH to 7 ± 0.2.Cotton plug the opening of the conical flask and sterilize the media by autoclaving at 121°C / 15 psi pressure for 15 minutes. The media turns metallic green colour upon heating and so it is cooled to 45°C to bring back its original colour (wine red/purple).

Enumeration of *Aspergillus niger*:

2ml of sludge (DE) sample collected from dyeing unit is taken for enumeration of *A.niger*. Potato Dextrose medium (PDA) is used for culturing *A.niger*. Based the need the PDA media is prepared and is autoclaved for 15minutes. After autoclaving, the media is kept inside the LAF chamber, it is then poured onto the sterile petri-plates. Before transferring the medium, the petri-plates are flame sterilized and then the poured media is allowed to solidify.

Once solidified, sludge sample is taken in a sterile pipette and slowly poured onto the solidified media. After which a flame sterilized L-shaped glass rod was used to spread the sample in the PDA medium. The petri-plates were sealed using paraffin tape to avoid contamination and is stored at room temperature. The cultured petri-plates are periodically examined for the growth. Generally the culture is incubated for 5-7days to obtain matured culture of *A.niger*.

Isolation of *Aspergillus niger* from onion:

Onions with fungal molds are selected for the study. PDA media and the glasswares were autoclaved at 121ºC at 15psi pressure. The autoclaved media is poured onto the petri plates and was allowed to solidify. Swap is made using non-absorbent cotton and a stick. The *A.niger* stains are swapped gently from onion's surface using the cotton swap. Then it is spread onto

the plates with PDA. The cultured plates are stored at room temperature (27ºC - 30ºC) in the incubator for 3-5 days.

Enumeration of *E.coli* from samples (Soil, sludge & Waste water):

100ml of Eosin – Methylene Blue Agar media was prepared. To 100ml of distilled water 3.59g of EMBA was dissolved. Prepared media autoclaved along with the petri-plates at 121ºC and 15 psi pressure for about 20 min. After cooling the media is poured into the petri-plates under aseptic conditions and is allowed to solidify.1g of soil sample is dissolved in 10ml of distilled water and was taken for inoculation. Once the agar is solidified the samples are inoculated on the respective plates by streaking method using a loop. After inoculation the plates are incubated at 37ºC in the inverted position. Results are to be observed after 24hrs of incubation.

Enumeration of Mixed Microbial Culture:

For obtaining mixed microbial culture, 13g of synthetic Nutrient Broth medium is dissolved in 1litre of distilled water. The prepared medium is autoclaved at 121°C at 15psi pressure for about 20minutes. 100mL of prepared Nutrient broth medium was transferred aseptically into four separate 250mL sterile flasks. 2mL of sludge and dye effluent , 2g of soil from dyeing unit and from STP were transferred to the flask containing sterile nutrient medium. The culture flasks are then incubated at 34°C for 24hrs and is used as the stock mixed microbial culture for further use.

c. Acclimatization

Biodegradation abilities of mixed microbial culture is increased by gradually introducing them to higher pollutants for treatment. This process is called acclimatization. Enhancing the capability of microbes to improve the decolorization process.

Sterile mineral salts medium (MSM); 1g NaCl, 0.1g Calcium chloride, 0.3g Magnesium sulphate heptahydrate, 1g Potassium dihydrogen phosphate & 1g disodium hydrogen phosphate and 3g yeast extract was dissolved in 1000ml of sterile distilled water. (8 flasks with microbial culture inoculated and 2 control flasks without culture for each dye)

100mL of MSM was aseptically added separately to each flask. The flasks are named from F1 to F10. F1 and F2 was used as control and added with blue dye and industrial dye separately. Flasks F3, F4, F5 & F6 are enriched with 50ml (100ppm concentration) of blue dye in each and four different stock microbial culture was added separately in each flask. Wherein, F7, F8, F9 & F10 are added with 50ml of industrial dye and four different microbial cultures were added separately. Initial absorbance was noted and further readings were taken after 24hrs of inoculation.

d. Decolourization Measurement:

The decolourization rate was measured by the percentage reduction of absorbance value . Original textile dye water is dark in colour as it is a mixture of various single dye. Percentage of decolourization was calculated using the formula;

$$\% \text{ of decolourization} = \frac{(Initial\ absorbance - Final\ absorbance)}{Initial\ absorbance} \text{ x } 100\%$$

UV-spec reading was taken at every 22hrs interval for all the 10 samples and colour removal efficiency was determined.

III. Experimental Investigation

a. Isolation

Culturing of *A.niger* from samples:

Aspergillus niger were isolated from the samples. It can easily identifiable species of the *Aspergillus,* genus with its white to yellow mycelial culture surface and black conidia. On Potato Dextrose Agar, conidial heads are large (upto 15 to 20 µm in diameter), globose, becoming loosen and will seperated into loose columns with age. The colour of the colonies were changed to white to dark brown in 5-7 days of incubation period (Figure 1).

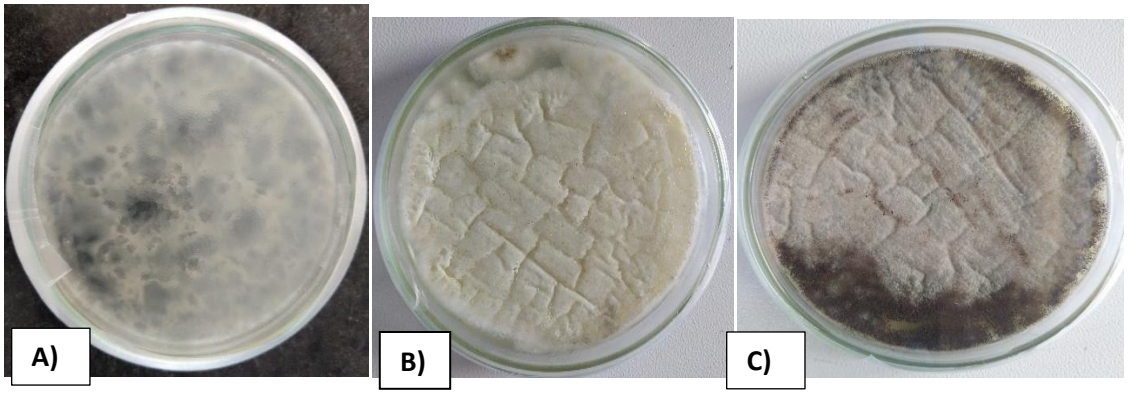


Fig 1 : a) 18 hours old A.niger culture on Potato Dextrose Agar. b) 3 days old A.niger on PDA – white mycelial culture. c) 7 days old mature black conidia of A.niger.

Microscopic observation of *A. niger:*

Microscopic images shows smooth and colorless conidiophores and spores. These, *Aspergillus niger* changes dark to dark brown spores from their heads. This is a unique characteristic of A. niger which was not seen on others. In microscopic view, the carbon black or brown color (as well as the conidia) shows the presence of *Aspergillus nige* (Figure 2). *r.*

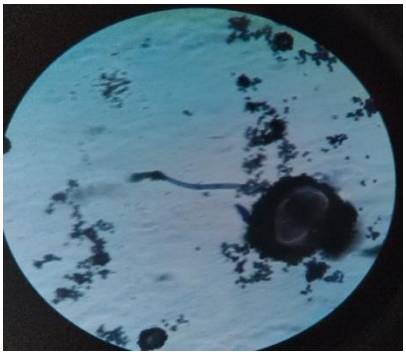


Fig 2 : Microscopic image of A.niger showing long tail like hyphae and global shaped conidial heads

Enumeration of *E. coli:*

Escherichia coli present in fecal matter is isolated by using selective media called Eosin methylene blue agar (EMB). Eosin Y and methylene blue are used as a pH indicators which forms a dark purple precipitate. This dyes will also inhibit the growth of most Gram-positive bacteria. Sucrose and lactose acts as a fermentable nutrient sources which increase the growth of microorganisms.

These nutrient sources such as lactose and sucrose which synthesis large amount of acid which will form the purple colour. These organisms will change the colour from dark purple to black. The fermenter (*E. coli)*, will produces a green metallic colour. Some weak fermenters will form mucoid pink colonies. Generally the less-colored or colorless colonies showed that the organism didnot ferments any nutrient sources (Lactose and Sucrose) and it is not a faecal coliform (Figure 3)..

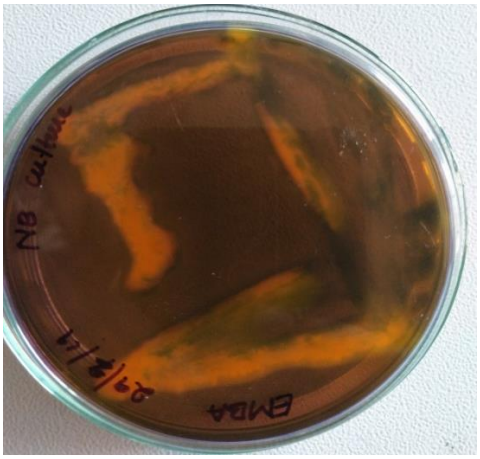


Fig 3 : Escherichia coli on EMBA

Enumeration of Mixed Microbial Culture:

After 24hrs of incubation the nutrient broth medium turned turbid indicating the growth of microorganisms. Bacterial growth can be identified as the gradual increase in the number of all bacterial cells. The cells will be divided into two equal daughter cells that are identical to the original one. The growth medium plays a key role in the growth rate. Growth rate increases with adequate amount of nutrients being available in the medium. The exponential increase in the bacterial cells will increase the turbidity of the nutrient broth medium. The four flasks containing different sources of inoculums serve as the stock solution and were stored at 4°C for further use (Figure 4)..

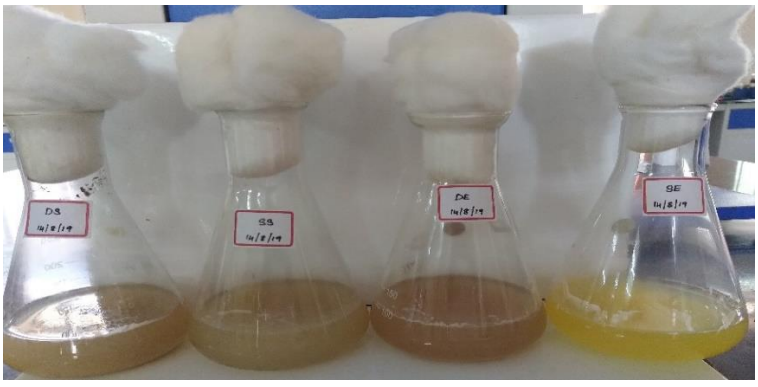


Fig 4 : 24hours old mixed microbial culture from four different sources (STP sludge, STP soil, Textile sludge & Textile soil)

b. Performance of mixed microbial culture in Blue dye and Industrial dye:

The maximum peak was found at the wavelength of 647nm for blue dye and 308.5nm for the industrial dye water. And the initial absorbance measurement was found to be 4.5453 AU for blue dye and 4.3599 AU for industrial dye (Figure 5)..

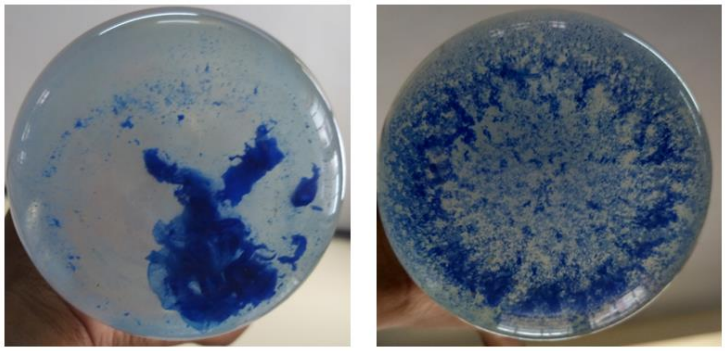


Fig 5. Blue dye adsorbed by the mixed microbial culture from DS and SS samples

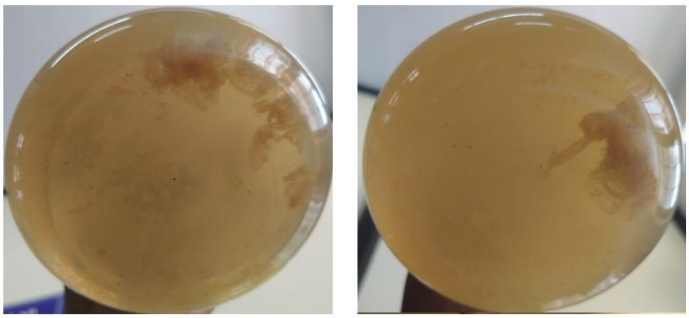


Fig 6. Industrial dye adsorbed by the mixed microbial culture from DS and SS samples

The above Figure (6) shows the acclimatized culture flasks of DS and SS samples in synthetic blue dye. The microbial culture agglomerated into clusters of varied shapes and sizes and absorbed the dyestuff contaminant from the wastewater. This shows that, the decolorization of the dye is due to adsorption on microbial biomass (biosorption).

IV. Results and Discussion

a. Performance of Mixed culture

Direct blue dye used for the study is an azo dye containing –N=N- as the chromophore group. It is formed by azo-coupling of o-dianisidine with the appropriate naphthalene disulfonate molecule. The microbial consortium produces azoreductase which catalyse the split of the –N=N- bond. Azoreductase produced uses two equivalents of NADH molecule to reduce one azo-compound which results in the formation of two other equivalents of aniline as the end product. The possible reaction mechanism is as follows;

(o-dianisidine)-N=N-(naphthalene disulfonate) + 2(NADH) → (o-dianisidine)-NH_2 + (naphthalene sulfonate)-NH_2 + 2NAD^+

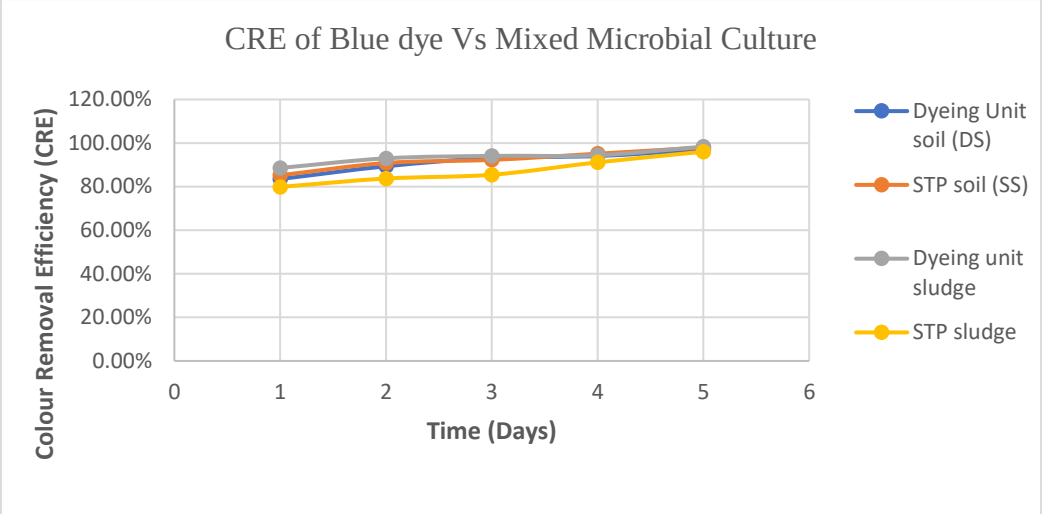


Fig7. Dye removal efficiency of mixed microbial culture against the blue dye

Figure 7 shows colour removal efficiency of mixed microbial culture against the blue dye. There was a gradual increase in CRE percentage and it showed maximum removal on the 5th day. Microbial culture enumerated from the dyeing bed soil showed the maximum CRE of 98.35%.The microbes from dyeing unit soil shows increased colour removal efficiency because these naturally prevail in the extreme environmental conditions such as pH (slightly basic – above 7.5 or 8), micronutrients (from the complex dye structures), etc. Whereas, the sludge samples from dyeing unit and STP have already passed through the treatment cycles which reduces the availability of the needed nutrients for microbial growth. Thus, shows a reduced CRE compared to that of the microbes from soil sources.

