Performance evaluation of developed bacterial consortium in MFC system for wastewater treatment and scale up

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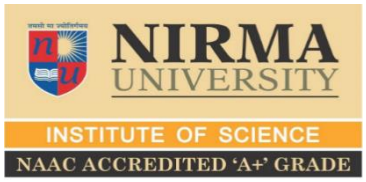

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CERTIFICATE

This is to certify that the thesis entitled **"Performance evaluation of developed bacterial consortium in MFC system for wastewater treatment and scale up"** submitted to the Institute of Science, Nirma University in partial fulfilment of the requirement for the award of the degree of M.Sc. in Microbiology, is a record research work carried out by **Mansi Kumar (21MMB013), Priya Mishra (21MMB015), Bansari Thakkar (21MMB032)** and **Nirali Thakkar (21MMB033)** under the guidance of **Dr. Nasreen S. Munshi**. No part of the thesis has been submitted for any other degree or diploma.

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DECLARATION

We hereby kindly declare that the work entitled "**Performance evaluation of developed bacterial consortium in MFC system for wastewater treatment and scale up**" is our original work. We have not copied from any other students' work or from any other sources except where due references or acknowledgement is made explicitly in the text, nor has any part been written for us by any other person.

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ABBREVIATIONS

AFM: Atomic Force Microscopy

BES: Bioelectrical System

CLSM: Confocal Laser Scanning Microscopy

COD: Chemical Oxygen Demand

CPCB: Central Pollution Control Board

GHG: Green House Gases

GPCB: Gujarat Pollution Control Board

MFC: Microbial Fuel Cell

MLD: Million Litres per Day

PAH: Polyaromatic Hydrocarbons

PEM: Proton Exchange Membrane

SEM: Scanning Electron Microscopy

UASB: Upflow Anaerobic Sludge Blanket Bioreactor

USEPA: United States Environmental Protection Agency

INTRODUCTION

“First impressions never have a second chance”

1. INTRODUCTION

Rapid industrialization and urbanization have contributed to huge energy requirements leading to endless use of non-renewable energy sources such as fossil fuels. As a consequence, these resources are getting exhausted. Moreover, burning of fossil fuels has remained the foremost contributor in releasing Green House Gases (GHG) particularly CO_2 in the atmosphere (Höök & Tang., 2012). This has put forth a requirement to find renewable energy sources that do not harm the environment. Another major issue due to the ever-increasing population and industrialization is the generation of millions of gallons of wastewater on daily basis. As per the Central Pollution Control Board (CPCB) annual report 2020-21, all the different states in India together generate about 72,368 MLD (Million Litres per Day) of sewage while the total installed capacity for its treatment across the country is only 31,841 MLD (http://sulabhenvis.nic.in). Changing human lifestyle has led to release of the bio-refractory contaminants in wastewater like pesticides, dyes, antibiotics, heavy metals, etc. (Ghangrekar et al., 2019). The absence of regulatory standards for their removal (Sofia & Lima., 2017) and their persistent release in the environment are leading to bio-toxicity, genetic mutations, bio-accumulation etc. (Gin et al., 2018). As per the report of USEPA (40 CFR Part 423, Appendix A), there are a total of 126 priority pollutants in wastewater out of which top 16 are Polycyclic Aromatic Hydrocarbons (PAH) which are potential carcinogens.

Conventional wastewater treatment technologies are unable to meet the standard limit for safe effluent discharge as prescribed by government and are energy consuming as well as cost-intensive. The conventional activated sludge method is the most widely used biological method that aerobically treats the wastewater. However, it demands a lot of energy (0.3-0.6 kWh m^{-3}) consumption (Tchobanoglous et al., 2004). Moreover, it is also inefficient to degrade a variety of pollutants especially aromatic hydrocarbons, most of which are amenable for biodegradation by anaerobic methods.

Some of the currently used methods that anaerobically treat wastewater like Fluidized Bed Reactor (Sattler, 2011) and Anaerobic Sequential Batch Reactor (ASBR) (Ghodeif, 2013) are highly expensive due to their higher maintenance cost. Other systems like anaerobic filters (Sattler, 2011) and Upflow Anaerobic Sludge Blanket (UASB) bioreactor (Hansen & Cheong 2017) have issue of clogging due to biomass accumulation. So, cleaning is required at regular intervals. Few other limitations of these methods are foaming, over-acidification, requirement

of higher temperature regimes etc. (Mersinkova et al., 2021). Hence, an alternative strategy is required to further remove residual organics such as high molecular weight aromatic hydrocarbons present in the industrial wastewater (Logan et al., 2006; Flimban et al., 2019). Recently, Bioelectrical System (BES) has been extensively studied for removal of such biorefractory contaminants including aromatic hydrocarbons. These systems are unique in terms of their ability to convert the chemical energy contained within organic wastes into electrical energy or hydrogen/chemical products (Pant et al., 2012).

Microbial Fuel Cell (MFC) is a type of BES where organisms oxidize the organics present in wastewater and convert the chemical energy of organic wastes into electrical energy (Ghangrekar et al., 2019). This oxidation occurs anaerobically in the anodic chamber. MFC has few advantageous characteristics like its ability to function at room temperature, has a great potential to generate green electricity, produces lower volume of sludge compared to the conventional treatment methods and above all offers the greatest advantage to anaerobically degrade the aromatic hydrocarbons. Additionally, the associated electrical installations are limited and no energy is consumed for aeration (He et al., 2017). Due to the higher treatment efficiencies associated with energy extraction (Mohan and Chandrasekhar, 2011), MFC could be considered as an alternative innovative technology for the traditional methods of biological wastewater treatment (Raghavulu et al., 2009).

Microbial fuel cell system is a biological method wherein biocatalysts play a major role. Biocatalysts can either be in the form of a pure culture or consortium. Consortium is more advantageous over pure culture as consortium can tolerate various stressful conditions, can withstand shock-loads and degrade varieties of complex pollutants (Haque et al., 2021). The biocatalysts function as exoelectrogens by attaching to the anodic surface mostly with the help of exopolysaccharides secreted by some of them. This attachment physically immobilizes the bacteria while at the same time offers them opportunity for cell-to-cell interaction and communication resulting in the formation of a biofilm (Read et al., 2010). Hence, in MFC, bacterial attachment to anode and the formation of a biofilm on the anode surface are essential for the efficient biological transfer of electrons between microbes and anode (Franks et al., 2010). This creates interest to visualize the structure of biofilm as well as the distribution of live and dead bacteria in developed biofilm (Saba et al.,2017).

The bioremediation potential of MFC has enhanced its research interest. Organic wastewater derived from municipal, industrial and other sources is rich in nutrients and has a yearlong

abundance thus serving as one of the most suitable substrates for MFC (Nawaz et al., 2022). Different types of wastewaters like Acidogenic food waste leachate (Li et al., 2005), wastewater from Chocolate industry (Patil et al., 2009), Petroleum refinery (Guo et al., 2016), Winery (Penteado et al., 2016), Tannery (Sawasdee et al., 2016) and CETP (Mukherjee et al., 2021), etc have already been used as anolytes in MFC.

Furthermore, continuous generation of wastewater requires a provision for its treatment on daily basis. Generally, batch mode of MFC system is used for treatment of wastewater. However, batch mode of the MFC system generates low current and power due to substrate depletion during longer incubation (Borole et al., 2016). This can be overcome by operating the MFC system in continuous mode which can be a realistic approach for further removal of residual organics present in treated wastewater. An effort has been made in the present study to operate MFC system in continuous mode using synthetic wastewater spiked with aromatic hydrocarbon as anolyte. The bioremediation potential of the system was evaluated based on electrochemical and biodegradation ability.

REVIEW OF LITERATURE

"Research is to see what everybody else has seen and to think what nobody else has thought."

2. REVIEW LITERATURE

2.1 Wastewater composition and global production

2.1.1 Composition of Wastewater

Wastewater contains 99% of water in addition to other components like solids, dissolved solids, microorganisms, nutrients, micropollutants, inorganic salts and heavy metals depending upon the sources of its origin like domestic, industrial, agricultural, mines, farms, etc. Domestic wastewater and municipal wastewater consist of high microbial loads while industrial wastewater contains higher concentration of chemical pollutants (Qadir et al., 2020).

The composition of different industrial wastewater varies according to the product which the industry manufactures. For example, wastewater released from iron and steel company contains oil, metals, acids, phenol, and cyanide while the wastewater generated from pulp and paper industry consists of chlorides, dioxins, suspended solids as well as organic substances (Hanchang et al., 2009).

2.1.2 Wastewater generation around the globe

Nearly 357 billion m^3 of wastewater is produced yearly around the globe and it is estimated to increase by 24% till 2030 as per the UN World Water Development Report (2017). This is almost five times more than the annual volume of Niagara Falls which has an average flow rate volume 2,407 m^3 per second (Qadir et al., 2019). Based on the anticipated increase in the urban population in the coming years, there would be an increasing demand of water supply. As water supply increases, the volume of wastewater produced would also increase than that of in use. This scenario conveys that higher volumes of wastewater will be generated in upcoming years for which new methods are needed to be developed for efficient treatment of this generated wastewater. As per the report by Qadir et al. (2019), the Asian continent alone produces 159 billion m^3 of wastewater annually which accounts for 42% of urban wastewater produced globally making it the largest producer of wastewater. By the end of 2030, Asia alone is estimated to generate 44% of world's wastewater. Other regions of world like North America and Europe produce 67 to 68 billion m^3 wastewater yearly. In contrast Sub- Saharan Africa produces 46 billion m^3 of wastewater annually, which is the lowest generation of wastewater per capita globally (Qadir et al., 2019).

2.1.3 Wastewater generation in India

The major cities of India generate 38,354 million litres of wastewater per day. There are a total of 234 sewage treatment plants in the country which account for the treatment capacity of 11,678 million litres of water per day which is only 30.5% of wastewater generated (Lal et al., 2012). According to the report introduced by Central Pollution Control Board (CPCB), industries in India discharge 13,468 million litres of wastewater per day (Kaur et al., 2010).

The per capita wastewater generation by class one cities and class two towns account for about 72% of wastewater generated by the total urban population in India. Delhi alone discharges 3,663 million litres of wastewater per day (MLD). Bihar produces 2,276 MLD and Assam produces 809 MLD. Maharashtra, Gujarat, Bengal, Delhi and Uttar Pradesh are the major contributors of wastewater generation which is about 63% (CPCB 2007).

As one of the potential treatment method for wastewater which is able to degrade the refractory compounds like aromatic hydrocarbons, MFCs are being explored worldwide and is being discussed here.

2.2 Working principle of MFC

In microbial fuel cell (MFC), the chemical energy of substrates can be transformed into electrical energy by the metabolic activity of microorganisms (Logan et al., 2006; Srikanth et al., 2011). A wide range of wastewater has been utilized as anodic fuels in MFC for the power generation as well as their vaporization (ElMekawy et al., 2014; Pant et al., 2010; Mukherjee et al., 2021). Coupling of anodic microbial oxidation with abiotic/biotic reduction at two distinct electrodes separated by an ion permeable membrane will drive the electron flow into circuit against the potential gradient (Srikanth and Mohan, 2012). The basic principle of the MFC system as show in Figure 1, is based on redox reactions in which biocatalysts oxidize or catabolize organic matter present in anodic chamber and generate electrons and protons (Gunes et al., 2022). Generated electrons are transferred from anode to cathode via an external circuit and electricity is generated. However, protons travel from anode to cathode via separators like salt bridge (Mukherjee et al., 2020), Nafion membrane (Min et al., 2005), Ultrex (Sun et al., 2010), clay material or ceramic separators (Ghadge and Ghangrekar 2015) where oxygen is reduced to form water.

Reactions

The following reactions summarize the biotic and abiotic activity in MFC where glucose is shown as a substrate.