The industrial dye effluent contains 30-40 individual disperse dyes being mixed together. This gives it a dark brown colour. Disperse dyes are water insoluble and are mostly azobenzene or anthraquinone dyes. The azobenze dyes contain two phenyl rings linked by a -N=N- bond and the anthraquinone dyes are polycyclic aromatic hydrocarbons. As disperse dyes are mostly azo dyes, the decolourization resulted would be due to the azoreductase mechanism.

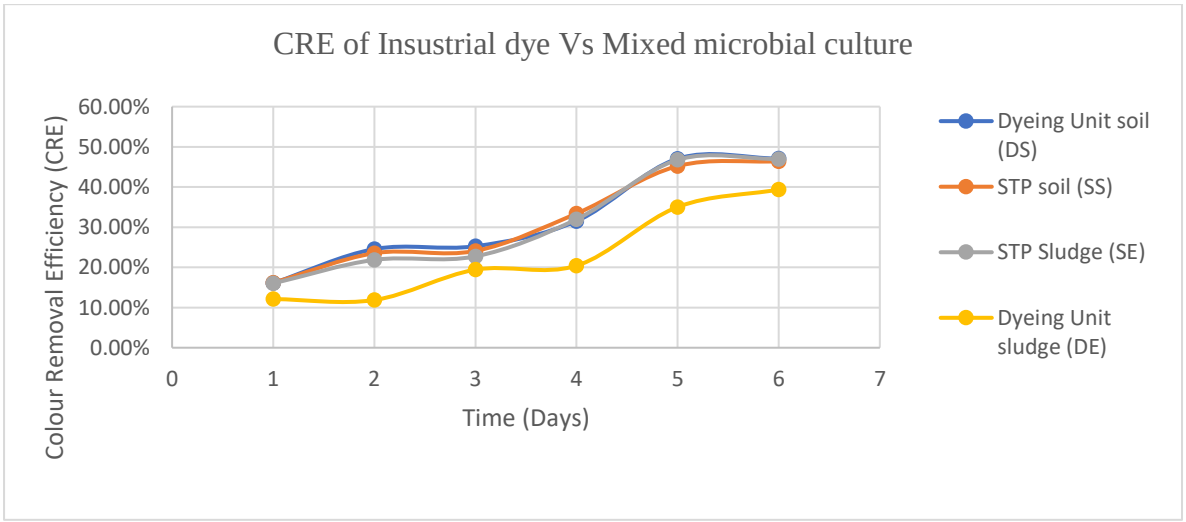


Fig 8. Dye removal efficiency of mixed microbial culture against the industrial dye

Figure 8 shows the CRE of mixed microbial culture against the industrial dye. The maximum removal was recorded on the 6th day. Microbial culture obtained from dyeing unit soil showed the maximum removal of 47.04%. This is because of the microorganisms prevailing in the dyeing unit soil is naturally acclimatized to the dye pollution. The microbes present in these sources utilize the dye stuff as carbon and nitrogen sources.

b. *E.coli* - Performance and Interpretation

Possible mechanism of dye removal by *Escherichia coli:*

Escherichia coli, shows the dye removal by azo- reductase activity. The electron donors will helps to reduce the azobond by Azoreductase. The azo bond breakdown which requires 2 mole of NADH to reduce 1 mole of a typical azo dye, into 2-aminobenzoicacid and N, N-dimethyl-p phenylenediamine (Ping-Pong Mechanism) (Dawkar et al., 2008). The enhancement of azoreductase during removal of azo dyes under stable condition was already reported (Dhanve et al., 2008). The Azoreductase enzymes synthesis some toxic amines. Despite the fact that, azo dyes were degraded only in anerobic conditions. This study reported the anerobic degradation of azodyes. Generally, anerobic reduction are sensitive to molecular oxygen but aereobic reduction has not inhibited by molecular oxygen. Hence, aerobic enzymes are called as oxygen insensitive azoreductases.

c. *Aspergillus niger* -Performance and Interpretation

Possible mechanism of removal by *Aspegillus niger:*

The decolourization of dye molecule may occur through the following steps which showed in the figure. The dye removal occurs by deamination and dehydrogenation process [Guibal *et.al,* 1995, Figure 9].

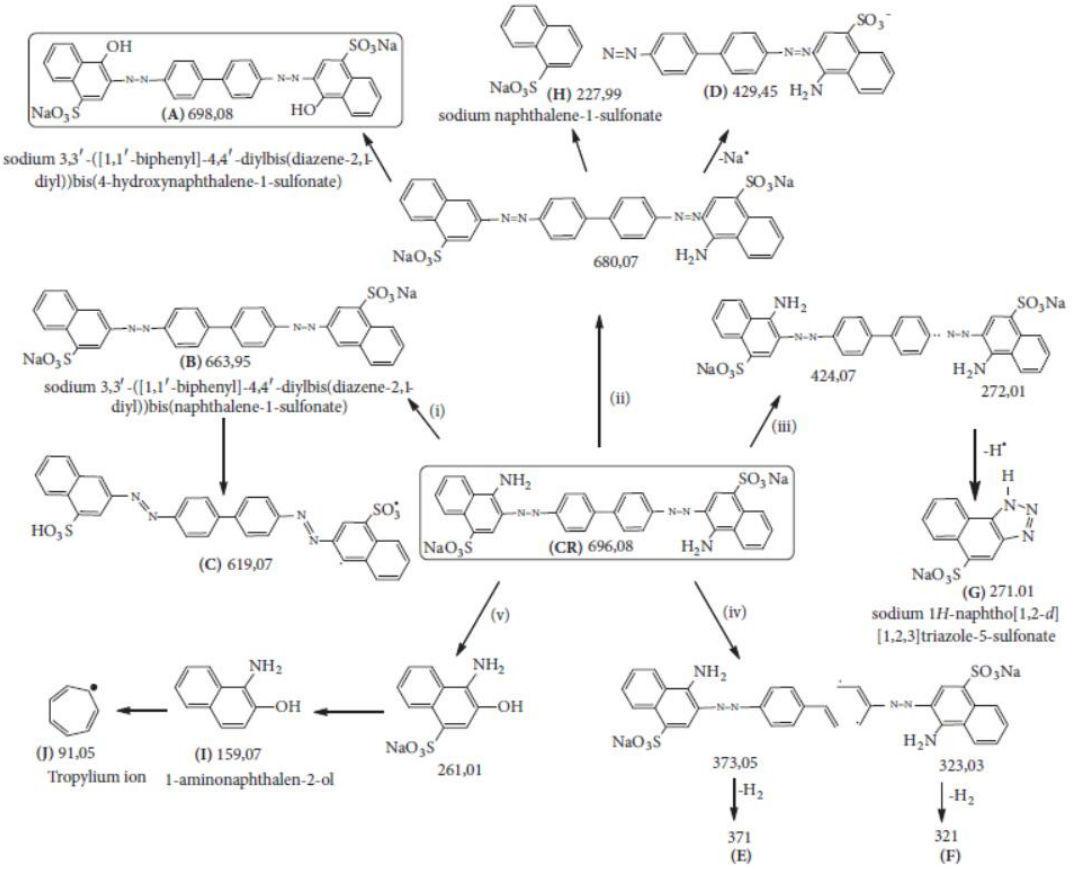


Fig 9: Biodegradation pathway of dye by A. niger (Asses et al., 2018)

d. *Bacillus* -Performance and Interpretation

Bacillus was isolated from the available eco-bio block made of volcanic ash & dormant *Bacillus* strain. Nutrient broth media was used to culture *Bacillus.* A piece from the eco-bio block was taken and crushed into powder to be used as the inoculation sample. The media and the glasswares were autoclaved before use (Figure 10)..

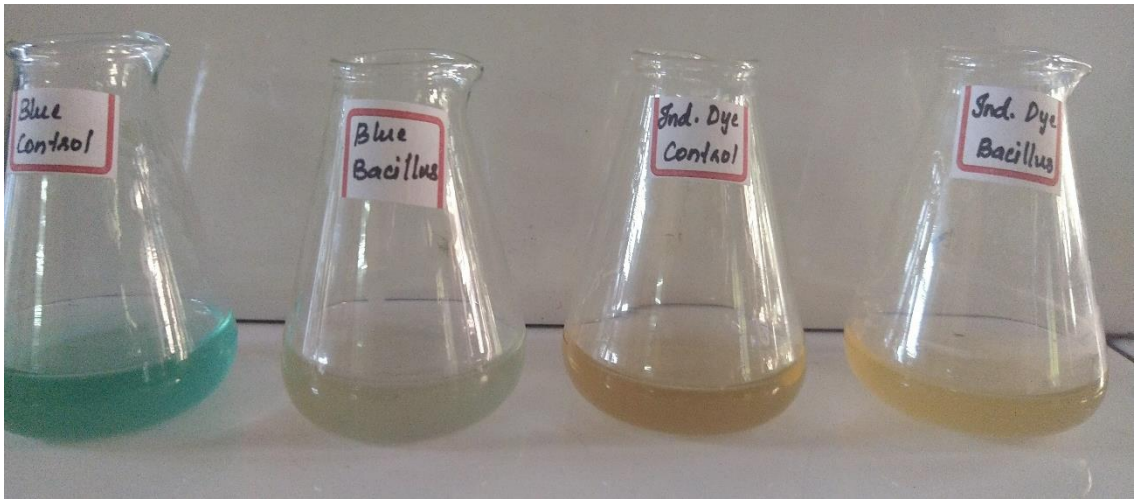


Fig 10: Colour Removal of different dye samples after 5 days of incubation.

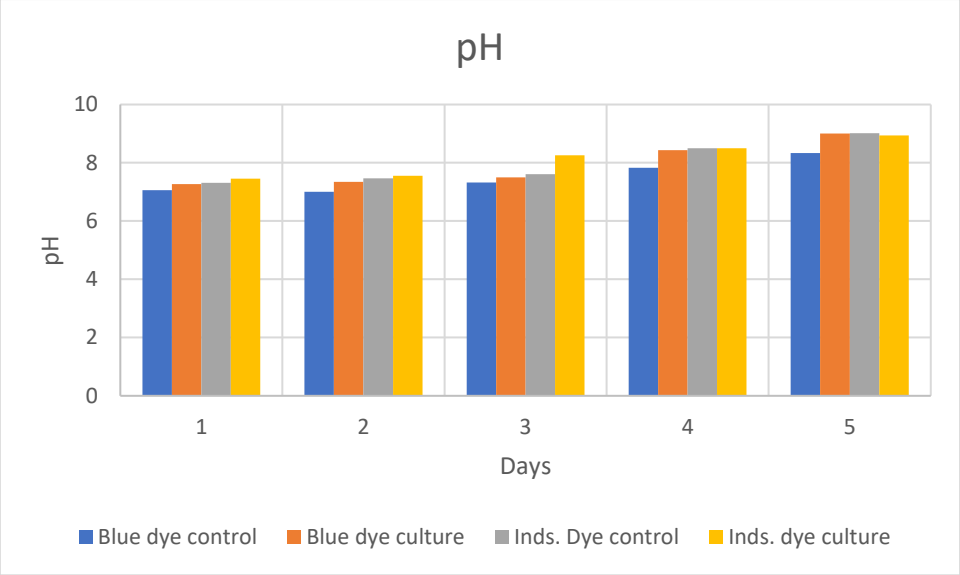


Fig 11: pH variation of different dye samples.

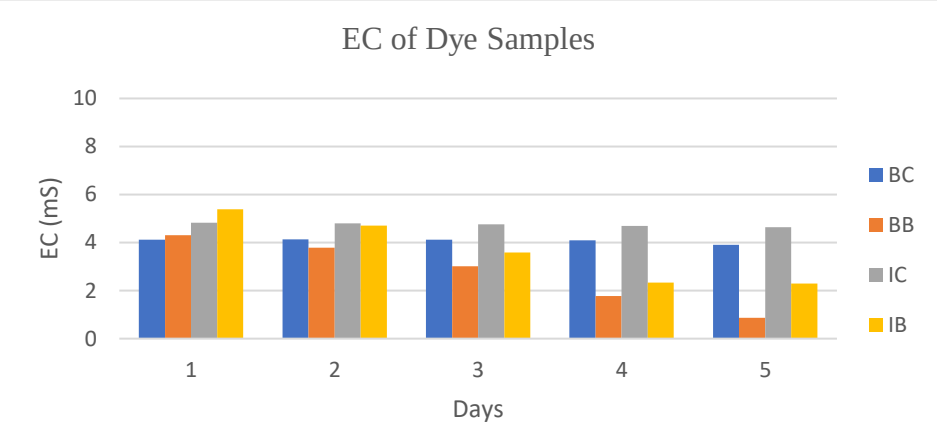


Fig 12: EC removal in different dye samples

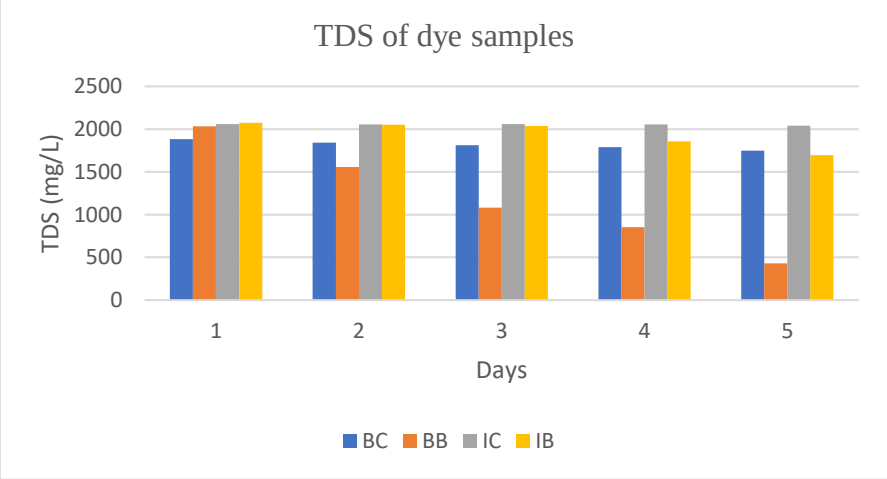


Fig 13: TDS removal in different dye samples

EC and TDS of blue dye treated with *Bacillus* culture samples showed considerable reduction. Whereas the culture samples with industrial dye water showed only fewer reduction. This was because the industrial dye waste consisted of more than 30-40 individual dyes that contributes to their complex nature (Figures 11,12,13).