Anodic reaction: $C_{12}H_{22}O_{11} + 13H_2O \longrightarrow 12CO_2 + 48H^+ + 48e^-$

Cathodic reaction: $O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O$

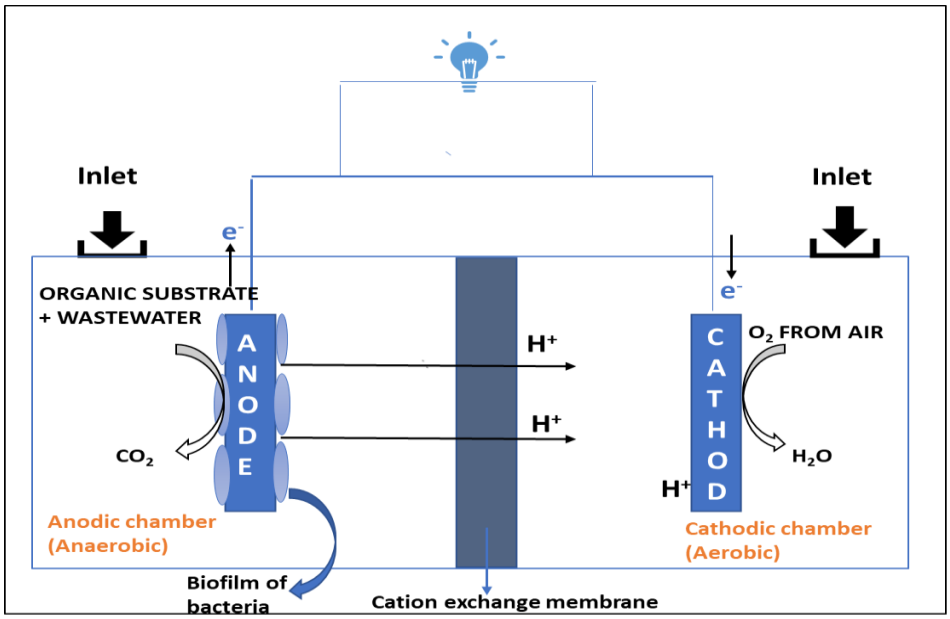


Figure 1: Working Principle of MFC

There are a variety of factors which affect the performance of MFC. The major factors such as types of electrodes, separators and biocatalysts are being discussed here.

2.3 Electrode

In Microbial fuel cell electrode materials play a very important role in the conductance of electrons generated by the bacteria (Mukherjee et al., 2021). As per different reports, electrode used in MFC can be divided into three categories (Zhou et al., 2011):

1. Anode
2. Cathode
3. 3D electrode

Many parameters of electrode materials affect the performance of MFC like good electrical conductivity, biocompatibility, low resistance, anti-corrosion properties, high surface area, high mechanical strength and toughness. Electrodes possessing the above listed characteristics

lead to maximizing the power density thereby improving the coulombic efficiency and minimizing the cost of microbial fuel cell system.

Electrodes can be further classified into two types based upon the electrode materials:

1. Carbon based electrode
2. Non-carbon based electrode

Carbon based electrode includes graphite rod, graphene, graphite brush, carbon cloth, carbon papers, carbon felt and reticulated vitreous carbon (RVC). Excellent electrical conductivity, chemical stability, biocompatibility and long durability are some of the characteristics possessed by carbon-based electrodes that make them more versatile for use (Zhou et al., 2011) Non-carbon based electrodes include those electrodes that are made up of materials like stainless steel, ceramic electrode, 3D electrode materials and advanced electrode etc (Zhou et al., 2011).

Some of the commonly used carbon based electrodes are Graphite, Graphene and Graphite rod with embedded nanoparticles, whose properties and efficiencies are described here.

2.3.1 Graphite

Graphite is a crystalline form of carbon which is a promising material as bioelectrode in MFC. Graphite is used in different forms like graphite rod, graphite brush, graphite foams, and graphite felts (Kalathil et al., 2017). Power and Current density detected from the graphite rod electrode as mentioned in one of the studies were 23±0.4 mW/m^2 and 303±7 mA/m^2 respectively (Barakat et al., 2022).

2.3.2 Graphene

Graphite is a raw material for production of graphene. Graphene has larger surface area than graphite (2630 $m^2 g^{-1}$). Graphene has a lower COD removal efficiency as compared to graphite which is 57% and 68.19% respectively (Zavala et al., 2023).

2.3.3 Graphite rod modified with different nanoparticles

Modification of electrode by coating with nanoparticles leads to improvement in properties of electrodes thereby enhancing the efficiency of the MFC system. Some studies showing the positive effect of nanoparticles on the electrodes have been briefly described below.

1. Fe/ Fe_2O_3 nano particles: Modification of electrode with iron nanoparticles results in formation of a thin layer of iron oxide on the surface of electrode which enhances surface wettability of electrode and also helps in degradation of inorganic compounds present in wastewater. Electrodes have been modified to overcome the limitation of their hydrophobic nature which negatively affects the microorganisms present in MFC system (Lin et al., 2022).
2. MnO_2 /HNT: These nanoparticles have been investigated as anodic catalyst in the form of a layer on the carbon cloth because of which power generation efficiency increased by 50% as compared to untreated electrode (Shen et al., 2016).
3. Graphite modified by graphite paste containing Fe_3O_4 achieved 1.5 to 2.2 times higher kinetic activity then pure graphite as reported by Lowy et al. (2006).

2.4 Separators

Separators play an important role in MFC system between anodic chamber and cathodic chambers and mediating the transfer of cations and anions (Mukherjee et al., 2021). A wide range of separators have been developed for MFCs like cation exchange membrane, anion exchange membrane, bipolar membrane, microfiltration, ultrafiltration, proton exchange membrane and salt bridge etc. (Ramirez-nava et al., 2021).

Performance of separators depend on the oxygen availability, substrate concentration, proton transfer efficiency, pore size, internal resistance as well as on power density and coulombic efficiency of MFCs. From the viewpoints of performance in dual chambered MFC and economic feasibility, the two most important separators are Proton Exchange Membrane (PEM) and salt bridge. PEM is a semipermeable membrane made up of ionomers which is specially designed to transfer protons from anode to cathode in microbial fuel cell.

2.4.1 Nafion membrane

It is a type of PEM. There are many types of Nafion membrane like Nafion 117 and Nafion 211(Peron et al., 2010). Nafion membrane has been used since its discovery due to its unique characteristic of hydrophilic/hydrophobic nanophase structure. Nafion 117 membrane possess high proton conductivity, mechanical and chemical stability in its hydrated state and ability to function at low temperature which makes it an excellent membrane for MFC systems (Peron et al., 2010).

2.4.2 Salt bridge

Salt bridge is a type of separator used specifically for dual chamber MFCs as these separators are cheap and more porous than PEM. However, a salt bridge consisting of 2% KCl and 10% agar sandwiched as a layer between two perforated plexi-glass plates was used as a separator in MFC (Kargi and Eker, 2007) which was compared with Nafion membrane. They observed that salt bridge offers a limitation of lower power output as compared to Nafion 117 membrane. However, oxygen diffusion was almost negligible in agar salt bridge (Min et al., 2005). Although salt bridge is simple and inexpensive, it offers low power density from MFC system due to its high internal resistance which poses limitation (Min et al., 2005; Wen- Swei LI., 2010).

2.5 Biocatalysts used in MFC

In MFC, the exo-electrogenic microorganisms play a vital role in the oxidation of organic materials anaerobically, existing in different types of wastewaters and subjected to selection in the fuel-cell environment. These groups of microorganisms transfer their electrons directly outside their cell under anaerobic or microaerobic condition thus, allowing them to function as electrogenic bacteria (Tahernia et al., 2020). Microbial composition present in wastewater varies when reach in MFC, depending on many factors such as the substrate used in MFC, mode of operation, anaerobic conditions in anodic chamber and severity of the conditions even in the cathodic chamber (Konovalova et al., 2018). Different biocatalysts have different electron transfer mechanisms and pollutant degradation capabilities, which can directly affect the performance of MFC in removal of pollutants and generation of green electricity. Hence, it is advisable to identify and screen microbes that can efficiently degrade pollutants as well as generate electricity while exploring the possible mechanisms of co-operation between different microorganisms (Guo et al., 2020).

The most widely used electrogenic microorganisms in the MFC system belong to *Shewanella*, *Geobacter* and *Pseudomonas* family (Sayed et al., 2017). Cells of anaerobically grown *Shewanella putrefaciens* were electrochemically active and they could grow in a fuel cell-type electrochemical cell in the absence of electron acceptors (Kim et al., 1999). Similar studies have been reported using Fe(III)-reducing bacterium, *Geobacter sulfurreducens* (Pham et al., 2003).

Bacterial communities were isolated from diverse environmental samples such as marine sediments (Bond et al., 2002), petroleum-contaminated soil, hot water springs and activated sludge (Al-Mamun et al., 2016). These organisms have been used as inoculum for most of the MFC systems (Sharma and Kundu, 2010). Bacterial strains such as *Shewanella oneidensis* DSP10 (Ringeisen et al., 2006), *Shewanella putrefaciens* (Kim et al., 1999; Park and Zeikus, 2002), *Pseudomonas aeruginosa* (Habermann and Pommer., 1991; Rabaey et al., 2005) are commonly used in MFC environments. Electrogenic microorganisms can utilize a wide range of substrates, depending on the species. For example, *Escherichia coli*, *Desulfuromonas acetoxidans*, *Geobacter sulfurreducens*, *Bacillus subtilis* use acetate as a substrate (Logan et al., 2010; Park et al., 2005; Holmes et al., 2004), *Klebsiella pneumoniae* uses lactose (Kumari et al., 2015) while bacterial strain *Geobacter metallireducens* can oxidize aromatic compounds such as benzoate and toluene (Bond et al., 2002; Lovley et al., 1993) and can generate electric current.

Several eukaryotic strains have been studied as biocatalysts in MFC with or without external mediator such as *Saccharomyces cerevisiae* and *Candida melibiosica* 2491 (Sayed et al., 2017). *S. cerevisiae* (Baker's yeast) which serves as a model system for many eukaryotes is considered as a good biocatalyst for MFC due to its various features such as its broad substrate spectrum, simple and fast mass cultivation, non-infectivity, low cost and possessing a longer shelf life in the dried state (Ramanavicius et al., 2016). *C. melibiosica* is a eukaryotic strain isolated from plant wastes. This organism was used in various studies as biocatalyst in a dual chamber MFC with or without the addition of mediators like methylene blue stain and using different carbon sources such as glucose, sucrose and fructose (Hubenova et al., 2010).

List of microorganisms which acted as biocatalyst in MFC system as reported in different studies are presented in Table 1.

Table 1: Various microorganisms used as biocatalyst in MFC

Sr. no.	Microorganism	Respiratory type	Substrate	Power generation ($mW\ m^{-2}$)	Current Production (mA)	References
1.	*Shewanella oneidensis* DSP10	Facultative Anaerobe	Lactate (anode) and buffered	10	20 mA	Ringeisen et al., 2007

			ferricyanide solutions (cathode)			
2.	*Shewanella affinis*	Facultative Anaerobe	Cysteine	19	ND	Logan et al., 2005
3.	*Geobacter sulfurreducens*	Obligate Anaerobe	Acetate	418–470	0.20-0.24 mA	Trinh et al., 2009
4.	*Bacillus subtilis*	Aerobe	Glucose, Sucrose, Starch, Acetate	ND	2.14 mA, 1.01 mA, 0.70 mA, 0.72 mA respectively	Bulchandani et al., 2012
5.	*Escherichia coli*	Facultative Anaerobe	Acetate	ND	1.4×10^{-3} mA/cm^2	Park et al., 1997
6.	*Geobacter metallireducens*	Anaerobe	Aromatic compounds (Benzoate, Toluene)	ND	ND	Bond et al., 2002
7.	*Geobacter sulfurreducens*	Obligate Anaerobe	Acetate	ND	0.4 mA	Lovley et al., 2002
8.	*Proteus vulgaris*	Facultative Anaerobe	Glucose	18	3.5 mA	Delaney et al., 1984
9.	*Saccharomyces cerevisiae*	Facultative Anaerobe	Glucose, Sucrose, Starch	ND	0.65 mA, 0.64 mA, 0.24 mA respectively	Bulchandani et al., 2012
10.	*Shewanella putrefaciens*	Facultative Anaerobe	Lactate	0.08	0.04 mA	Kim et al., 2002
11.	*Clostridium beijerinckii* SR1	Obligate Anaerobe	Starch	0.73	0.8 mA	Ibrahim et al., 2019
12.	*Bacillus cereus*	Facultative Anaerobe	Disaccharides	400	ND	Sreelakshmy et al., 2022

13.	*Corynebacterium sp.* MFC03	Facultative Anaerobe	Glucose	7.3	ND	Liu et al., 2010
14.	*Rhodoferax ferrireducens*	Facultative Anaerobe	Glucose	0.25	0.48 mA	Chaudhuri et al., 2003

* Note: ND = Not determined

2.6 Scale up of microbial fuel cell

Batch mode of MFC system is efficient for treatment of wastewater but it has limitations of low current and power generation due to substrate depletion during longer incubation. To overcome this issue, semi-continuous or continuous mode of operation may prove useful.

In a study by Rahimnejad et al. (2011), dual chambered air–cathode MFC was fabricated for the purpose of wastewater treatment. Graphite plates were used as electrodes and glucose was used as a substrate with an initial concentration of 30 g/L. This system was operated in batch mode as well as continuous mode. During batch mode operation, the current and power density were 410 mA /m^2 and 133 mW/ m^2 respectively. During the continuous mode of operation, the effect of hydraulic retention time (HRT) on performance of MFC was examined. The optimum HRT was found to be around 7 h where the maximum current and power density of 1210 mA m^{-2} and 283 mW /m^2 were observed.

Microbial fuel cells (MFCs) operated in continuous mode had been found to be more efficient than the system operated in batch or fed-batch mode for both COD removal and power generation (Sevda et al., 2015) Moreover, if the flow rate of the electrolyte exceeds the rate of substrate consumption and microbial growth, power and substrate degradation eventually decrease even when MFC is operated in continuous mode (Sevda et al., 2015).

In laboratories, parameters which affect the power output of MFCs had been investigated including the presence and absence of the membrane, varying ionic strength, pH, configurations, hydraulic retention time (HRT) and organic loading rates (Chang et al., 2020).

The hydraulic retention time (HRT) refers to the average interval that a soluble compound (eg. the substrate) remains in the microbial fuel cell. It is usually expressed in hours (h) and it is estimated by the ratio between the electrolyte volume (V) and the electrolyte flow rate (Q)

(Sevda et al., 2015). Generally greater HRT leads to more efficient treatment in anodic chamber which ultimately generates greater power and coulombic efficiency (Sevda et al., 2015)

Treatment of low strength wastewater using microbial fuel cells (MFCs) had been effective at hydraulic retention times (HRTs) similar to aerobic process, but treatment of high strength wastewater would require longer HRT (Kim et al., 2016). In a study by Ye et al. (2020), a two-chambered MFC was constructed, and operated in a continuous flow with artificial municipal wastewater as a substrate. The effect of hydraulic retention time (HRT) on the recovery of nutrients by MFC were studied. The COD removal rate was 92% and it was not influenced by varying HRT from 0.35 to 0.69 d. Highest power density of 253.84 mW /m^2 was found with 0.69 d HRT.

In another study by Fazli et al (2018), MFC was used to treat spent caustic wastewater with variation in HRT. MFC performance was evaluated in terms of voltage production, COD and sulphide removal efficiency. HRT range tested were 7, 8 and 9 days. This study had shown that HRT of 9 days was optimum for the MFC operation with the highest COD and sulphide removal efficiency being 98% and 98.98% respectively. The removal efficiency had increased with increasing HRT from 7 to 9 days. In terms of voltage production, the highest achievable voltage was 82.1 mV produced at HRT of 9 days.

Organic loading rate is the quantity of substrate entering through the influent to the digester per unit time which is termed as OLR. It is measured in g /L d (Labatut and Pronto, 2018). Tamilarasan et al. (2017) studied upflow anaerobic microbial fuel cell operated with surgical cotton industry wastewater at different OLR like 0.7–1.2 g COD/L d, 1.9 g COD/L d and >1.9 g COD/L d. The highest total COD and suspended COD removal were obtained to be 78.8% and 69%, respectively which were accomplished at an optimum OLR of 1.9 g COD/L d.

In another study, tubular air cathode stacked MFC was constructed with high scalability as well as low material cost and operated under continuous mode for real wastewater treatment along with bioelectricity generation. It was operated using two OLRs- 1.2 and 4.9 kg COD/m^3d respectively. Here, five non-Pt MFCs were connected in series and parallel circuit treating swine wastewater which could enable an increase in generation of voltage and the current. The parallel stack retained high power output and the series connection underwent energy loss due to the substrate cross-conduction effect. It was observed that in the parallel stack, 83.8% COD removal and 90.8% of NH_4^+-N removal were achieved at 1.2 kg COD/ m^3 d while 77.1% COD removal and 80.7% NH_4^+-N removal were achieved at 4.9 kg COD/m^3 d.

To boost MFC voltage, different approaches have been used which include connecting multiple MFCs in series or parallel or connecting electrical components such as capacitors and DC-DC booster. Dong et al. (2015) observed that in a system fed with synthetic wastewater having COD 1000 mg/ L operated in continuous mode for more than three months at room temperature (~25°C), the voltage output was increased to 5±0.4 V using a capacitor-based circuit. Several studies carried out by operating MFC system at different flow rates along-with their bioremediation ability in terms of COD removal has been summarized in Table 2.

Table 2: Different types of MFC systems and their efficiency

Sr no	Volume capacity	Flow rate	Current /power generation	COD removal	BM/CM	Design type	References
1.	0.25 L	200 mL/h	225 ± 1.4 mW m^{-2}	ND	Batch (22 days) + continuous (78 days)	Single chamber mediator less	Srikaanth et al., 2016
2.	3.6 L	49.8 mL/h	47 mW m^{-2}	87%	CM	Three chamber	Chang et al., 2021
3.	0.29 L	12.9 & 51.7 mL/h	4.9 OLR=175.7 W m^{-2} 1.2 OLR=54.4 W m^{-2}	77.1 %	BM for 12 months; CM	Tubular air cathode stacked MFC	Zhuang et al., 2012
4.	700 L	2500 mL/h	NM	86 % - 87 %	CM for 60 days	Eighteen stacked MFCs (Dual chamber)	Linares et al., 2019
5.	350 mL	0.35, 0.47, 0.59, 0.70 mL/h at different time interval	1.179±0.031 W m^{-2}	92.4 %	CM for 120 days	Two chambered	Ye et al., 2019

6.	498 mL	600 mL/h (trial 1) 180 mL/h (trial 2)	22.03 mW m^{-2}	26.88 %, 55.56 % and 47.53 % on day-3, 6, and 9 respectively	CM	Dual chamber	Ponnaiah & Chinnaraj, 2021
7.	100 mL	600 mL/h	979 mW m^{-2}	10 %	BM+CM	Five MFC series	Daniel et al., 2009
8.	250 mL	16 mL/h	536 mW m^{-3}	34 %	BM+ CM	Dual chamber	Zhuwei et al., 2008
9.	850 mL	18 mL/h	481 mW m^{-3}	77.9 %	CM	Upflow membrane less MFC+ Photobioreactor	Hai-ming et al., 2013
10.	20 L	30 mL/h	6000 mW m^{-3}	89.6±3.7 %	CM for 40 days	MFC+ membrane bioreactor (MBR)	Li et al., 2012
11.	3.7 L	2 mL/h to 2.8 mL/h	12.83 mW m^{-2} 9.4 mW m^{-2}	71.5 % 76.5 %	BM for 11 days and CM for 11 days	NM	Zhao et al., 2013
12.	ND	273 mL/h	281.30±32.31 mW m^{-2}	ND	CM	Tubular Dual chamber MFC	Yousefi et al., 2016
13.	1700 mL	15 mL/h initially and increased upto 21 mL/h	6800 mW m^{-2}	60 %	CM	Single chamber air cathode	Garcia, Martinez et al., 2016

14.	250 mL	30 mL/h	49 W m^{-3}	ND	CM for 1 month	Parallel MFC	Verstraete et al.,2005

* Note: NM: Not Mentioned; ND: Not Determined; BM: Batch Mode; CM: Continuous Mode

2.7 Physico-chemical factors affecting performance of MFC

The two major factors which are known to affect the performance of MFC are pH and temperature. These factors are discussed here along with probable options to overcome the adverse environmental conditions.

2.7.1 Effect of pH

Continuous operation of an MFC causes acidification at the anode and alkalinization at the cathode. Acidification results by accumulation of protons generated by microbial oxidation of organic compounds. On the other hand, alkalinization is accomplished due to the continuous consumption of protons and absence of replaceable protons from the anodic oxidation reaction. This process leads to increase in pH in the cathodic chamber causing significant decrease in current generation in MFC (Oliveira et al., 2013). Generally, bacteria require pH closer to neutral for their optimal growth and respond to the pH changes in the internal and external environment by adjusting their activity (Biffinger et al., 2008). Changes in pH can cause variation in several physiological parameters such as concentration of ions, membrane potential and biofilm formation (Jadhav and Ghangrekar., 2009). In anodic microenvironment pH plays a crucial role in MFC by influencing the substrate metabolism thereby affecting the mechanism of electron and proton generation. Shifts in pH lead to decline in bacterial activity and ultimately affects the biofilm performance (Behera et al., 2009; Yuan et al., 2011). MFCs operated at low anodic pH conduct proton transfer at higher rates thereby, increasing the availability of protons at the side of cathode resulting in the formation of a pH gradient (Raghavulu et al., 2009). However, development of a pH gradient between anodic and cathodic chamber leads to drop in voltage efficiency in MFC systems. Maintaining the pH of MFC system can rectify this problem which can be done by different ways such as acid/base dosing or addition of chemical buffer like phosphate to sustain steady current production thereby providing optimal environment for growth of microorganisms (Cheng et al., 2007). Despite the use of different types of buffers in MFCs and their advantages in MFC operation and performance, chemical buffers have not been of practical use for real applications due to a

significant cost and impacting power generation due to their abilities to generate varying conductivities in different solutions (Nam et al., 2010). To overcome this problem and to guarantee a continuous feed of a buffer at a low cost, some research studies had been carried out where gaseous CO_2 was added in the cathodic chamber to maintain acidic pH (Torres et al., 2008). This gas combines with hydroxide ion in the cathodic chamber to produce carbonate and bicarbonate, creating a carbonate or bicarbonate buffered catholyte system resulting in an increase in power density and cell voltage, with decreased pH imbalance (Fornero et as., 2010).

2.7.2 Effect of Temperature

Changes in temperature may affect system kinetics and mass transfer (activation energy, mass transfer coefficient, and conductivity of the solution), thermodynamics (free Gibbs energy and electrode potentials) as well as nature and distribution of the microbial community (different species having different optimum temperature) (Martin et al., 2010; Min et al., 2008; Wang et al., 2008). Temperature is a vital parameter in the MFC performance, resulting in an increase in power output and COD removal with an increase in temperature (Martin et al., 2010; Jadhav and Ghangrekar., 2009). Increase in power density with an increase in temperature may relate to the enhancement of the microbial metabolism and membrane permeability accompanied by an increase in ionic conductivity and decrease in ohmic resistance in MFC system (Guerrero et al., 2010). Analysis of microbial activity can be carried out in terms of biofilm development at the anode creating an impact on anodic biocatalytic activity. High temperature leads to stable biofilm and MFC operation in a short period of time and consequently a higher performance is observed in the temperature range of 30 to 45 °C (Patil et al., 2010). Lower temperature results in decrease in the metabolic rates thereby leading to decomposition of whole biofilm and inactivation of the bacterial metabolic activity. On the other hand, higher temperature above 45ºC leads to decline in efficiency of MFC performance. Different electrogenic bacteria have different optimum temperature ranges and grow at different temperature, where the temperature during the initial growth phase of biofilm determines the abundance of the various microbial species as well as their distribution within the biofilm matrix. After the successful formation of biofilm, the microbial species are capable of adapting their metabolism at different temperatures (Liu et al., 2011).