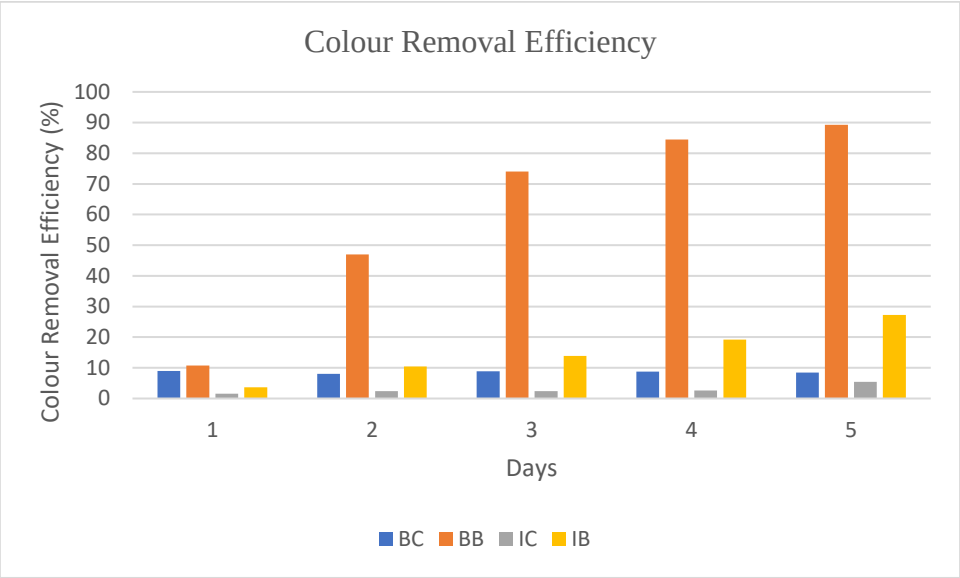


Fig 14: CRE in different dye samples

The above graph shows the CRE of *Bacillus* culture against the blue dye and industrial dye. The maximum removal was recorded on the 5th day in flask containing Blue dye and culture of about 89.25%. Flask containing industrial dye and culture samples showed only less removal of about 27.28%. This is because of the industrial dye samples exhibit a complex nature as it contains 30-40 dyes together (Figure 14)..

Possible mechanism of dye removal by *Bacillus sp.:*

The removal of dye stuff from effluent is caused by the presence of the enzymes oxido-reductase from *Bacillus sp.* This heme protein helps for the breakdown of lignin by oxidation. Peroxidases (LiP), catalyze phenolic substrates which results in radical formation by using hydrogen peroxide as the electron donor. By combinig of MnP and LiP another peroxidases, versatile peroxidases (VP), was produced. These Versatile Peroxidases can oxidize Mn2+ but phenolic and non-phenolic aromatic compounds called dyes. These peroxidases are produced by the series of reactions such as reductive cleavage. Peroxidases utilizes the azo-reductase enzymes to cleave/break the N=N azo bond. The resulting product undergoes desulfonation and deamination which further metabolize the organic complex compounds to much simpler forms (Figure 15)..

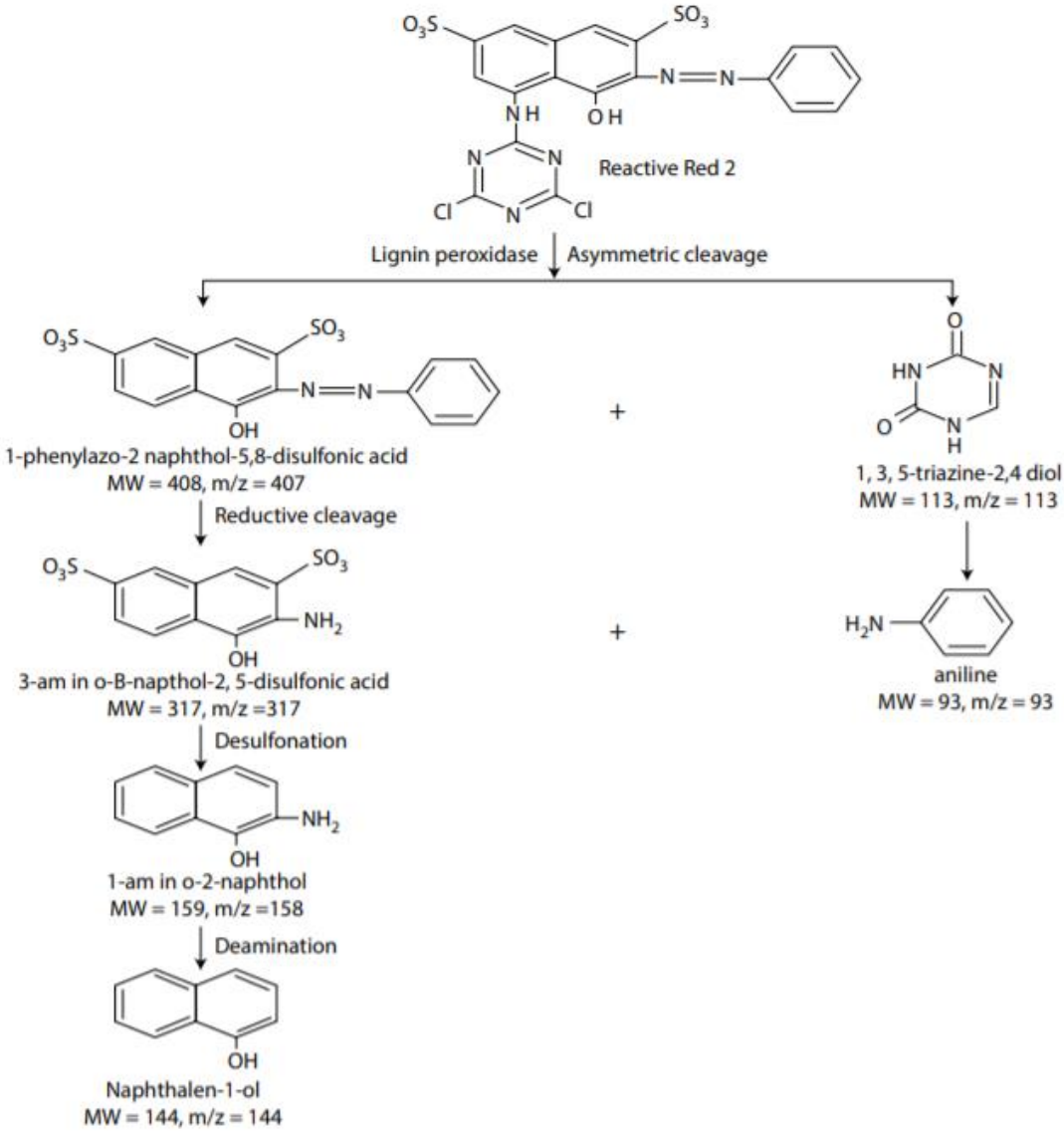
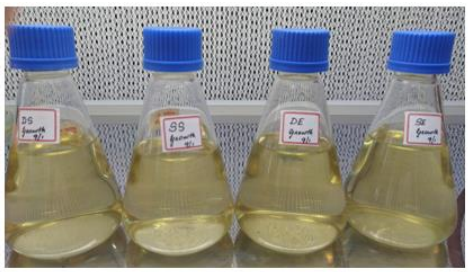


Fig 15: Biodegradation pathway of Bacillus sp. (Khandare and govindwar 2016).

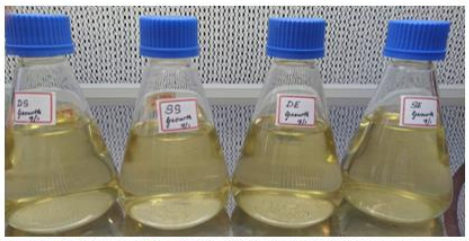
e. Growth rate:

The bacterial growth curve includes various stages of growth of bacteria such as Lag phase, Exponential (log, logarithmic) phase, Stationary phase. Death phase (exponential or logarithmic decline)

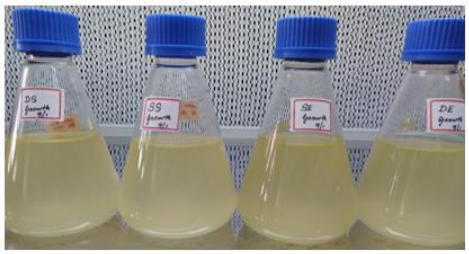
In lag phase, the bacterial cells adapt themselves to the new medium. The cell size will increased but no changes in cell number. In log phase, the bacteria divide rapidly. The cell size and number increased. The curve grows linear in this phase and the cells are enzymatically most active. During the stationary phase, as the nutrients drained, and waste were formed and secondary metabolic products will produced, the growth will equals to the death rate, hence live cell number is same. Then the cell will goes to the death phase. In the death phase, cell density will decreases fastly because the cell lacks nutrients increases. The death of cells in the population exceeds the formation of new cells (Figure 15,16,17).



a) Culture flasks at 0th hour



b) Culture flasks 4 hours



c) Culture flasks 8 hours

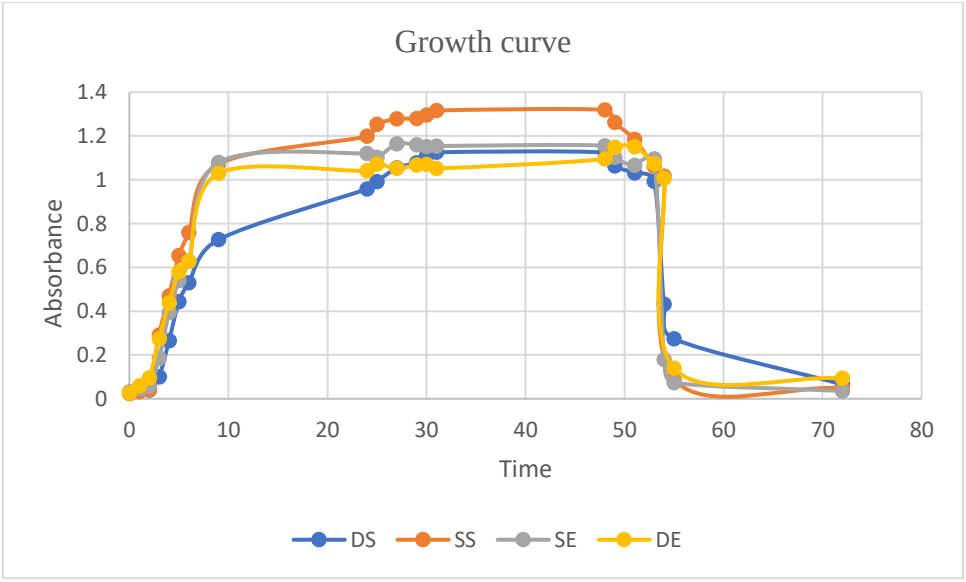


Fig 16. Growth curve of DS, SS, SE & DE samples at different time intervals.

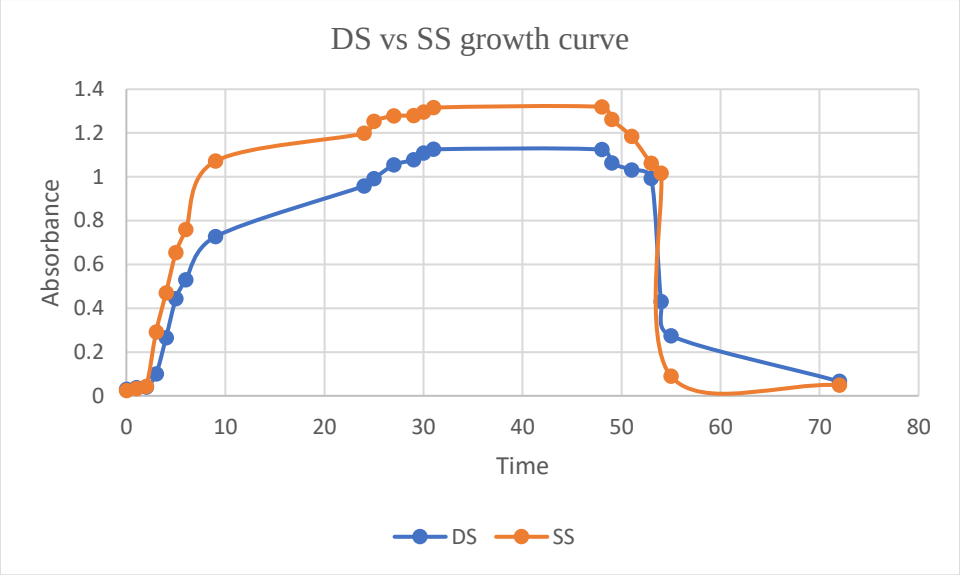


Fig 17. Comparison of DS & SS growth curve

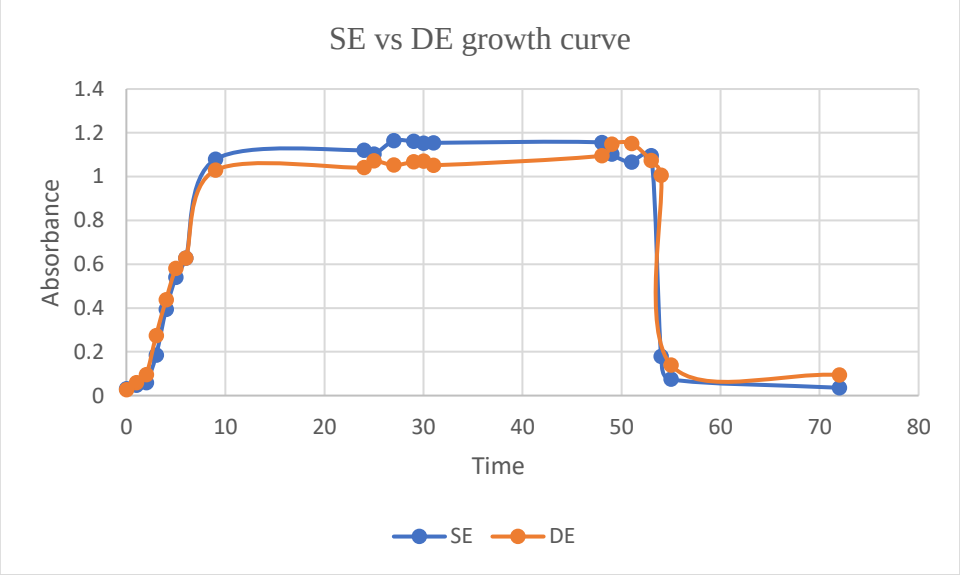


Fig 18. Comparison of growth curve of SE & DE samples

V. Summary and conclusion

The bacterial biodegradation is proved to be a viable treatment for the contaminated textile dye wastewater. The acclimatized culture studies showed that the microbial consortium is naturally accommodated to the dyestuff environment. The major difficulty encountered in the continuous soil column studies was in maintaining the steady flow-rate of inflow dye wastewater sample. The column studies were continued further after making modification in flow-rate and other experimental setups.