2.8 Microscopic analysis of biofilm

MFC is a type of Bioelectrical System (BES) wherein microorganisms function as catalysts. These electrochemically active microorganisms (EAMs) are usually found to be attached to the anodic surface forming an electroactive biofilm (EABFs) (Borole et al., 2011). The biocatalysts are called EAMs due to their ability to transfer electrons to the anode which maximizes the current density of MFC system. The attachment of these microorganisms to the anodic surface mediates electron transfer directly from the cell across the cell envelope or via nanowires to the anode thus increasing the efficiency of electron transfer. Therefore, increase in the power density of the MFC system depends upon the ability of the organism to attach to the anodic surface as a biofilm (Borole et al., 2011). Moreover, understanding the structure and composition of the biofilm may lead to better conductivity of MFC system (Schechter et al., 2014). Different types of microscopic methods have been used to visualize the anodic biofilm structure on using different types of electrodes. An overview of such studies is listed in the Table 3.

Table 3: Different types of microscopy used for anodic biofilm analysis

Sr No.	Type of Microscopy	Type of Anode	References
1.	CLSM	ITO (Indium-Tin Oxide)	Holtmann et al., 2022
2.	SEM	Carbon paper	Liu et al., 2012
3.	SEM	Carbon cloth	Naeem Ali et al., 2017
4.	FE-SEM	Graphite	Watanabe et al., 2008
5.	Confocal Resonance Raman Microscopy (CRRM)	Glassy Carbon Plate	Virdis et al., 2014
6.	SEM	Tin coated copper mesh	Taskan & Topcu., 2020
7.	SEM	Carbon cloth	Kim & Lee., 2010
8.	CLSM	Platinum	Yoon et al., 2012
9.	FE-SEM	Modified Graphene	Chen et al., 2015
10.	SEM, CLSM	Fe(II) Molybdate nanocatalyst coated graphite	Mohamed et al., 2020
11.	SEM, CLSM	Graphite Plate	Alagarsamy et al., 2018
12.	AFM, CRRM	Glass electrode	Lebedev et al., 2014

Confocal Laser Scanning Microscopy (CLSM) is one of the most widely used method as it offers an advantage to visualize the three- dimensional structure of biofilm by taking optical sections leaving the biofilm structure undisturbed (Holtmann et al., 2022). It also facilitates the visualization of live and dead cells in the biofilm (Mohamed et al., 2020). Another widely used method is Scanning Electron Microscopy (SEM) which provides highly resolved images of biofilm with the morphological details and topographical structure (Schechter et al., 2014). The micro and nano structures like nanowires produced by the exoelectrogens can be visualized by Atomic Force Microscopy (AFM) (Li et al., 2016).

Since the conventional methods for wastewater treatment are unable to remove **aromatic hydrocarbons** if present in wastewater, as an alternative technology, MFC has been shown to play an important role in both treating wastewater along with electricity generation (Abourached et al., 2014). Thus, a **prototype** of batch mode of MFC system with a **bacterial consortium** containing ***Psuedomonas stutzeri, Bacillus subtilis, Shewanella xiamenensis*** and ***Stenotrophomonas maltophilia*** was successfully developed to degrade **sodium benzoate** as a model hydrocarbon in our laboratory. As the wastewater is generated continuously on the daily basis, it requires **continuous treatment**. The conventional treatment plants at CETP are not able to meet the disposal standards most of the times and hence such water can be treated by MFC in continuous mode. Hence, in the present study, an effort was made to scale up the current batch mode system to continuous mode which is suitable to function at field level. Since the activity of MFC is dependent upon successful growth of organisms and biofilm formation, it is required to optimize the growth conditions for these organisms as well as observe their localization on anodic surface.

OBJECTIVES

"No project is completed until its objective has been achieved."

3. OBJECTIVES

Following objectives were designed to address the research problem.

I. Performance evaluation of bacterial consortium and biofilm for wastewater treatment and electricity generation
   (a) Optimization of growth parameters of bacterial strains present in consortium
   (b) Visualization of biofilm structure using CLSM technique

II. Scaling up of microbial fuel cell system to treat aromatic hydrocarbon containing wastewater through continuous mode of operation and performance assessment

MATERIALS AND METHODS

"Science is all about the process; it is not about the conclusion."

4. MATERIALS AND METHODS

4.1 Development of bacterial consortium using screened isolates from Kharicut canal

The bacterial cultures had been isolated from four different sites of Kharicut canal, Ahmedabad.

4.1.1 Isolation and screening strategy for electrogenically active strains

Total 120 bacteria were isolated from four different sites of Kharicut canal using serial dilution method. Further, the organisms were screened using O-F test. Out of them, 81 organisms were found to be Oxidative-Fermentative positive. MBRT was performed to check their electrogenic potential. Organisms which were capable to reduce methylene blue in shorter time were selected. 23 organisms out 81 organisms were found to be MBRT positive. Each of the twenty three isolated organisms were then individually inoculated in MFC system to check their exo-electrogenic potential where nine organisms were found to be efficient which were then combined in all possible ways to form a total of 32 different consortia.

4.1.2 Strategy for development of the consortium

Three different criteria were chosen for the selection of each bacterial strain like:

1. Exoelectrogenic ability of the organisms
2. COD reduction capability
3. Ability to produce exopolysaccharides (EPS)

- Each consortium was also investigated in MFC system and the consortia with the best potential for COD Reduction and voltage generation was selected.

4.2 Fabrication of dual- chambered MFC system

4.2.1 Overview of structure and components

Two identical plastic jars each of 1.2 L volume capacity were used as cathodic and anodic chambers that were connected by a PVC pipe filled with slurry made up of agar and NaCl which functioned as a salt bridge for transfer of protons. The cathode contained Phosphate Buffer Saline (PBS) (Annexure I) which served as a conductive catholytic fluid with oxygen as an electron acceptor. The anode contained synthetic wastewater (Annexure I) spiked with

aromatic hydrocarbon (sodium benzoate) as anolyte which was prepared artificially in the lab using different salts. Both the chambers contained activated graphite electrodes with dimensions 15.0 cm × 0.5 cm × 3.0 cm. The anolyte was then inoculated with cell loading of the selected consortium for the bioremediation of hydrocarbon from the wastewater. Each chamber was connected externally with the help of connector to the Data Logger system (GTek, India) which recorded the output voltage of each system.

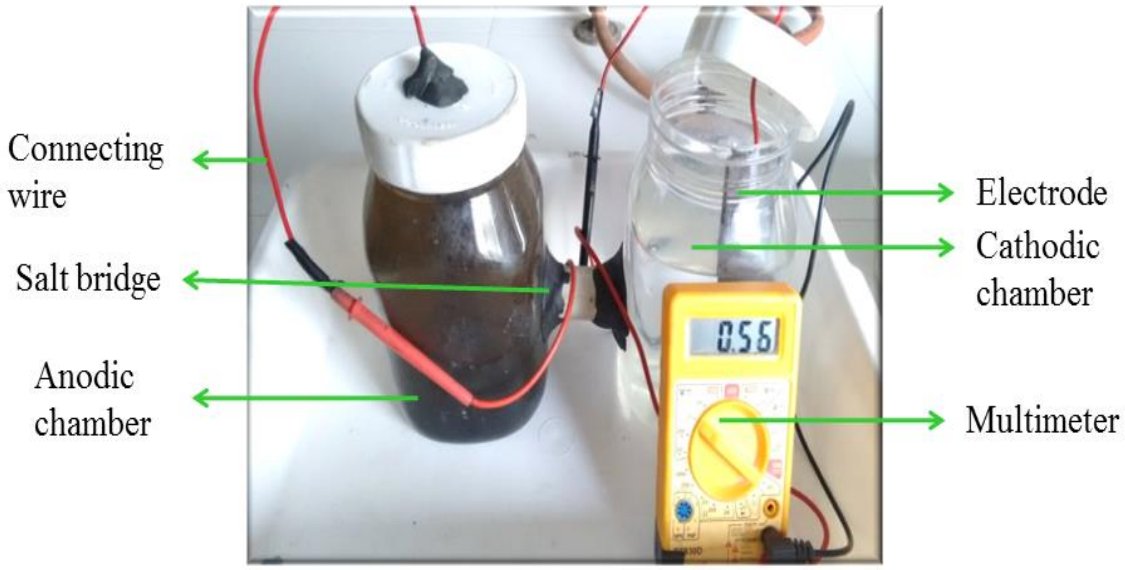


Figure 2: A prototype of batch mode system developed in the laboratory

4.2.2 Preparation and Assembly of different components of MFC system

4.2.2.1 Preparation of Catholyte

Phosphate Buffer Saline was used as a catholyte (Annexure I).

4.2.2.2 Preparation of Anolyte

Synthetic wastewater spiked with aromatic hydrocarbon (sodium benzoate) prepared in lab was used as anolyte. It consists of basal media and salt solution. The composition of basal media is given in Annexure I.

4.2.2.3 Substrate used in MFC system

Synthetic wastewater was spiked with 5 mM Sodium Benzoate as a substrate.

4.2.2.4 Culture activation and cell harvesting

The procedure for cell harvesting is as follows:

i. Organisms involved in the consortia (KRTO021, KO014, EPS026 and EPS040) were inoculated in luria broth and nutrient broth.
ii. These were then incubated at 28ºC for 24 h.
iii. The activated culture was now subjected to cell harvesting by centrifuging the media at 7500 rpm and 25ºC for 10 minutes to pellet down the cells.
iv. The supernatant was discarded and the cells in the pellet were washed with N- saline (0.85% NaCl in distilled water) twice to remove the traces of nutrient broth.
v. The washed cells with an $OD_{560\ nm}$ of 0.6 to 0.8 were finally suspended in synthetic wastewater. Each cell suspension was mixed in equal proportion to form the consortia.

4.2.2.5 Salt bridge formation

The procedure for salt bridge formation is as follows:

i. Salt bridge was prepared by dissolving 11.6% NaCl and 10% agar agar in distilled water. The beaker containing this solution was then heated in a water-bath at about 95ºC to form the slurry.
ii. This slurry was filled in small PVC pipes and stored in the refrigerator until assembled.

4.2.2.6 Electrode Activation:

The graphite electrodes were activated by keeping in hot air oven at 70ºC overnight.

4.3 Optimization of different growth parameters of bacterial strains present in the consortium

Optimization of different growth parameters is required to obtain suitable conditions for rapid growth of individual organism of consortium which needs to be developed each time for setting up new MFC systems.

4.3.1 Optimization of carbon source utilization by bacterial strains of consortium using GEN III Biolog® plate

To identify the favourable substrates of bacteria under study, GEN III Biolog® plates were used. One plate per culture was used. GEN III Biolog® plate is a 96 well microplate which provides 94 biochemical tests to identify a broad range of gram positive and gram negative

microorganisms. It enables the evaluation of utilization of 71 carbon sources by individual strain. Utilization of carbon sources as well as resistance to sensitive compounds is determined phenotypically by change in the color of the incorporated tetrazolium dye from colourless to purple. The schematic for methodology followed is depicted in Figure 3.