VI. References:

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Oil Remediation by PRP Powder

1. Introduction

Indian textile industry is one of the leading industries in the world. The Textile dyeing industries have the greater potential to cause water pollution. Dyeing is the most important step in the textile industry. Most of the dye molecules do not bind with the fabrics. As a result, it will lose in the water as wastewater which leads to pollutants. During finishing 10-15% of dyes were lost in wastewater in the textile industries. Dyeing in the textile industry generates a large quantity of effluents that have a higher concentration of mineral salts and dye molecules. Nearly, 700000 tons of dye effluents were discharged worldwide. An estimated textile industries release the dye stuff in the range of 10 to 100 mg/l which is visible. These dyes are synthetic and are more complex. It is having an aromatic structure that is more stable and difficult to disintegrate. The mostly used dyes are azo, anthraquinone, and phthalocyanine (Aksu and Donmez 2005). These dye stuff and their products are highly carcinogenic and cause mutagenicity to flora and fauna. These dyes interfere with photosynthesis in the marine ecosystem. The dark visible color in the water will make the dye difficult to penetrate. It also has some harmful aromatic, metals, and chlorides (Pandey et al 2007). These dye effluents were difficult to treat because this effluent water is having high BOD, COD, Colour, and pH. The most problematic environmental problem is the improper disposal of dye wastewater from the textile industry. Since textile dye effluents contain heavy metals such as Zinc, Cadmium, and lead. When the wastewater containing dyes is used for irrigation, these heavy metals get aggregated into the various plant parts and will cause severe health problems to humans and animals (Basha and Rajaganesh 2014). Textile wastewater contains various structurally complex synthetic dyes when released into the aquatic environment, reduce the dissolved oxygen concentration which will create anoxic conditions that are harmful to aquatic ecosystems. Generally, dye wastewater was treated by traditional physical and chemical methods. Physical treatment methods include filtration, sedimentation, and adsorption. These methods are having some drawbacks. Some chemical treatment methods include coagulation or flocculation techniques along with activated sludge treatment. These methods possess some disadvantages such as the formation of sludge, costly (Senen et al 2003). Some other treatment methods include ozonation, UV, or Hydrogen peroxide will generate hydroxyl radicals which are reactive oxidants. But this method is having certain drawbacks such as consumption of high energy and raw materials. Adsorption by using Activated carbon and nanofiltration will also remove dyes from textile wastewater. The disadvantage of this method is the accumulation of

solid waste which requires additional treatment for disposal. The sludge from treated water will form a secondary level of land pollution. (Aamr and Cuiling 2012). Biological treatment methods are cost-effective and eco-friendly method which is an alternative treatment method for dye removal. This bioremediation is a pollution control method that uses bacteria, fungi and algae etc., for the color removal and disintegration of certain harmful chemicals to less effective forms (Sriram et al 2013). Many works have been reported for the bioremediation of textile wastewater. Various types of microorganisms have been grown in the dye-rich water and soil.

1.1 Treatment by Bacteria

Bacteria decolourization of dye is faster method than other biological method. Many bacteria capable of degrading dyes has been studied. Some bacteria isolates such as Bacillus subtilis and Aeromonas hydrophilia are capable of degrading dyes from 1970. Recent studies have revealed that some bacteria strains can degrade dye molecules under aerobic conditions. Many bacterial strain requires additional carbon source as they cannot completely utilize dye as a sole energy source (Naresh et al 2013).

1.3 Oil Remediation by PRP Powder

The oil spills from the industries to the water bodies creates the major threat to the environment. From 20^{th} to 21^{st} century more than 7 million tonnes of oil from industries was discharge into the environment from over 140 massive oil based industries (Ornitz and champ 2002). The oil spills is usually termed as a leakage of petroleum hydrocarbons into the environment especially into the water bodies due to human activities or by natural disasters (Uzma et al 2008). Scientists found that the, consumption of oil contaminated water leads to the severe DNA damage and it leads to cancer. This will also cause extreme psychological stress. Petroleum based products consists of a complex mixture of hydrocarbons which consist of benzene ring based aromatic and some short-length aliphatic hydrocarbons. The chemical compounds such as xylene, toluene, benzene and polycyclic aromatic hydrocarbons which may cause an adverse effects to humans (Bahadar et al 2014). Frequent exposure of high concentration of Volatile organic compounds may lead to the damage in central nervous system which cause symptoms such as headaches, fatigue and dizziness. This will also lead to oxidative stress and decrease in the white blood cells count which weakens the immune system. Particularly, benzene is a carcinogenic substance (Kim et al 2013). Polycyclic aromatic hydrocarbons such as naphthalene affects the formation of red

blood cells which leads to anemia. The major symptoms are nausea, vomiting skin and eye irritation. The petroleum hydrocarbons treatment methods can be classified into physical, chemical and biological method. Physical mechanical methods uses high temperature booms which enable the burning of oil in-situ. Skimmers are also used to remove oil from water surface. In chemical methods, chemicals sprayed into the oil spill that will break it up into small droplets. These methods have some disadvantages have many limitations like costlier, require additional cleanup (Dave and Ghaly 2011). Biological treatment method is very effective which utilize the microorganism for the treatment. It has been found that most of the crude oils are biodegradable. The microorganisms uses hydrocarbons for its growth and metabolism and degrade the contaminants. However the availability of nutrients for biodegradation is limited. The application of additional nutrients will enhance the biodegradation ability. The Petroleum remediation product powder is a commercial bioremediation product. It was made up of hollow microsphere of beeswax which act as a nutrients source for the oil-degrading bacteria. It is up to 100 micron in size. The PRP powder is highly oleophilic as well as hydrophobic so, Water cannot penetrate into the microsphere. The microorganisms are encapsulated into the sphere are capable of degrade the petroleum products. The advantage of this method after degradation of hydrocarbons into simpler forms such as Co_2 as a residue. The releases residue is environmental friendly and can be utilized by aquatic environments (Lee et al 1997).

The aim of this study is to check the biodegradation of the textile dye effluent by bacteria, algae isolated from textile effluent and chlorella vulgaris. The bioremediation of oil such as petroleum hydrocarbons by using Petroleum Remediation Product and bacteria.

2. Literature Review

Dyeing is the most important step in the textile industry. Most of the dyes are lost as wastewater. The treatment of dye wastewater is one of the challenging environmental problems. The treatment of textile dye effluent follows various physical and chemical methods. Mc. Mullan et al (2001).

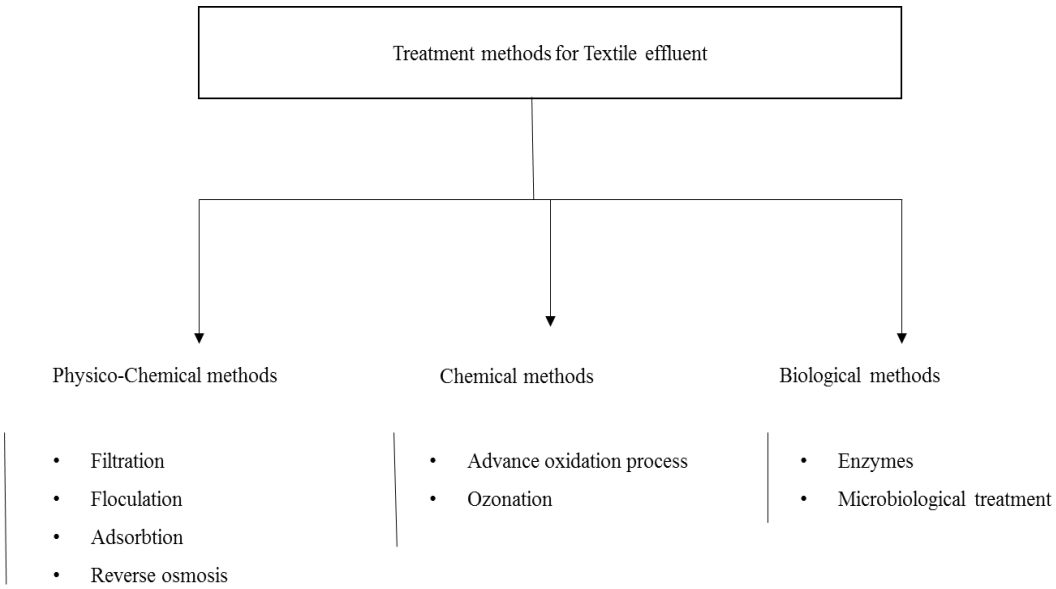

Figure 2 Methods of Textile effluent treatment (Radia and Romana 2019)

2.1 Bacterial Bioremediation

Dyes are structurally complex and its decolorization was very challenging. Some microorganisms such as bacteria, fungi, and algae have the ability to degrade complex dye molecules. The effective dye treatment depends on the activity and sustainability of microorganisms. Generally, Microorganism disintegrate textile dyes by using two methods i) adsorption by microbial biomass ii) disintegration of dyes by using enzymes. The use of biomass as adsorbent is useful only when the textile effluent was more toxic and does not helps bacterial growth. Bacteria, fungi and algae can be used as an adsorbent. Adsorption methods does not disintegrate the complex dye molecules into smaller fragments. Whereas the method involves the complete transformation of dye into smaller forms. So this method is more practical (Han et al 2012). Microbial degradation of azo dyes involves the breakdown of azo bonds by using enzymes azoreductase which helps for the transfer of four electrons to azodyes. The azo dyes are electron acceptors, which results in the dye decolorization and formation of some intermediate products such as metabolites. These intermediates are degraded further by aerobically or anaerobically.

From 1870's *Bacillus cereus, Bacillus subtilis.* and *Aeromonas hydrophillia* are effective in decolorization of a number of dyes (Sriram et al and, Shah 2013, Wuhrmann et al 1980). The dye decolorization bacteria were isolated from the textile effluents and it shows good colour removal efficiency (Aktar et al 2019). The microbial consortiums have good dye

degradation efficiency when compared with the individual microbial strain. Phuagre et al (2011) *Providencia sp.* and *pseudomonas aeuroginosa.* Occurred as a consortium. The dye removal was observed within 1hr incubation in the consortium and it detoxifies the dyestuffs. Lopez et al (2006) isolated the ligninolytic microorganisms from wastes for dye removal. This lignolytic system of microorganisms helps them to degrade complex dye molecules. The aerobic degradation of dye stuff from the effluent is caused by the presence of the enzymes oxidoreductase. The heme protein present in the enzymes helps for the breakdown of lignin by oxidation. Peroxidases (LiP), catalyze phenolic substrates which result in radical formation by using hydrogen peroxide as the electron donor. By combining Manganese peroxidase and LiP, versatile peroxidases (VP), was produced. These Versatile Peroxidases can oxidize Mn2+. These peroxidases are produced by a series of reactions such as reductive cleavage. Peroxidases utilize the azo-reductase enzymes to cleave/break the N=N azo bond. The resulting product undergoes desulfonation and deamination which further metabolizes the organic complex compounds to much simpler forms.

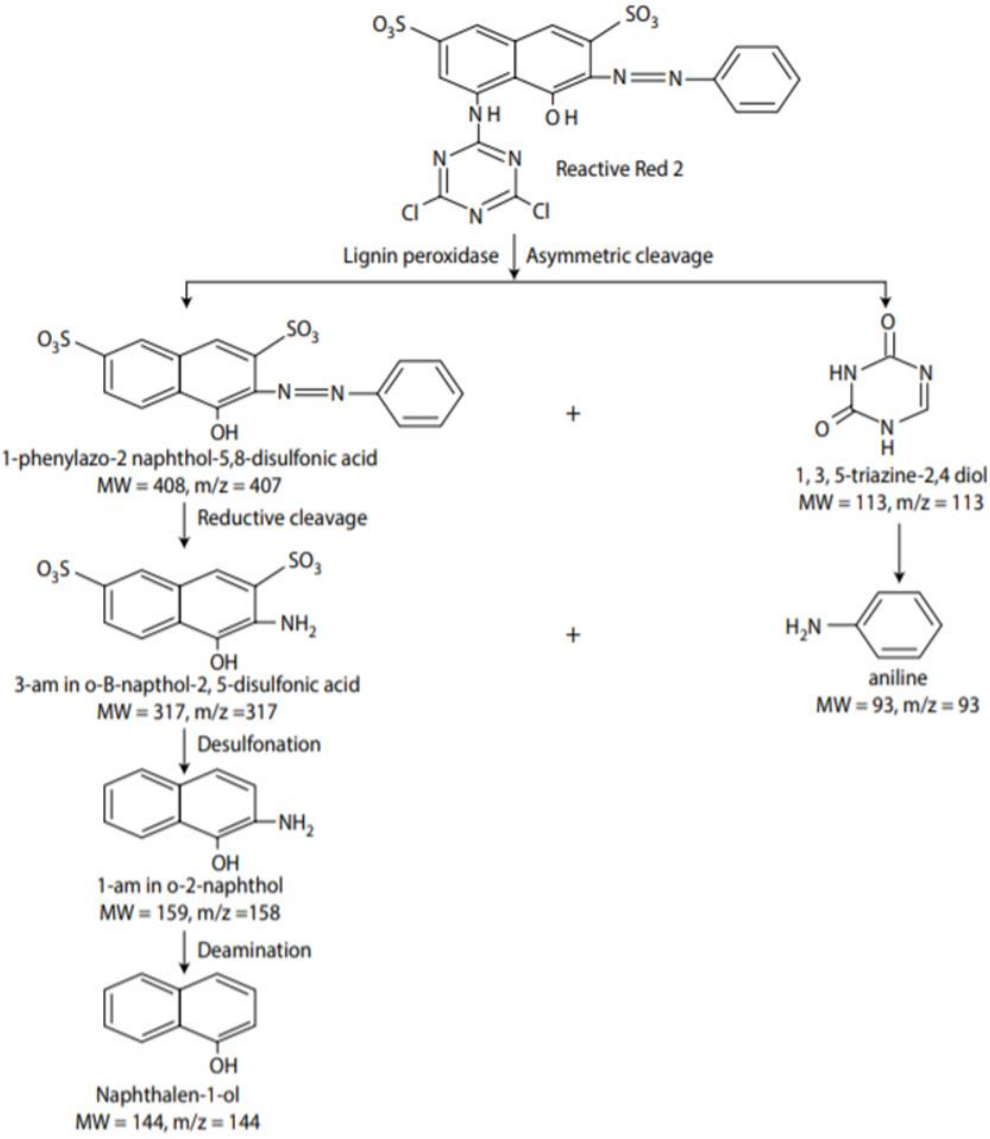

Figure 3 Biodegradation of dye by bacteria (Bhatia et al 2017)

The biological method of dye removal such as bioremediation is of minimum cost and it saves energy. This is feasible, eco-friendly and it produces less amount of solid sludge. It removes synthetic dye by breaking the stable bond and make them less toxic. The biological methods can reduce the Chemical Oxygen Demand and turbidity (Bhatia et al 2017).

2.3 Oil spills Bioremediation

when large amount of petroleum based hydrocarbon released in to the environment from leakage from the pipeline, storage tanks, drilling process and non-suitable waste disposal.

This oil spills may be caused by the accidents in the industries, release of residues from the environments (Li et al 2017). The discharge of petroleum hydrocarbons in marine and terrestrial ecosystem leads to the major threat to humans and aquatic life forms. This will pollute the environment, volatile hydrocarbons cause the risk of explosion, affects the quality of air and water, wastes the non-renewable resources and have a large economical loss. The carcinogenic and mutagenic effect of oil spills in the aquatic environment have been proved (Cheng 2017). In Marine ecosystem, this oil spills will prevents the penetration of sunlight in to the deep water of the sea. It will affect the photosynthetic effects of the aquatic plants (Bovia et al 2017).