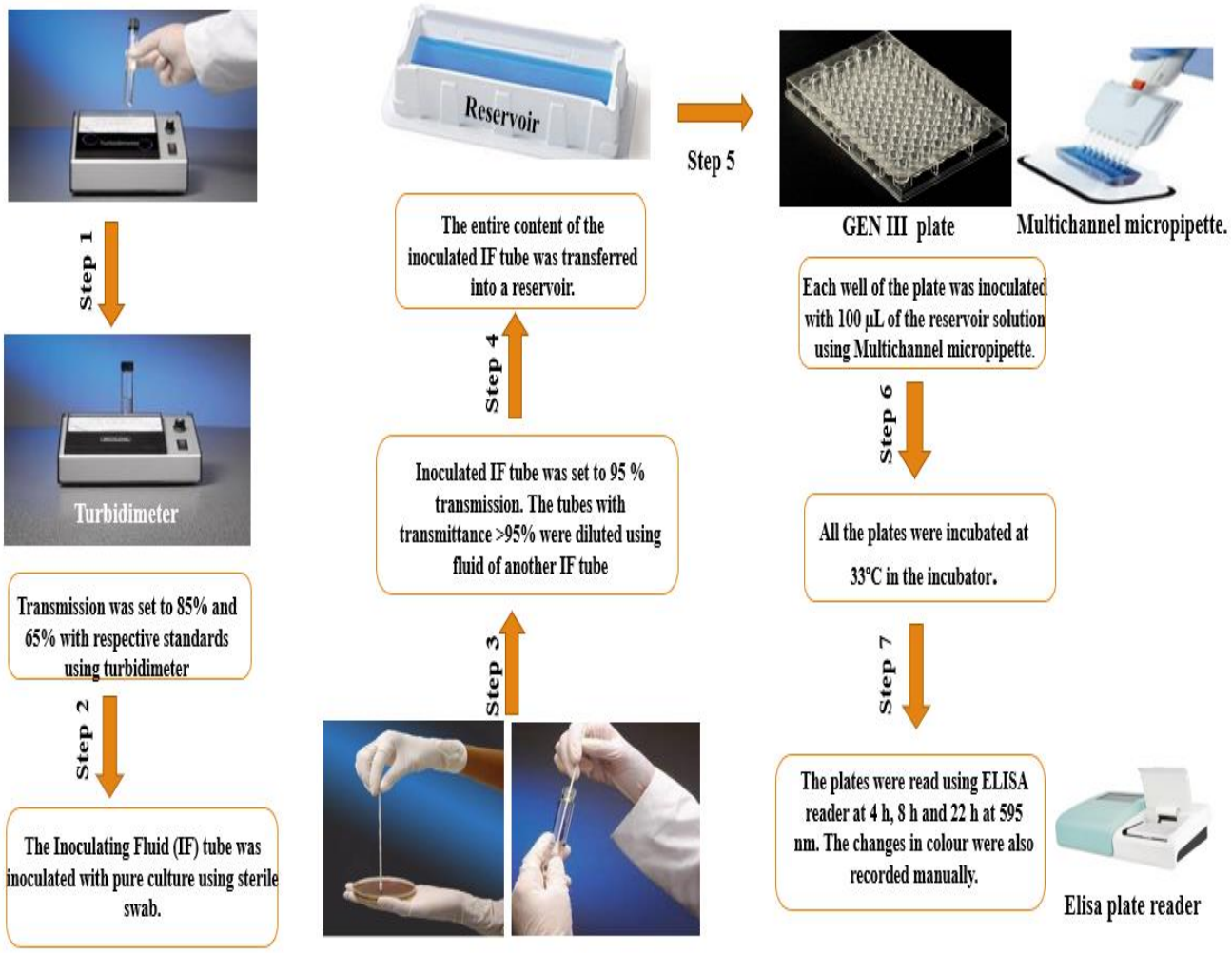


Figure 3: Methodology for analysis of carbon source utilization by bacterial strains using GEN III plate

4.3.2 Growth curve of different strains of the bacterial consortium

Growth curve provides the information about cell physiology and growth kinetics. Growth curve also allows to determine how bacteria respond to variable environmental conditions.

Procedure for performing growth curve

1. All the four strains of the designed consortia namely *Pseudomonas stutzeri, Stenotrophomonas maltophilia, Bacillus subtilis* and *Shewanella xiamenensis* were inoculated individually in three different flasks containing sterile nutrient broth.
2. These inoculated flasks of each culture were then exposed to three different conditions: (a)Aerobic and shaking condition, (b)Aerobic and static condition and (c) Anaerobic and static condition.
3. Anaerobic condition was maintained in the flask by overlaying sterile paraffin oil on the broth. One mL aliquot was collected from each inoculated flasks immediately after inoculation in sterile microcentrifuge tubes for 0 h reading at 560 nm.
4. The flasks inoculated with organisms for aerobic and shaking conditions were incubated in shaker incubator at 28˚C and at a speed of 120 rpm. The other two flasks were incubated at the same incubation temperature at static condition.
5. One mL of culture suspension was withdrawn from each flask after an interval of every 2 h till 24 h.
6. The growth of organisms in different conditions was determined by measuring the optical density (O.D.) at a wavelength of 560 nm using a spectrophotometer after every two hours of time interval.
7. At the end of the experiment, a graph of time versus $O.D._{560\ nm}$ was plotted to obtain the growth curve of individual organisms.

4.4 Microscopic analysis of the anodic biofilm in MFC system

Microscopic analysis helps to indicate the structure and function of microorganisms in the biofilm. Microscopic analysis of the anodic biofilm was done using CLSM (Confocal Laser Scanning Microscopy). Pure culture of *Stenotrophomonas maltophilia* (KO014) was visualized in live and dead condition for the comparison of cells in the biofilm.

4.4.1 Microscopic analysis of anodic biofilm using CLSM

CLSM enables the visualization of 3D structure of the biofilm. A dual chambered MFC system was set up and run for 20 days.

Sample preparation method for CLSM analysis

(a) Cell harvesting

1. 20 ml of sterile nutrient broth was inoculated with pure culture of organisms and incubated in shaking condition at 28˚C for 24 h and 120 rpm.
2. This was then followed by harvesting of cells by centrifuging the media containing the activated culture at 7500 rpm and 25ºC for 10 minutes to pellet down the cells. The pellet of cells was then resuspended in 2 mL of fresh normal saline.
3. One mL of this suspension was added in 20 mL N-saline for live and 20 mL 70% Isopropanol (IP) to make the cells dead.
4. These samples were incubated at room temperature for one hour with an intermittent mixing at every 15 minutes.
5. This was followed by centrifugation at 7500 rpm and 25ºC for 10 minutes to pellet down the cells.
6. The obtained pellets of both live and dead cells were resuspended in 20 mL N- saline and centrifuged at 7500 rpm and 25ºC for 10 minutes followed by resuspension in 10 mL N-saline.

(b) Preparation of staining dye

1. LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit of Thermofisher Scientific was used for staining the samples.
2. The kit contained a staining dye made up of two components: Component A and Component B in addition to mounting oil.
3. For preparation of the staining reagent, 5 µL of component A was taken with 5 µL of component B. Both the reagents were mixed thoroughly.

(c) Staining of the sample

1. One ml of the previously prepared cell suspension in N- saline was mixed with 3 µL of the prepared staining reagent mixture.
2. The cells were allowed to take up the stain by incubating it at room temperature in dark for 15 minutes.

For microscopic analysis, 5 µL of the above stained culture suspension was taken onto a clean glass slide and fixed with the coverslip followed by analysis under the Confocal Laser Scanning Microscope LEICA DMi 8 Wetzlar, Germany.

4.5 Optimization of MFC system using different sodium benzoate concentrations

Individual MFC systems were run with four different concentrations of SB like 5 mM, 10 mM, 15mM and 20 mM to optimize the substrate concentration. Initially culture was activated followed by electrode activation, salt bridge formation, membrane activation and cell harvesting. Each concentration to be investigated were added in synthetic wastewater and all the systems were run in duplicates. The set up of the entire system is shown in Figure 4. These systems were connected to the datalogger and voltage output was monitored daily. The treatment efficiency was determined in terms of COD reduction and SB degradation. The growth of organisms was also depicted by spectrophotometric analysis.

Figure 4: Batch mode MFC systems containing different SB concentrations such as 5 mM, 10 mM, 15 mM and 20

4.5.1 Procedure for COD estimation

1. Samples were collected in clean and labelled microcentrifuge tubes
2. COD cells were filled with COD solution A (2.20 mL) and solution B (1.8 mL)
3. One mL sample collected in the microcentrifuge tube was added to each of the respectively labelled COD cells and one mL double distilled water was added to the cell to set reagent blank.

4. The COD cells were then incubated at 148°C for 2 h in Spectroquant TR 420 thermal cycler (171201, Darmstadt, Germany) for digestion.
5. After 2 h, cells were removed from thermal cycler and allowed to cool down.
6. Reagent blank was set in COD- BOD Analyzer using the COD cell containing double distilled water as a control.
7. Other cells with different samples were then analyzed using COD analyzer (173017, Darmstadt, Germany) shown in Figure 5 and readings were noted down.

Figure 5: Spectroquant prove 300 COD BOD Analyzer

4.5.2 Procedure for estimation of sodium benzoate concentration

1. A standard graph was plotted for concentration of SB vs. OD_{230} readings. It was used for determining SB concentration in samples collected from MFC at various intervals.
2. Samples were collected in clean and labelled microcentrifuge tubes.
3. Samples were centrifuged at 7500 rpm for 10 min at 25^{o}C to pellet down the cells.
4. Twenty µL of centrifuged sample was taken and added to 980 µL synthetic wastewater without SB to make 20:1000 dilution.
5. OD_{230} nm was measured in UV-VIS spectrophotometer and compared with standard graph to find the concentration of the sample.

4.5.3 Estimation of cell density using spectrophotometer

Cell density was determined spectrophotometrically at 560 nm using UV-visible spectrophotometer.

4.6 Performance evaluation of developed consortium in MFC system.

The performance of the developed consortia was evaluated for the treatment of wastewater and electricity generation. The experiments were performed in duplicates. The synthetic wastewater spiked with 5 mM sodium benzoate was subjected to treatment by inoculating it with the developed consortium. The treatment performance was analysed in terms of organic load reduction potential by measuring COD reduction and SB degradation. Total viable count was also evaluated.

4.7 Use of CETP wastewater as substrate in MFC

Inlet wastewater samples were collected from 'The Green Environment Services Co-operative Society Limited' (GESCSL) CETP plant situated at GIDC Vatva, Ahmedabad, Gujarat (Figure 6). The wastewater was analyzed based on various physico-chemical characteristics like colour, odour, pH, electrical conductivity, total dissolved solid (TDS) and COD within 24 h of sampling.

a)  b) 

Figure 6: CETP situated at Vatva, Ahmedabad: (a) GESCSL, CETP plant, Vatva, Ahmedabad (b) Inlet pipeline and Outlet pipeline in GESCSL Plant.

This wastewater from CETP Inlet was subjected to treatment in dual chambered MFC system by the developed consortium and the treatment was evaluated in terms of COD reduction and generation of voltage.

4.8 Development of continuous mode of microbial fuel cell system for wastewater treatment

Synthetic wastewater spiked with aromatic hydrocarbon was subjected to treatment in the continuous mode of MFC operation by maintaining a constant flow rate using peristaltic pump.

4.8.1 PEM membrane

The continuous mode of MFC system was designed where PEM membrane was used as separator. Nafion Membrane 117 was used as a proton exchange membrane (PEM) due to its unique characteristics such as high selectivity for ions, chemical and thermal stability, and high proton conductivity due to availability of sulphonic acid groups and higher degree of hydration (Ramirez-Nava., 2021).