Figure 4 The Exxon Valdez Oil Spill - The Atlantic (Baniasadi – Mousavi 2018)

Various physical and chemical methods are available to remediate the oil contamination. The major methods are ozonation, dredging and electrochemical degradation. But these are more expensive and requires higher energy. The traditional physio-chemical methods are very effective but they produce various hazardous compounds which are more immunotoxic and carcinogenic. It requires costliest chemicals for good removal of oil contaminants (Jain et al 2011). Biological method is very effective methods among the various methods. The advantages of using biological methods over physical methods are,

a) Biological methods detoxify the hazardous substances into the less toxic forms, Wheras physical methods transfer the substance to another environment. b) Biological method is less excavation method to the environment.

Bioremediation use microorganism to degrade the organic contaminants. These microorganisms utilize these contaminants as sole carbon sources for its growth. Biological

treatment is low cost and doesnot produce any secondary contaminants very effective in removing toxic components. In case of petroleum hydrocarbons, microorganisms use hydrocarbons as a substrate for its growth, produce biomass and decompose the pollutants into harmless compounds (Balba et al 1998). The adaptive nature of microorganisms and their resistants to grow in extremely polluted environment is one of the advantages of bioremediation. The indigenous microorganism present in the oil spills will natural degrade the contaminants. For effective biodegradation, addition of materials into the site will speed up the natural degradation process. The addition of nutrients into the contaminant site will enhance the growth and the acivity of the native microorganisms will induce the hydrocarbon degradation.

Jafarinejad (2017) reported that the microbes use hydrocarbons as a growth medium or used as a cometabolism. Cometabolism is the contaminants may be used as an extra source of nutrients along with the supplementes growth medium. Also, various physical and chemical parameters affects the bioremediation. The physical parameters such as contaminated site, Temperature, pH and pressure and chemical parameters such as nutrient and oxygen availability, contaminant nature, acidity, alkalinity and salinity. More than 200 different types of species of bacteria, fungi and yeast have the ability to degrade various petroleum based contaminants. These microorganisms are indigenous organisms which was naturally found in fresh water, marine environment and soil. Atlas (1995), isolated the bacteria from the soil and check its efficiency for the degradation of different compounds of petroleum hydrocarbons. The indigenous soil bacteria can degrade the different compounds of petroleum hydrocarbons.

2.3.1 Petroleum Remediation Powder

Although, the indigeneous microorganism can utilize organic compounds from petroleum hydrocarbons for its growth and it will detoxify the petroleum products into non-toxic forms. Head and Swannell (1999) reported the addition of supplemented nutrients into the contaminant sites will stimulate the microorganisms to degrade the petroleum hydrocarbons.

PRP consists of tiny spheres of treated wax, which contain nutrients for microbial growth. When a wax sphere contact with contaminated oil, it will interacts with the hydrocarbons. Nutrients inside the wax sphere helps the indigenous microorganisms present in

the environment to nourish until they eat every droplet of oil. This powder have the ability to remediate the oil over 20 percentage of its weight.

Petroleum Remediation Product powder used for various oil, fuel and other liquid petroleum hydrocarbon clean up applications such as fuel or oil spilled on land or ground, oil spills on shorelines, waterways or marinas, hydraulic fluid spilled in industrial plants, vehicle leaks, gasoline or fuels flowing into storm water runoff, heating oil in home storage tanks, railroad ballasts, oil wells & oil fields, transformer vaults, maintenance facilities, vehicle rollovers, marsh and wetlands, car wash or parking garage washing usage or areas where oil, diesel fuel, gasoline, liquid petroleum or other hydrocarbon clean up and natural bioremediation requirements.

This powder helps for the biodegradation of fuels and it reduce the presence of aliphatic and aromatic hydrocarbons. This powder binds with oil present in the contaminant will form a matrix, and produce a solid mixture that is stable. This powder remediate the petroleum products by absorbtion.

3 Materials and Methods

3.1 Bacterial Bioremediation

3.1.1 Source of Microorganisms

Textile soil was collected from the nearby textile industry in zip-lock bags.

3.1.2 Isolation of bacteria from Textile soil

The nutrient medium and glasswares were autoclaved @15psi pressure or 121^{o}C. The bacteria were isolated from the soil by the serial dilution method. Soil from the textile industry was suspended in 10ml of sterile distilled water and it was mixed well for 15 minutes. Then the suspension was serially diluted in the range of 10^{-1} to 10^{-6}. The nutrient agar medium was poured into a Petri plate and allowed it to solidify. Then the suspension was spreaded on the plate. The samples were pipette out and it was spreaded with glass L rod and incubated for 24hrs at 37^{o}C. After 24 hours, different colonies of bacteria were grown. The colonies were selected and it was streak plated on the sterile nutrient agar medium. It was incubated overnight and pure culture of bacteria was obtained.

3.1.3 Characterization of bacteria

3.1.3.1 Gram staining

The loopful of culture was taken. Thin smear was prepared on a clean dry glass slide. It was air-dried and fixed by gentle heating. The slide was flooded with gram's crystal violet and it was drained out. Then, the smear was flooded with gram's iodide. It was decolorized by a decolorizer. It was washed with water. Then, it was counterstain with 0.5% safranin. Again it was washed with water. The slide was air-dried and viewed under a microscope.

3.1.3.2 16sr RNA sequencing

The isolated bacterial strain was identified by 16sr RNA sequencing (Yaazh Xenomics, Coimbatore).

Ribosomes are complex structures found in all living cells which functions in protein synthesis machinery. Basically ribosome's consists of two subunits, each of which is composed of protein and a type of RNA, known as ribosomal RNA (rRNA). Prokaryotic ribosomes consist of 30S subunit (small sub unit) and 50S subunit (large sub unit) which together make up the complete 70S ribosome, where S stands for Svedberg unit non-SI unit for sedimentation rate. 30S subunit is composed of 16S ribosomal RNA and 21 polynucleotide chains while 50S subunit is composed of two rRNA species, the 5S and 23S rRNAs. The presence of hyper variable regions in the 16S rRNA gene provides a species specific signature sequence which is useful for bacterial identification process. 16S Ribosomal RNA sequencing is widely used in microbiology studies to identify the diversities in prokaryotic organisms as well as other organisms and thereby studying the phylogenetic relationships between them.

3.1.4 Bacterial growth determination

The growth curve of bacteria isolates was determined in Nutrient Medium containing blue dye and Textile effluent. The Bacteria grown without dye medium was taken as control. 50ml of the medium was taken and it was inoculated with bacteria. The culture was incubated in a shaker at 100rpm. An aliquot of culture was taken at regular intervals of time. Absorbance was measured at 600nm. It was plotted graphically.

3.1.5 Dye removal efficiency of bacteria

Dye decolorization experiments were carried out in a flask containing nutrient medium with dye and textile water. The dye (10ppm) and Textile water without a medium were also taken for studies and the culture was inoculated. The dye removal efficiency was calculated by

measuring the absorbance of the decolorization flask. % of Dye removal was calculated as follows.

$$\textbf{Dye Removal \% } = \frac{(\textit{Initial absorbance} - \textit{Final absorbance})}{\textit{Initial absorbance}} \textbf{ *100}$$

3.3 Bioremediation of oil by PRP powder

Three different oil samples having petroleum hydrocarbons have been taken from different settling tanks such as settling tank 2, settling tank 4 and settling tank 6. Two sets of samples were taken. In the first set 1gram of Petroleum Remediation Product powder was weighed and added to the samples. In the second set, Petroleum Remediation Product and bacteria was inoculated.

3.3.1 Charaterization of PRP Powder

3.3.1.1 X-Ray Diffraction

The Morphology and the crystalline structure was determined from characteristic peaks obtained from XRD image. The characterization of petroleum remediation product was carried out by X-ray diffractometer for the crystallographic structure analysis. X-ray analysis was done using XRD shimadzu 6000. The X-ray generator was operated at a voltage of 40kV and a current of 30mA, where the samples were subjected to cu radiations at a speed of 5 per min and drive axis 0f 2. Further, the images obtained were compared with the joint committee on power diffraction standards library to account for the crystalline structure of the particle.

3.3.1.2 SEM with EDaX analysis:

The Petroleum Remediation product powder was characterized for their size using scanning electron microscope. EDaX (Energy Dispersive X-ray) analysis of PRP powder was carried out to confirm the elemental composition of the sample.

3.3.2 Determination of Bacterial growth in PRP Powder

1.3g of PRP Powder was weighed and added into 100ml of distilled water. Then it was autoclaved. The culture was inoculated into the medium. The organism inoculated into nutrient media is used as a control. The growth of the bacteria was estimated by measuring the absorbance @600nm.

3.3.3 Parameters

Eight parameters including the pH, Electrical Conductivity, Total Dissolved Solids, Total Suspended Solids, Chemical Oxygen Demand and Fluid Properties such as Surface Tension, Thermal Conductivity and Viscosity were tested for investigate their effects on the degradation of fluids using the bacteria growing in PRP Powder.

4 Results and Discussions

4.1 Bacteria

4.1.1. Isolation of Culture from soil:

The microbial culture was isolated purified and screened for the dye degradation

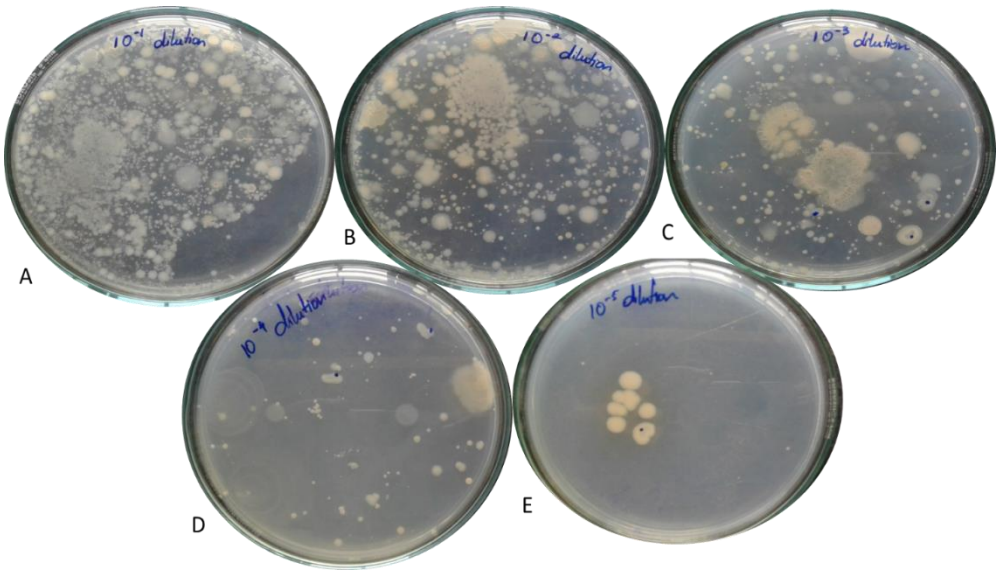

Fig 5 Serial dilution A) 10-1 dilution B) 10-2 dilution C) 10-3 dilution D) 10-4 dilution E) 10-5 dilution

Saranraj and sivasakthivelan (2014) isolated the dye decolourizing bacteria from dye contaminated soil by using the serial dilution pour plate method. They isolate six different dye degrading bacteria and examined the dye removal efficiency. These bacteria from dye soil can decolorize the textile dye-containing water.

The colonies were picked up and it was purified by streak plate method. Streaking in an agar plate was a method used to isolate a pure strain of bacteria. The colonies were taken and it was streak on the new plate.

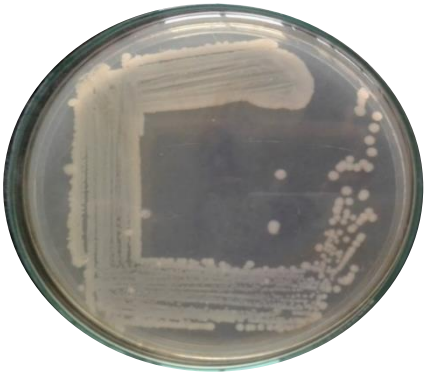

Fig 6 Streak plate

Pillai et al (2014) showed the indigenous microorganism has the ability to decolorizing dye which was isolated from the textile dye degrading bacteria from the soil by serial dilution method. They isolate the pure culture of *Streptomyces spp.* and screen for its dye removal efficiency. The isolated bacteria can degrade the dye within 48hr of incubation. Bioremediation using indigenous microorganisms was not harmful and it has a significant dye removal capability.

4.1.2 Characterization of Bacteria

The isolated bacteria were characterized by the gram staining method.

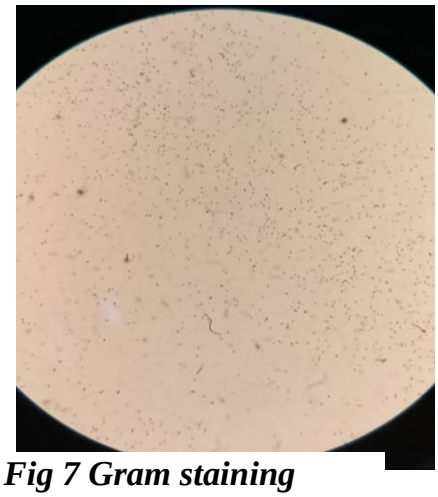

Fig 7 Gram staining

The gram staining results showed that the isolated bacteria are gram-negative rod-shaped bacteria. It takes safranin color while staining and it appears as a pink colour.

Aeromonas caviae are rod-shaped, facultative anaerobic and gram-negative bacteria. It's generally presented in groundwater and the aquatic environment.

The Gram stain, the most widely used staining procedure in bacteriology, is a complex and differential staining procedure. Through a series of staining and decolorization steps, organisms in the Domain Bacteria are differentiated according to cell wall composition. Gram-positive bacteria have cell walls that contain thick layers of peptidoglycan (90% of cell wall). These stain purple. Gram-negative bacteria have walls with thin layers of peptidoglycan (10% of wall), and high lipid content.. The performance of the Gram Stain on any sample requires four basic steps that include applying a primary stain (crystal violet) to a heat-fixed smear, followed by the addition of a mordant (Gram's Iodine), rapid decolorization with alcohol, acetone, or a mixture of alcohol and acetone and lastly, counterstaining with safranin. The rod-shaped Gram-negative bacteria isolated from textile effluents. Under a light microscope, these gram-negative bacteria appear as a pink color. Because this strain could not take up the crystal violet stain and it retains the counterstain safranin. So it was visualized as a pink color Zhang et al (2004).

4.1.3 Sequencing

The isolated bacteria were identified by using 16srRNA sequencing.

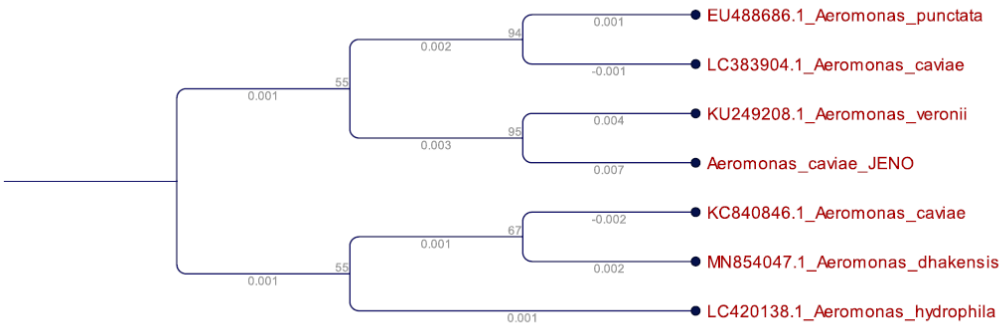

Figure 8 16sr Rna sequencing of isolated strain

The results showed that the bacteria isolated from textile effluent was *Aeromonas caviae*. Generally, *Aeromonas caviae* present in groundwater and aquatic environments.