- **The procedure for activation of Proton exchange membrane is as follows.**
  1) PEM membrane was added in a beaker containing 3% H_2O_2 solution and boiled for 1 h (Alkaline treatment).
  2) Then it was boiled for 30 min in double distilled water to remove traces of H_2O_2.
  3) It was then boiled in 0.5 M H_2SO_4 solution for an hour (Acidic treatment).
  4) At last, this membrane was washed again in boiling double distilled water for 30 min to remove traces of H_2SO_4.

Designed MFC system had ten chambers in which first 5 chambers were named as IN chambers as wastewater entered to these chambers at a constant flow rate of 10 mL/h from the reservoir. The other five chambers were named as OUT chambers as wastewater came out of these chambers at a constant flow rate of 10 mL/h to the discarder.

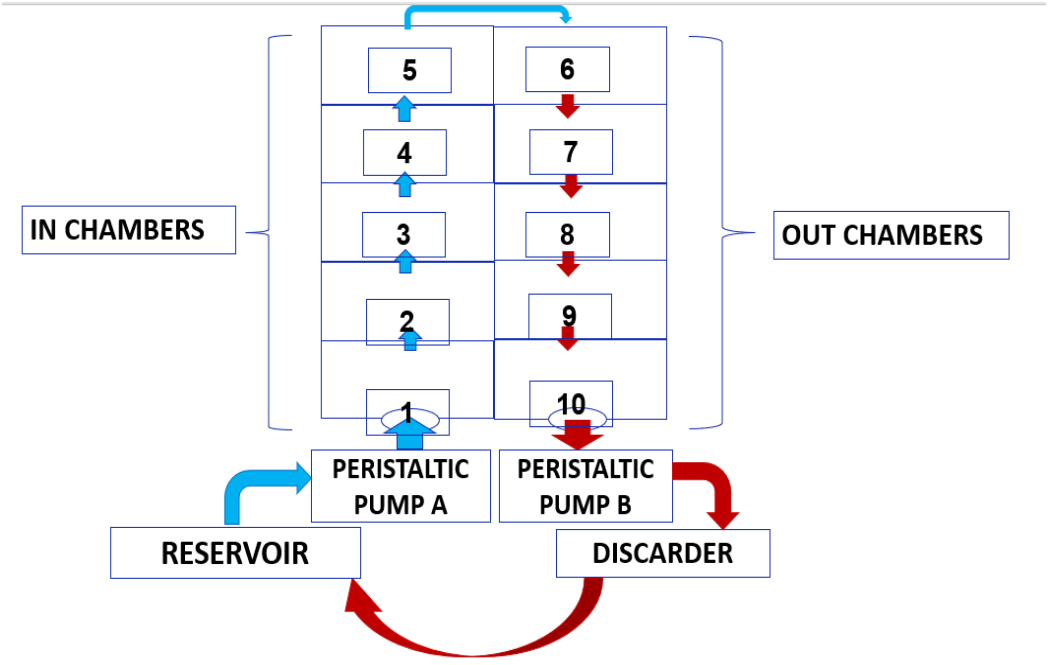


Figure 7: Schematic diagram of ten chambered continuous mode MFC system

The reservoir passed the wastewater to be treated to the MFC system with the help of first peristaltic pump (Peristaltic Pump A) which had been adjusted to a desirable flow rate of 10 mL/h. Another peristaltic pump (Peristaltic Pump B) mediated the removal of treated water at the adjusted flow rate of 10 mL/h to the discarder. This water collected in the discarder was recycled back into the MFC system till maximum treatment efficiency was achieved. The path followed by the wastewater to pass through all the chambers initiating from the reservoir and ending to the discarder has been shown in Figure 7.

This MFC system was operated for longer period of time. Current and voltage output were monitored with the help of either Datalogger (GTek, India) or digital multimeter at regular intervals. Moreover, samples were collected each day for evaluating the treatment performance using different parameters such as sodium benzoate degradation, pH and COD reduction analysis on the basis of standard methods (APHA, 1998). Growth of microorganisms was also monitored on daily basis by spectrophotometry as well as by measuring the total viable count. The actual structure of the system alongwith its different parts have been represented in Figure 8.

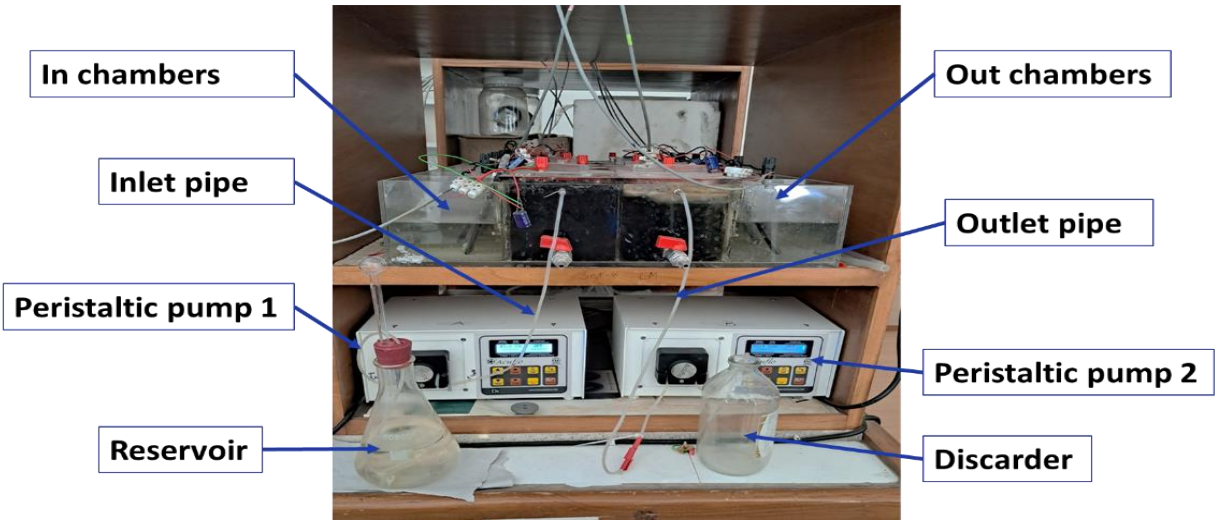


Figure 8: Set up of MFC system in continuous mode

4.8.2 Data Analysis

Average value from replicate experiments were considered for analysis. The differences in readings were represented by error bars in graphs or standard deviations with readings. The

significant differences in various treatments were obtained by performing ANOVA and indicated by p-value as obtained.

RESULTS AND DISCUSSION

"A little progress each day adds upto a big result."

5. RESULTS AND DISCUSSION

5.1 Characterization of bacterial cultures isolated from Kharicut canal and used for consortium.

Organisms of the consortium were selected based on their ability to produce EPS at moderate level, exoelectrogenic ability and potential to reduce organic load or COD in MFC through earlier studies.

The selected isolates forming the consortium along with their gram staining reaction and other characteristics are presented in Table 4.

Table 4: Characteristics of organisms in the developed consortium

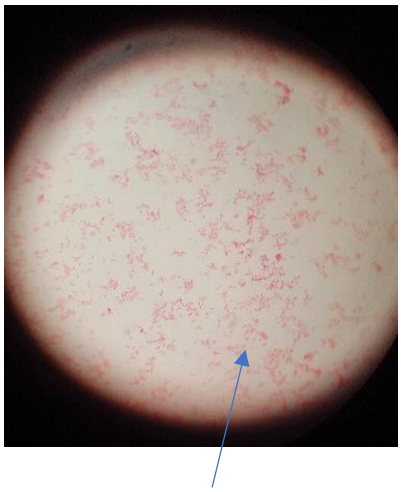
Sr No.	Strain Designation	Culture name	Shape	Gram's reaction	Major characteristics of culture
1.	KRTO021	*Shewanella xiamenensis*	Short rod	Gram negative	Moderate EPS production and COD reduction potential
2.	KO014	*Stenotrophomonas maltophilia*	Short rod	Gram negative	Highest (43%) COD reduction potential among screened isolates
3.	EPS040	*Bacillus subtilis*	Rod	Gram positive	COD reduction potential (33%) and moderate EPS production
4.	EPS026	*Pseudomonas stutzeri*	Short rod	Gram negative	Stable voltage generation (0.8 V)

Moderate EPS producers produce EPS in the range of 1200 to 2960 mg/ 100 mL. The organisms that produce EPS in the range of 0 to 1200 mg/ 100 mL are low EPS producers. Organisms producing EPS greater than 2960 mg/ 100 mL are high EPS producers.

5.1.1 Gram's staining of bacterial strains forming the consortium

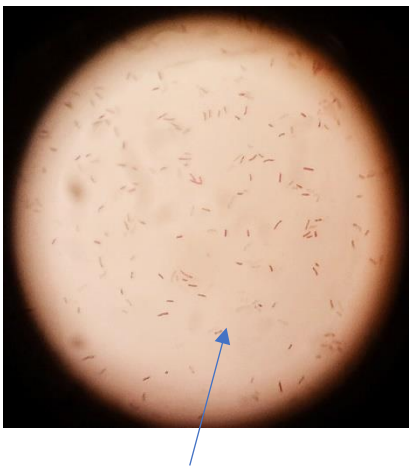
The individual strains in the consortium were characterized phenotypically by Gram's staining and the results are displayed in Figure 9.

1) *Stenotrophomonas maltophilia* (KO014)



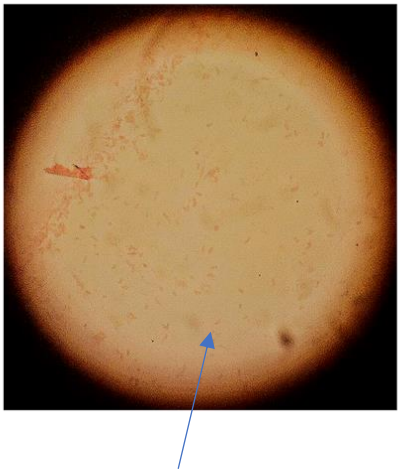
Gram negative short rod

2) *Shewanella xiamenensis* (KRTO021)



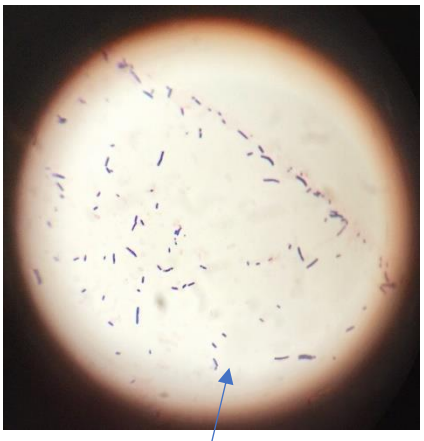
Gram negative short rod

3)*Pseudomonas stutzeri* (EPS026)



Gram negative short rod

4) *Bacillus subtilis* (EPS040)