Aeromonas sp. from the dye-containing water. The efficiency of *Aeromonas sp.* for degrading industrial dyes was checked. *Aeromonas sp.* has the potential to degrade dye molecules.

4.1.4 Growth curve determination

Bacteria will grow in a predictable pattern, resulting in a growth curve composed of four distinct phases of growth: the lag phase, the exponential or log phase, the stationary phase, and the death or decline phase.

4.1.4.1 Lag Phase

The lag phase is an adaptation period, where the bacteria are adjusting to their new conditions.

4.1.4.2 Exponential or Log phase

Once cells have accumulated, they proceed into cell division. The exponential or log phase of growth is marked by predictable doublings of the population, where one cell become two cells, becomes four, becomes eight etc.,

4.1.4.3 Stationary phase

The number of new cells produced is equal to the number of cells dying off or growth has entirely ceased, resulting in a flattening out of growth on the growth curve.

4.1.4.4 Death or Decline phase

The last phase of the growth curve, the death or decline phase, the number of viable cells decreases in an exponential manner.

The growth curve pattern was studied by growing the organism in dye and textile waster and comparing it with the control culture where no dyes were added. The growth of the bacteria was determined for 74hours.

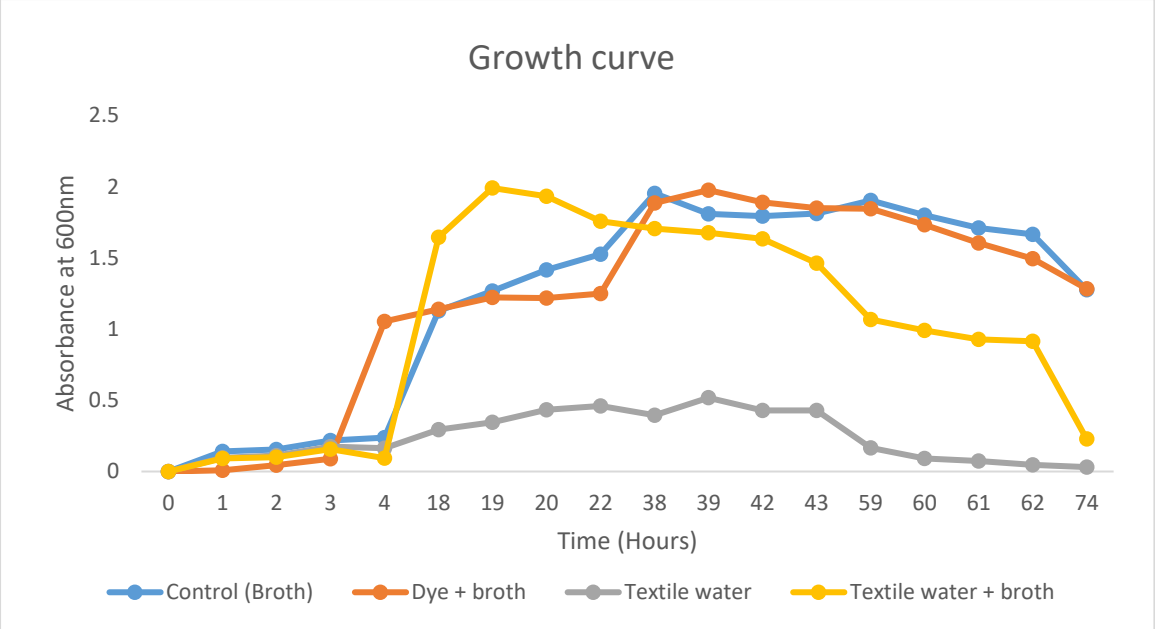

Figure 9 Growth curve of the bacteria

The graph shows the growth of the bacteria in different culture conditions. The growth pattern was gradually increased after 15hrs of inoculation. The bacteria obtain their maximum growth in a nutrient medium amended with synthetic dye when compared with the control. The growth rate was lower in the textile water as compared to the control and synthetic dye. This strain utilizes dye as carbon and energy sources for its growth. It was evident that the growth of the organisms in the textile effluent was lower due to the presence of toxic metals or non-hydrolyzed functional groups in the effluents which may retard the bacterial growth.

4.1.5 Determination of Total suspended solids

The total suspended solids were determined for the bacterial culture growing in the dye and textile water-containing medium.

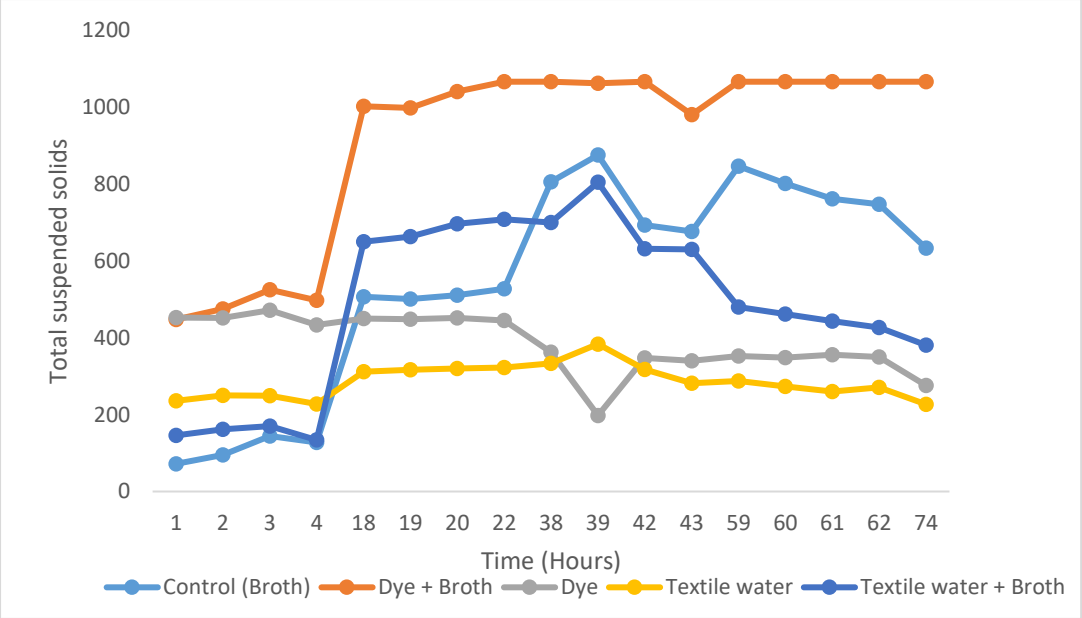

Figure 10 Total suspended solids

It was observed that the total suspended solids were reduced in the culture growing in dye and textile water. The initial total suspended solids of the untreated synthetic dye and textile water are 453mg/l and 236mg/l respectively. After treatment, the concentration of suspended solids was reduced up to 276mg/l for synthetic dye and 227mg/l for textile water.

4.1.6 Bacterial Dye removal efficiency

The percentage of dye removal efficiency was measured for different medium. The bacteria was grown in textile water and synthetic dye.

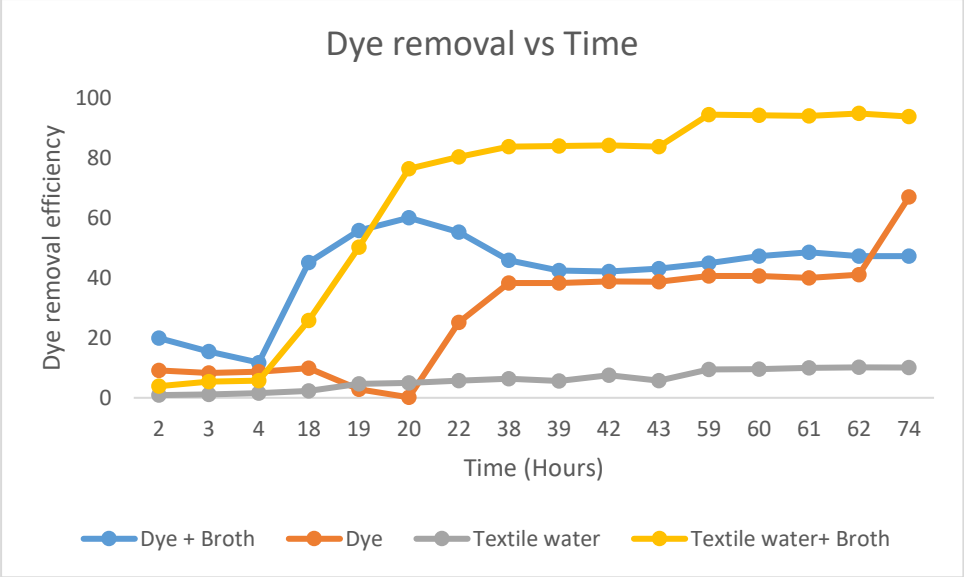

Figure 11 Bacterial Dye removal efficiency

The above results show the percentage of color removal against the industrial dye. The maximum peak was found at the wavelength of 650nm for synthetic blue dye and 310nm for textile water. The initial absorbance of synthetic blue dye at 650nm was 0.6255 and for textile water at 310nm was 4.321. The nutrient medium and textile water exhibit the maximum color removal of 94%. The maximum color removal efficiency of medium with dye is 47%. For Synthetic dye and Textile water, the color removal efficiency was found to be 66% and 10% respectively.

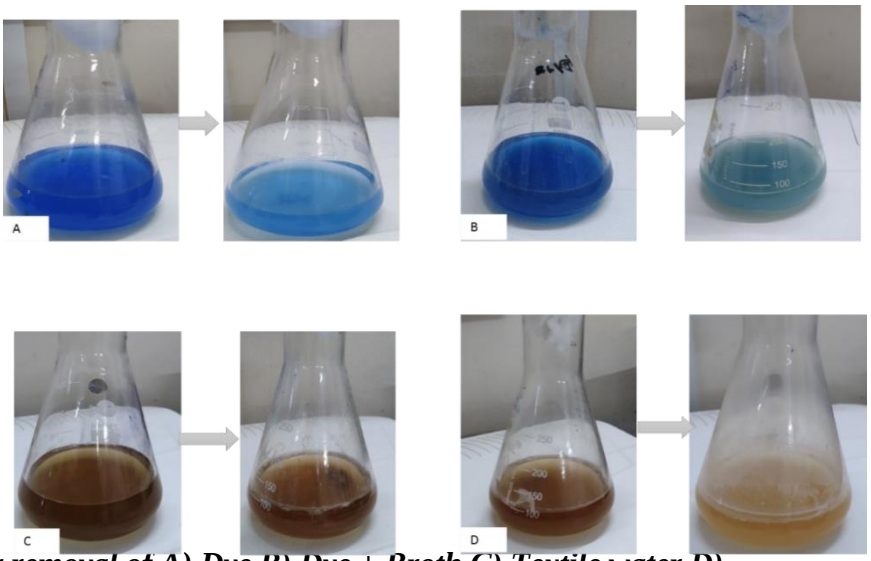

Figure 12 Colour removal of A) Dye B) Dye + Broth C) Textile water D) Textile water + Broth

The textile water amended with medium showed good color removal. The organisms utilize textile water as nutrient sources for their growth. The low colour removal

was observed in bacteria grown in dye and textile water alone. Dyes are deficient in carbon sources so, biodegradation of dyestuff without any extra carbon content was very difficult. So the bacteria growth in a dye and textile water supplemented with nutrient medium shows the efficient dye removal (Kumar et al., 2014). The bacteria cannot utilize dye as a sole energy source and requires an additional carbon source. The bacteria were grown in dye supplemented with nutrient medium (Koleker et al 2008).

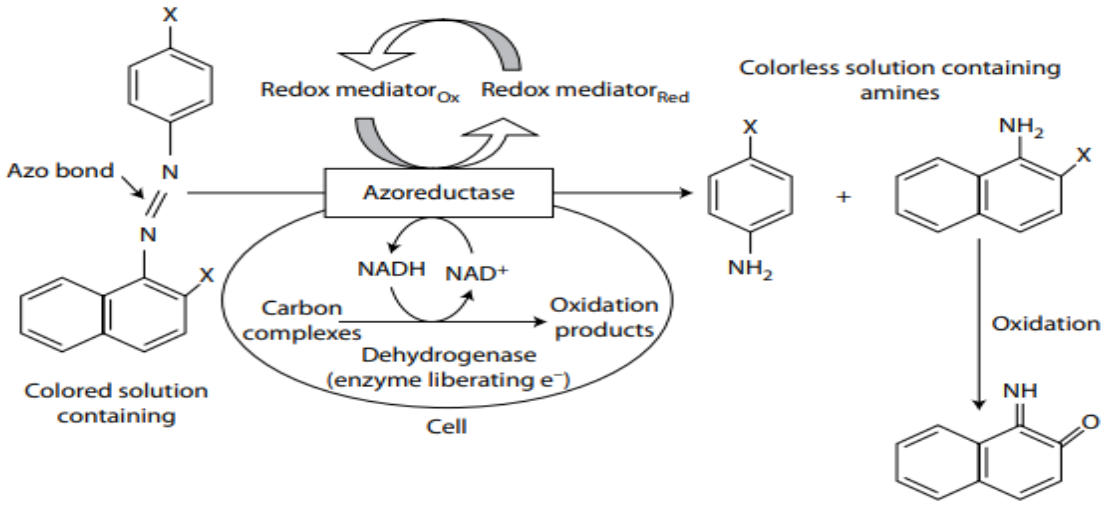

Figure 13 Bacterial degradation of azodye (Kishore et al 2018)

4.4 Bioremedition by using Petroleum Remedition Powder:

Fluid wastes were taken from different fluid tanks. The wastes consists of complex mixtutre of oil, water, suspended solids, and dissolved solids.

4.4.1 Characterization of PRP powder

4.4.1.1 X-Ray Diffraction

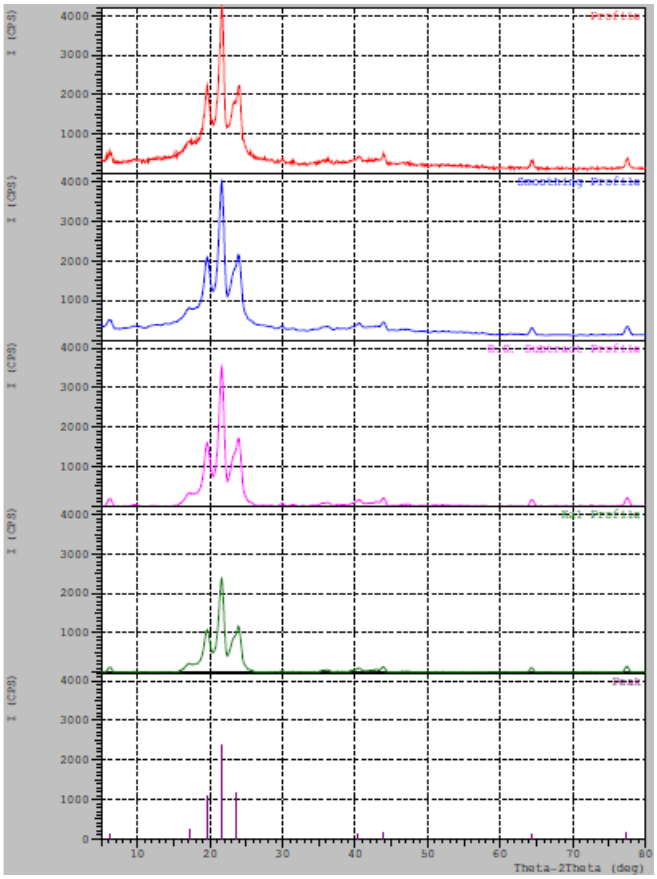

Figure 21 X-Ray Diffraction analysis of PRP powder

Petroleum Remediation product powder was demonstrated by the characteristic peaks observed in X-Ray Diffraction (XRD) image. XRD confirmed the crystalline nature of the particles and show intense peaks at the 2Θ value which index at 21.53°C, 23.55°C and 19.63°C with d spacing values of 4.12228, 3.77350 and 4.5173 respectively. The average naocrystallite size calculated from scherrer formula was found to be 9.3nm.

Dinker et al (2017) reported earlier, the characteristic peaks of compounds from beeswax was observed at 2Θ = 21° and 24° with d spacing values of 4.1892 and 3.7690 respectively.

4.4.1.2 SEM with EDaX

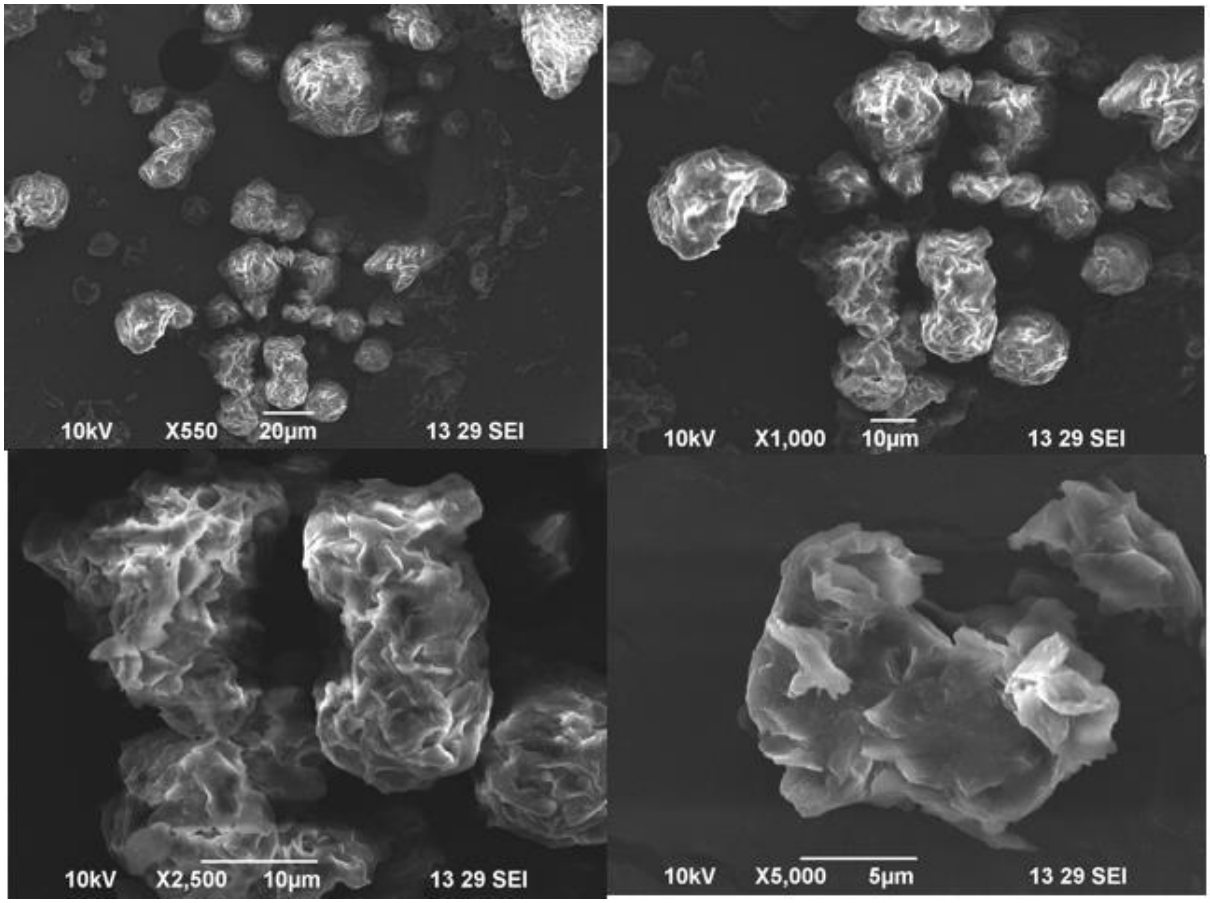

Figure 22 SEM image of PRP powder at different magnification X500, X1000, X2,500 and X 5,000

The above figure depicts the diiferent magnification of Scanning Electron Microscope of Petroleum Remediation product powder. The low magnification SEM results showed that the products are well defined microsphere. From the high magnification SEM images the products are found to be the surface of the PRP powder looks like a fluffy micron sized particles due to amorphous nature of the product. The elemental composition of the PRP powder was determined by Energy dispersive X-ray microanalysis.

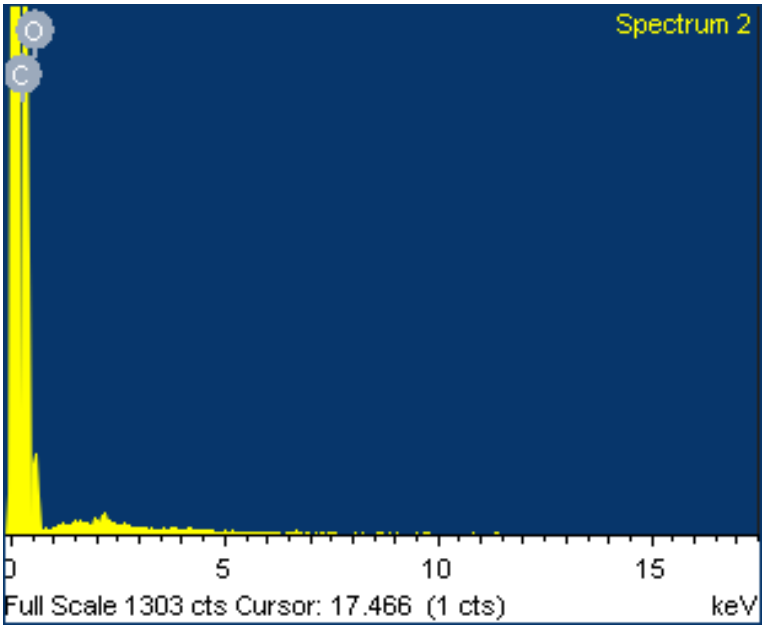

Figure 23 EDaX analysis of PRP powder

The SEM energy dispersive X-ray microanalysis spectra of the PRP. The strongest peaks observed in the spectrum related to carbon and oxygen. The elemental constitution of PRP powder with major peaks found to have a weight percentage of 72.39% of Carbon and 27.61% of oxygen. The EDaX analysis confirms the presence of carbon and oxygen in the sample.

Since, Petroleum Remediation product powder majorly composed of hollow microspheres of beeswax. Hossain et al 2009 characterize beeswax powder for petroleum applications by Sem with EDaX. The analsysis confirmed that carbon and oxygen are the main component of beeswax. On weight basis the % of carbon varies from 40 -70 % and oxygen varies from 10 – 30 %. SEM results of beeswax showed the visible ridges and valleys indicates the beeswax sample is fragile and it is not hard. It appears like a colloidal and cloudy structure due to the amorphous and heterogenous nature of the polymer (beeswax).

4.4.2 Growth of bacteria in PRP powder

The bacteria was grown in a medium supplemented with Petroleum remedition powder. The bacteria have the ability to grown in a medium formulated with PRP powder. This bacteria utilize PRP powder as a nutrient source for its growth. This was compared with control which was grown in nutrient medium.

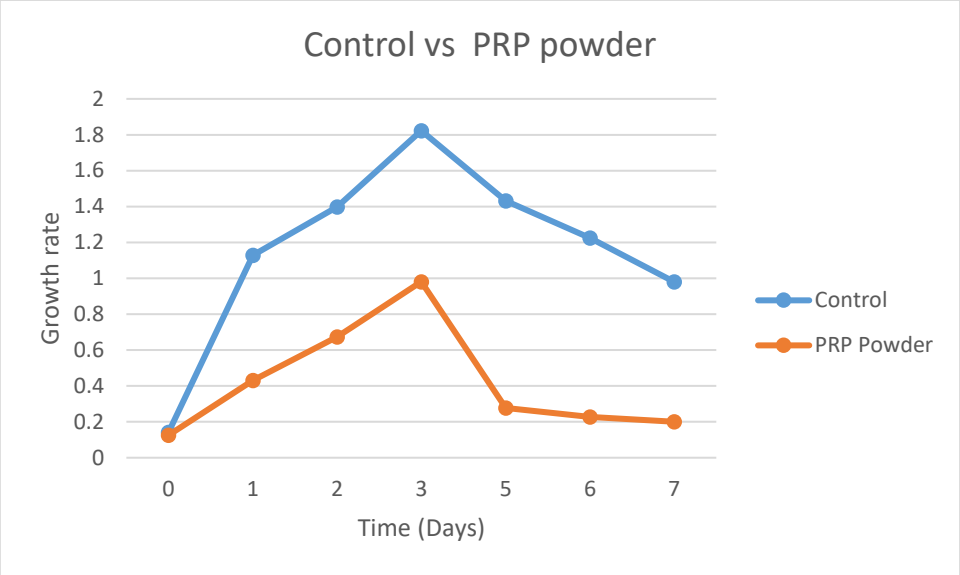

Figure 24 Growth of Bacteria in PRP Powder medium vs control

The growth of the bacteria was higher in nutrient medium than the PRP powder medium. Since bacteria required time to adapt the new medium. The bacteria can able to grow in PRP powder supplemented with medium. The PRP powder made up of beeswax can spread over contaminated water and will acts as nutrient for indigenous petroleum remediating microorganism. This PRP powder absorb the contaminants present in the surrounding environment.

4.4.3 Parameters

The following parameters were assessed.

4.4.3.1 pH

The pH of the initial fluid samples were detertmined. The initial pH of the samples taken from different tanks were almost neutral.

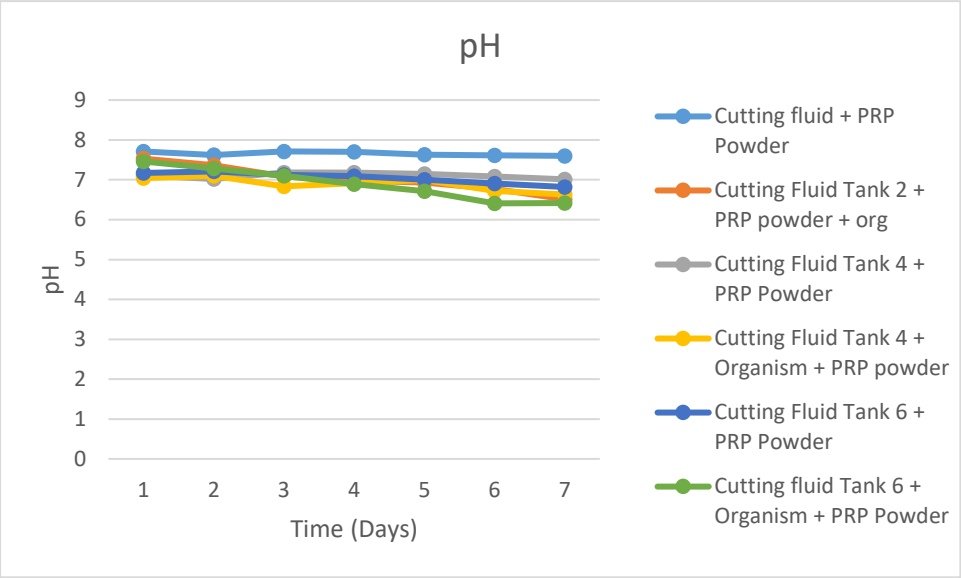

Figure 25 pH of Fluid Wastes incorporated with PRP Powder

Six different samples were maintained. The pH of the medium was gradually decreased from the initial pH. From this graph, it was observed that fluid samples inoculated with organisms shows the decrease in the pH than the other samples. This is due to the metabolism of microorganisms. While growing, bacteria can produce metabolites during the log and stationary phase. The metabolites were acidic in nature. When this metabolites are excreted from the cells, it will increase the pH of the medium (Jin and Kirk 2018).

4.4.3.2 Electrical conductivity

Electrical conductivity is the ability of the materials to conduct electrical charges. It is a measure of fluids electrostatic chargeability. The unit of Electrical conductivity was µs/cm.

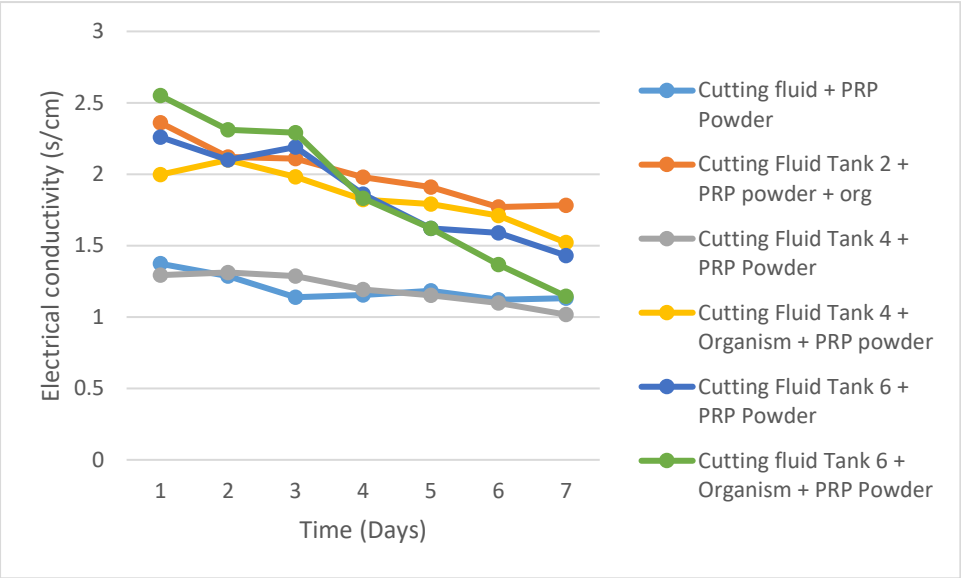

Figure 26 Electrical conductivity of fluid wastes with PRP Powder

The electrical conductivity of six different samples with PRP powder were determined for seven days. The electrical conductivity was decreased from initial values.

It was observed that, the conductivity of the fluids was higher if the impurities or salt concentration was higher.

4.4.3.3 Total Dissolved Solids

Total Dissolved Solids is a measure of organic and inorganic materials present in the fluids.