Gram positive rod

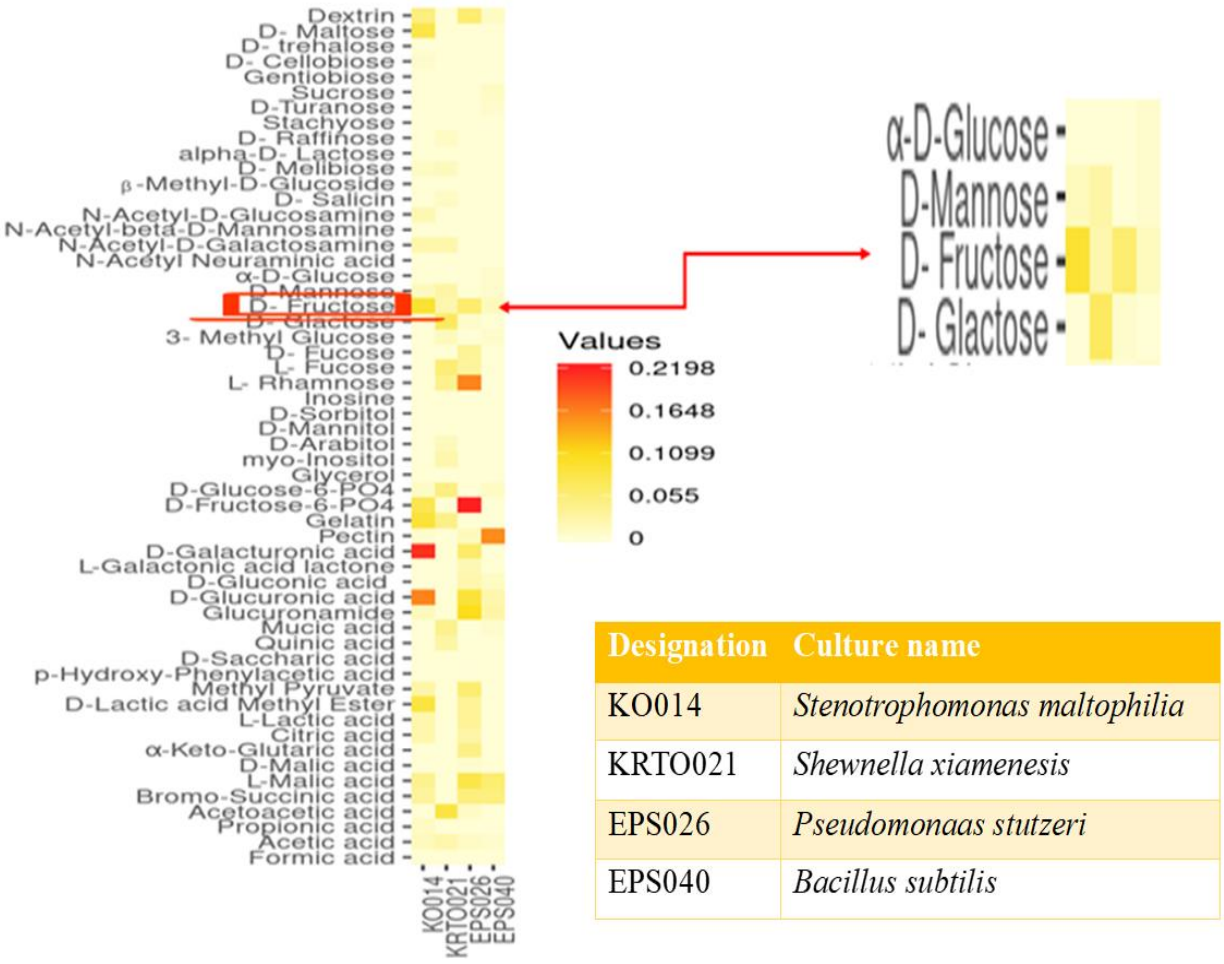
Figure 9: Gram's staining of the different bacterial strains of the consortium

5.2 Determination of preferred carbon source for bacterial strains of the consortium

The utilization of carbon source was analysed by plotting a heat map from the values of optical densities of growth obtained at 8 h and 22 h in ELISA reader at 595 nm and phenotypically by noting colour changes due to reduction of tetrazolium dye in the GEN III Biolog® plate.

5.2.1 Carbon source utilization at 8 h in GEN III Biolog® plate

Among all four organisms, *Pseudomonas stutzeri (EPS026)* was observed to utilize the highest number of carbon sources at 8 h. The common carbon sources utilized efficiently by all four organisms of the consortium at 8 h were D-Fructose and Acetic acid (Figure 10). The utilization of D- Fructose has been magnified in figure for clear appearance.

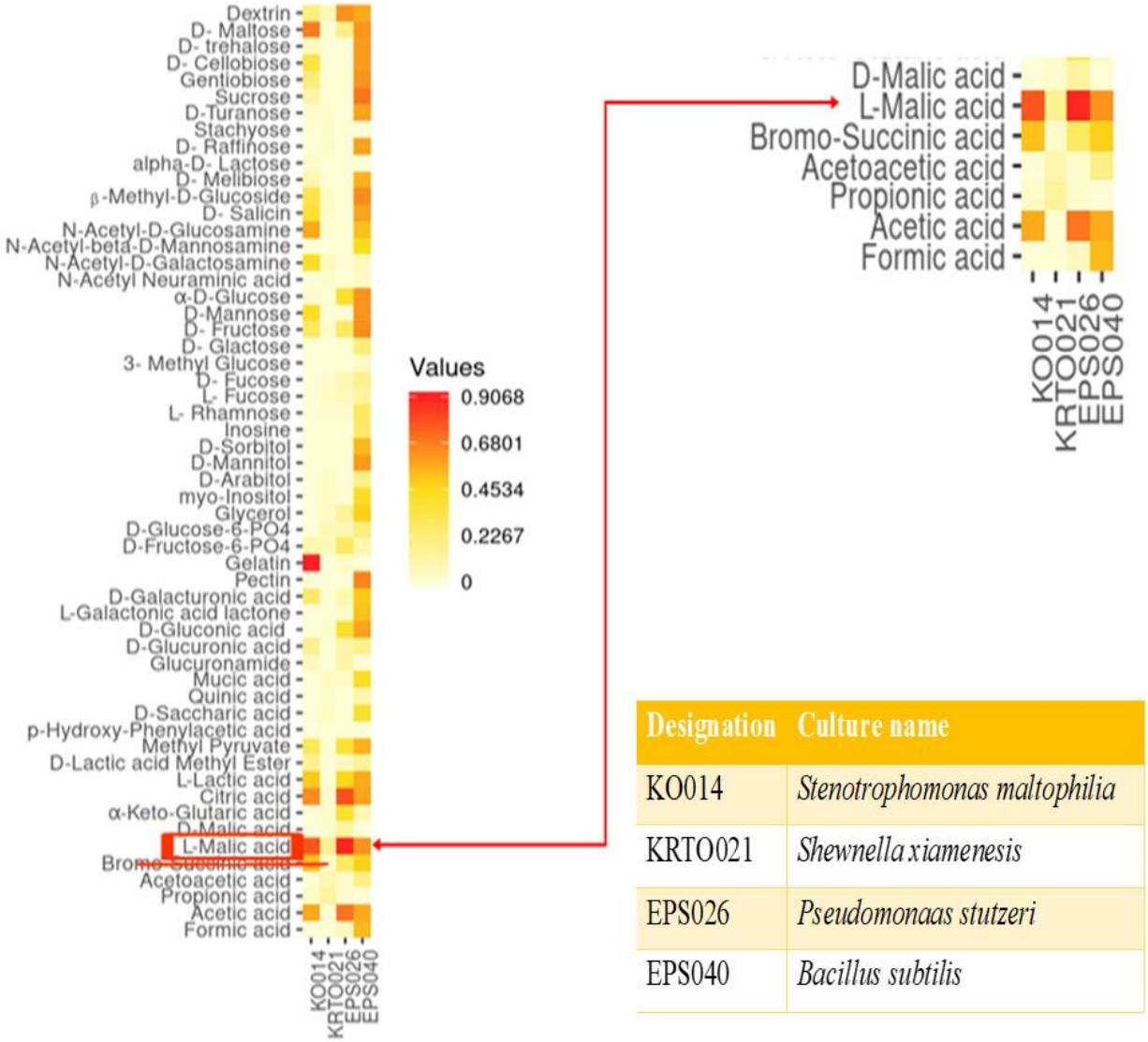


Designation	Culture name
KO014	*Stenotrophomonas maltophilia*
KRTO021	*Shewnella xiamenesis*
EPS026	*Pseudomonaas stutzeri*
EPS040	*Bacillus subtilis*

Figure 10: Heat map showing carbon source utilization by individual strain of the consortium at 8 h.

5.2.2 Carbon source utilization at 22 h in GEN III Biolog® plate

At 22 h, *Bacillus subtilis* (EPS040) utilized the maximum number of sugars among all the four organisms of the consortium. Moreover, the common carbon sources utilized by all the four organisms at 22 h were Dextrin, D- melibiose, D-fructose, glucose-6-phosphate, fructose-6-phosphate, galacturonic acid, citric acid and bromosuccinic acid. However, the common carbon source utilized most efficiently by all the four bacterial strains at 22 h was L- Malic acid as seen in Figure 11.



Designation	Culture name
KO014	*Stenotrophomonas maltophilia*
KRTO021	*Shewnella xiamenesis*
EPS026	*Pseudomonaas stutzeri*
EPS040	*Bacillus subtilis*

Figure 11: Heat map showing carbon source utilization by individual bacterial strain of the consortium at 22 h.

5.3 Growth curve of bacterial strains of the consortium

The growth curve analysis would assist in determining the time required for biomass development of the organisms for inoculation in MFC system. The individual bacterial strains were exposed to three different conditions for analysing their growth: (a) Aerobic and shaking condition, (b) Aerobic and static condition, (c) Anaerobic and static condition. The growth of the individual organisms was determined by measuring the optical density of the inoculated media at an interval of every 2 h at a wavelength of 560 nm in a spectrophotometer. This was repeated till 24 h. The results were analysed by plotting a growth curve of incubation time (h) versus $O.D_{560nm}$.

5.3.1 Growth of organisms at aerobic and shaking conditions

All the organisms had shown a very small lag phase. *S. maltophilia*, *S. xiamenensis* and *B. subtilis* had a log phase starting from 2 h and had been continued even after 20 h. *P. stutzeri* had shown a log phase from 4 h to 18 h after which it had entered to the decline phase (Figure 12).

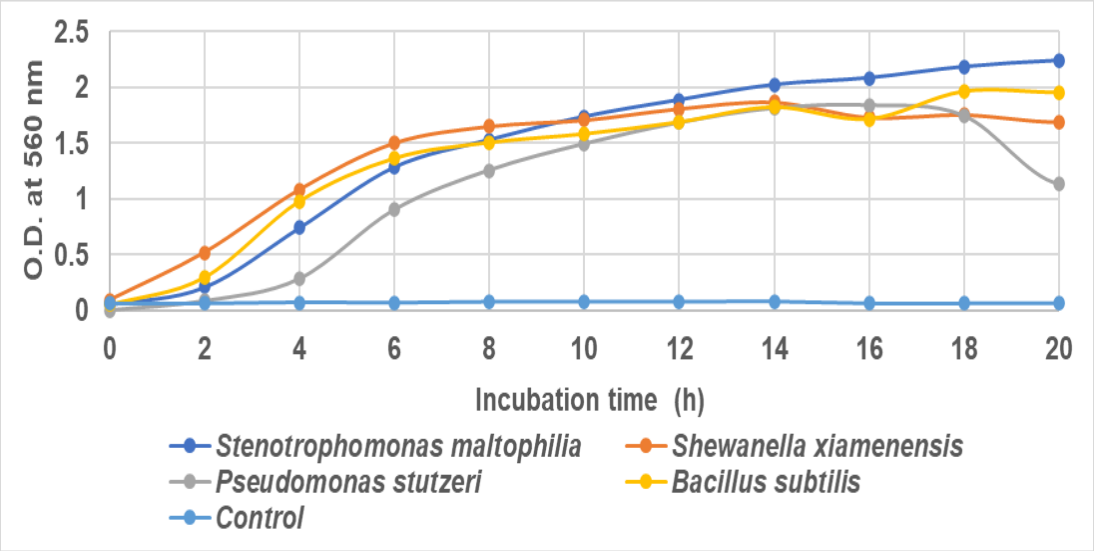


Figure 12: Growth curve of organisms at aerobic and shaking conditions

5.3.2 Growth of organisms at aerobic and static conditions

All the organisms had shown a longer lag phase (4-8 h except *P. stutzeri*) compared to aerobic and shaking condition (Figure 13). However, *B. subtilis* had immediately entered into the log phase which had a period of 0 h to 6 h. *S. xiamenensis* had a log phase starting from 8 h while *S. maltophilia* and *P. stutzeri* had a log phase starting from 4 h and all three organisms had their log phase extending above 20 h. The most efficient growth under aerobic and static condition was observed for *S. maltophilia*.