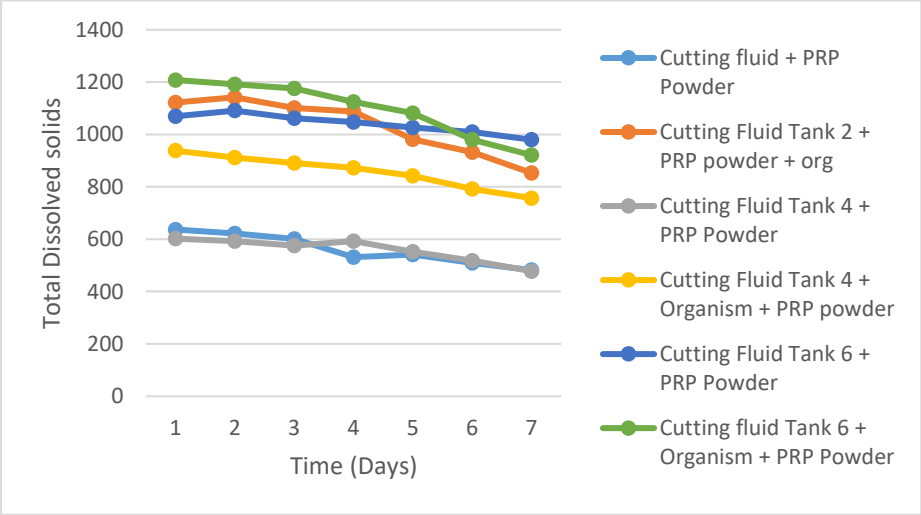

Figure 24 TDS of Fluid Wastes incorporated with PRP Powder

The Total dissolved solids were determined. The total dissolved solids were declined gradually day by day. The sample taken from cutting fluid 6 inoculated with organisms having highest initial Total Dissolved solids 1208 mg/l will reduced upto 921mg/l. The cutting fluid tank 6 wihout organism have Total Dissolve solids of 1070mg/l and reduced upto 980mg/l. The Cutting Fluid tank 2 with and without organisms have initial TDS of 637mg/l and 1122mg/l respectively to 482 mg/l and 853mg/l on 7th day. Similary, the sample from cutting fluid 4 inoculated with organisms have TDS of 938mg/l and it will reduced to 757mg/l.

Ranieri et al (2016) reported, that the oil contaminant water having high concentration of Total Dissoved Solids. In Bioremediation microorganisms utilize or absorb Total Dissolved Solids as nutrients and minerals to support their physiology and metabolisms and significantly reduce the TDS in fluids.

4.4.3.4 Total Suspended solids

The presence of suspended solids in a water increase the turbidity. The suspended solids may consists of some air-borne particulates, colloidal organic particles and other microscopic organisms.

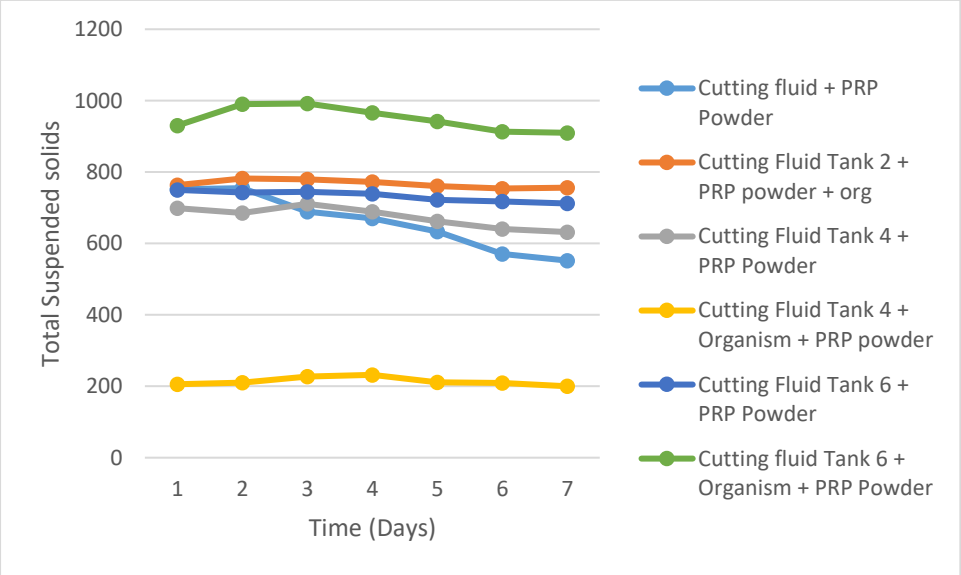

Figure 28 TSS of Fluid Wastes incorporated with PRP Powder

The Total Suspended Solids were higher on the cutting fluid tank 6 inoculated with organisms and PRP powder and it was observed as 930mg/l. After 7 days the TSS were reduced upto 910mg/l. The Cutting fluid Tank 4 have lower initial TSS. The highest TSS removal was observed in sample from Cutting fluid I from 752mg/l to 552mg/l.

The decrease in the rate of the suspended solids was due to the presence of microorganisms. These bacteria feed on the suspended solids present in the oil samples and decrease in the amount of TSS in the samples. The bacteria acts as a synergistic way and capable of degrading Suspended solids in the oil wastewater. The bacteria utilize the organic matter for the production of energy by cellular respiration and for synthesis of protein for cellular growth (Curds 1968).

4.4.3.5 Chemical Oxygen Demand

Chemical Oxygen Demand is the total amount of oxygen required to chemically oxidise the biodegradable and non-biodegradable organic matter.

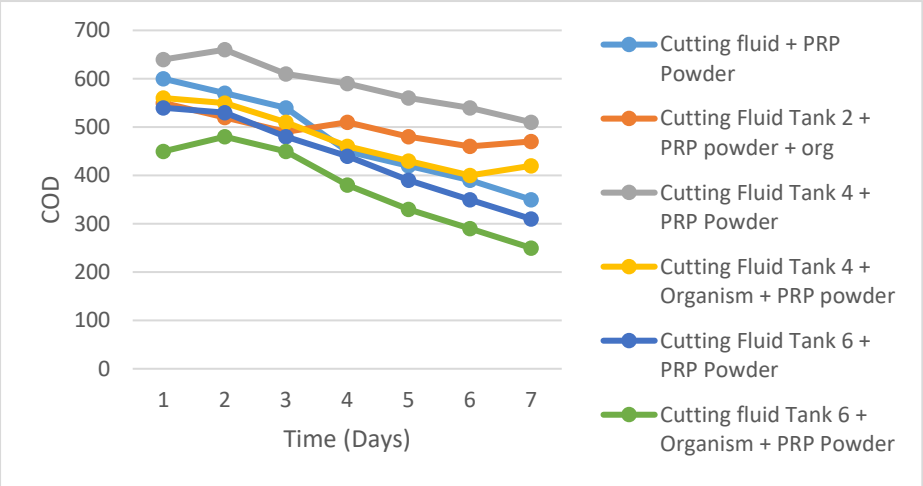

Figure 29 COD of Fluid Wastes incorporated with PRP Powder

The highest removal of COD was observed in Samples from cutting fluid tank 2 supplemented with PRP Powder. The sample from cutting fluid tank 4 inoculated with organisms having highest removal of COD from 560mg/l to 420mg/l. The initial COD of tank 6 having COD of 450mg/l will reduced up to 250mg/l.

Eckenfelder (1980) treated the petrochemical waste water through biological treatment. Initially, the Chemical Oxygen Demand of sample containing petroleum hydrocarbon was higher. After the biodegradation of the petroleum hydrocarbon there was a significant decrease in the COD value. The decline in COD value is due to the degradation of organic matters by bacteria.

4.4.2.6 Thermal conductivity

Thermal conductivity is the ability of the material to transfer or conduct heat. The thermal conductivity of fluid taken from tank 2,4 and 6 were determined.

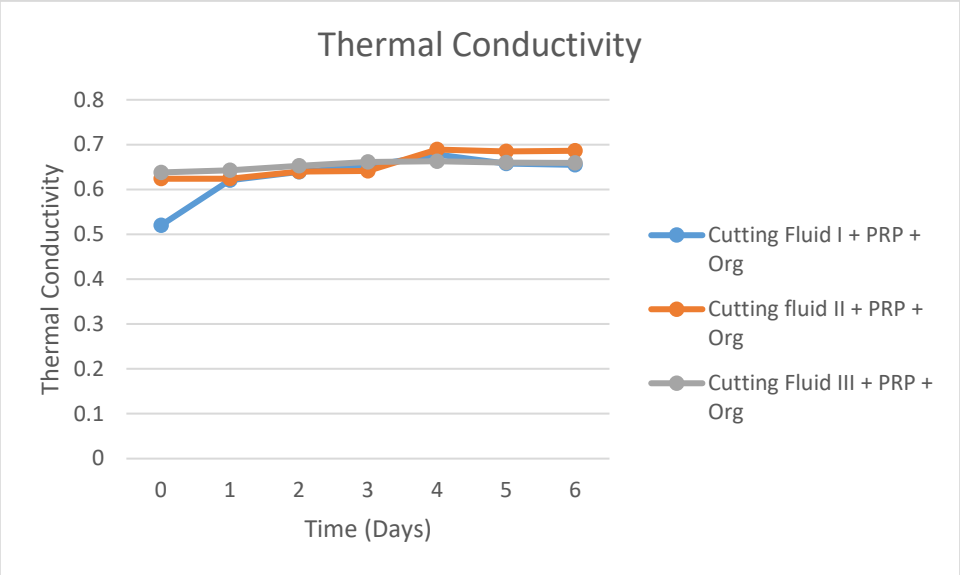

Figure 30 Thermal conductivity of Fluid Wastes incorporated with PRP Powder

The results depicts the thermal conductivity of fluid samples were increased after the inoculation of bacteria. The bacterial biodegradation of petroleum hydrocarbons increases the thermal conductivity of the samples. The fluid sample from tank 2 have initial thermal conductivity of 0.52 and it was increased up to 0.655. The fluid sample from tank 4 have the initial thermal conductivity of 0.624 and it was increase upto 0.686. The fluid sample 6 have highest thermal conductivity of 0.659.

Nakanishi et al (2017) reported the increase in the thermal conductivity of the petroleum hydrocarbon contaminated water bioremediation. The degradation of petroleum hydrocarbon by bacteria increase the thermal conductivity of the fluids.

4.4.3.7 Viscosity:

Viscosity is a measure of oils resistant to flow. It decreases with increase in the temperature and increases with decrease in the temperature. Some microorganisms have the ability to degrade the oil. As a result, the viscosity of the crude oil has been reduced.

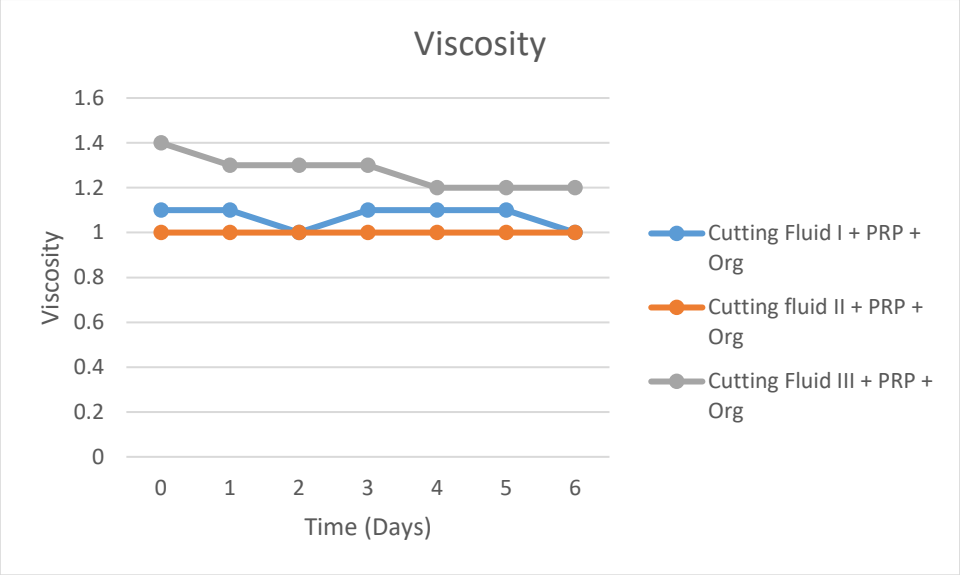

Figure 31 Viscosity of Fluid Wastes incorporated with PRP Powder

It was observed that there was a gradual decrease in the viscosity of the samples. The sample taken from fluid tank 4 doesnot show any changes in the viscosity. The cutting fluid from tank 6 have initial viscosity of 1.4cp will reduced up to 1.2cp.

Roy et al (2014) study the effect of bacterial treatment on oil biodegradation which inferred from changes in viscosity. It was found that the treatment of bacterial strain enhance the fluidity of oil samples.

4.4.3.8 Surface tension

The surface tension refers to the tendency of the fluid surface which make it acquire large surface area. The property of the surface of the liquid that allow it to resist the external force, due to the molecules cohesive nature. The surface tension was measured by using Tensiometer.

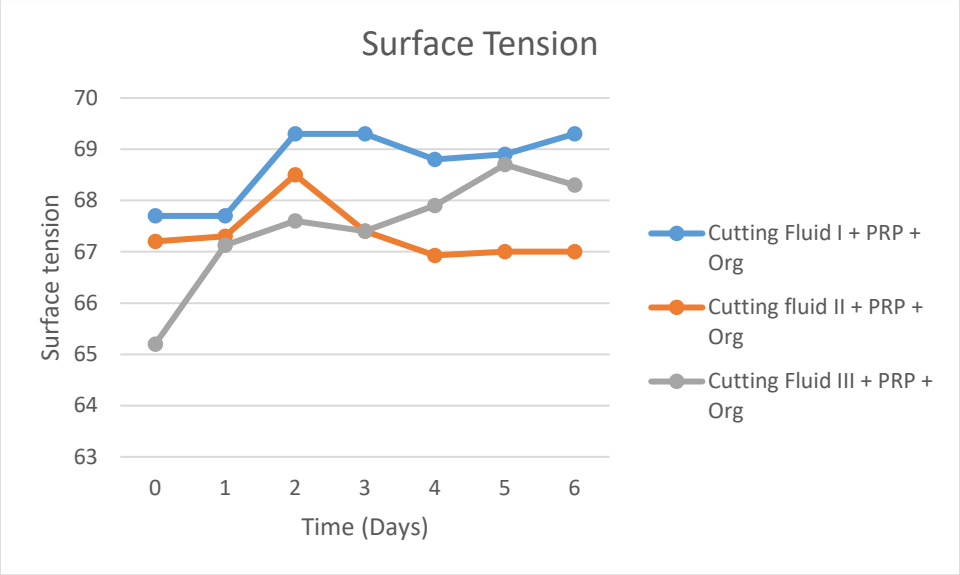

Figure 32 Surface Tension of Fluid Wastes incorporated with PRP Powder

The results depicts the increase in the surface tension of the fluid samples. The initial surface tension noted on 0^{th} day. After 2 days there was a gradual increase in the surface tension.

It was observed that the surface tension of the fluid was increased after the inoculation of culture. Larson, cantwell and Hartzell studied the effect of surface tension on the bacterial growth. The surface property of the bacteria in a liquid can change in growth conditions and envoironmental factors. The factors such as growth medium and dilution rate influence the surface tension of the fluid. The oils affects the surface tension of the fluid.

Akhavan et al 2008 observed that the microbial community reduce the surface tension which indicates the bacterial strain produces biosurfactant. They observed a changes in surface tension by bacteria. They stated the positive correlation between the reduction of surface tension and population of microbes which indicates the microbial growth increase the biodegradation process.

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