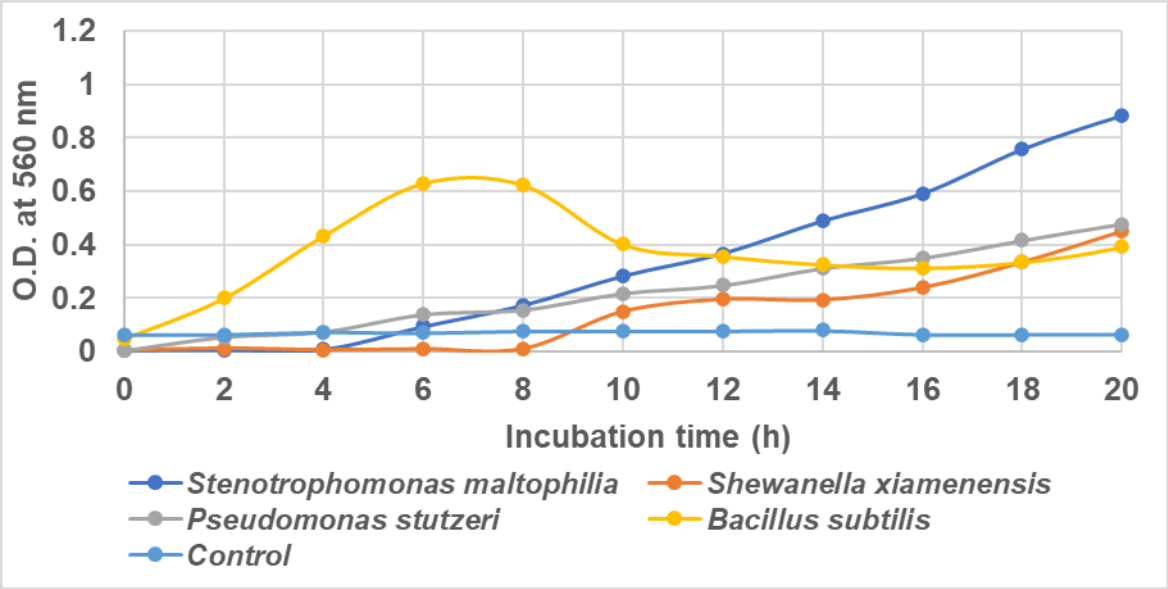


Figure 13: Growth curve of organisms at aerobic and static conditions

5.3.3 Growth of organisms at anaerobic and static conditions

As seen in Figure 14, organisms had shown a longer lag phase compared to aerobic conditions due to absence of oxygen. Though the growth rate for all cultures was low, the growth behaviour shown by all the organisms had been observed to be quite similar to aerobic and static conditions. This indicates their efficiency to function in both aerobic and anaerobic environments in a similar manner. Due to similarity of their growth pattern in aerobic as well as anaerobic conditions, these organisms can be expected to work together and reduce the organic load of wastewater coupled with voltage generation when inoculated in the anaerobic anodic chamber of MFC system.

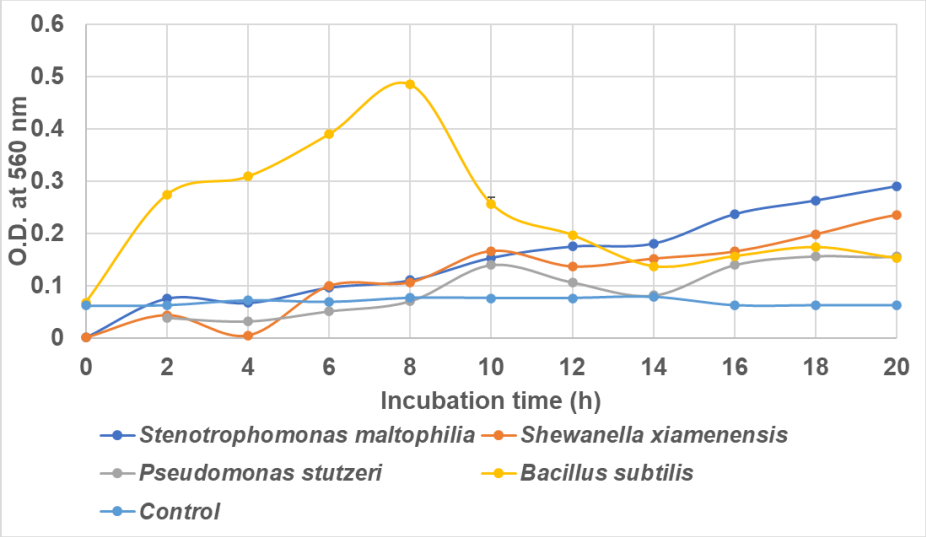
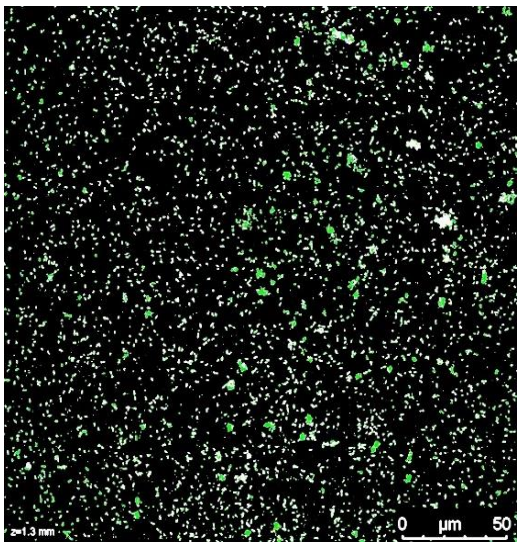


Figure 14: Growth curve of organisms at anaerobic and static conditions

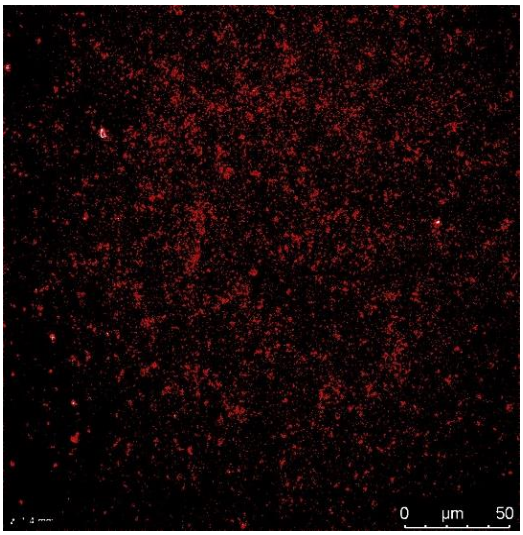
5.4 Microscopic analysis of pure culture and anodic biofilm

LIVE/ DEAD® *Bac*Light™ Bacterial Viability Kit containing Syto 9 dye and Propidium Iodide was used for staining live and dead bacterial cells respectively. The images were taken with a LEICA DMi 8 (Wetzlar, Germany) equipped with 63X oil immersion objective lens. Syto 9 was excited at 488 nm and detected from 471 to 570 nm whereas Propidium Iodide was excited at 501 nm and detected from 570 to 702 nm. The live cells emitted green fluorescence at 488 nm while dead cells emitted red fluorescence at 498 nm. The CLSM images of live and dead cells of *Stenotrophomonas maltophilia* are shown in Figure 15.

a)



b)



c) d)

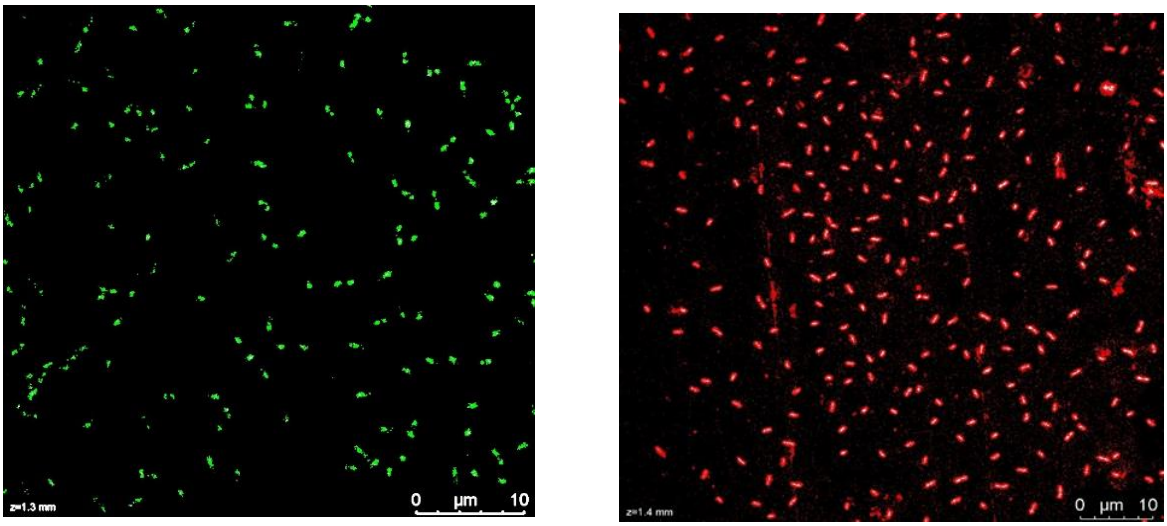


Figure 15: CLSM analysis of live and dead cells of *Stenotrophomonas maltophilia*: a) and c) Live cells emitting green fluorescence; b) and d) Dead cells emitting red fluorescence

Morphology of *Bacillus subtilis* EPS040 was studied using Gram's staining and CLSM. The organism is gram positive and rod shaped as shown in Figure 16.

a) b)

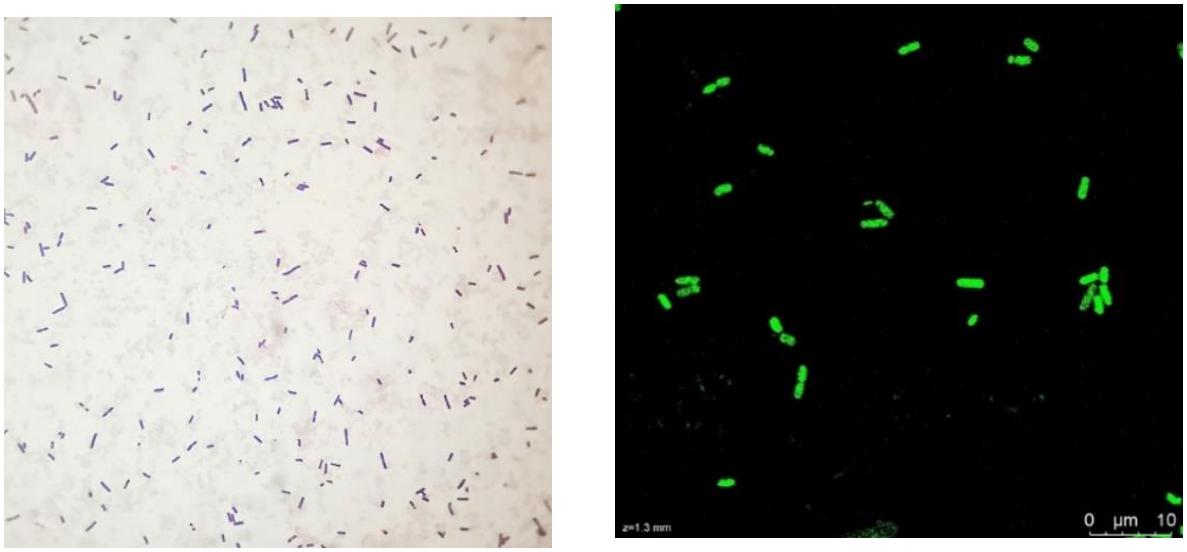


Figure 16: Microscopic visualization of *Bacillus subtilis*: a) Gram's staining b) CLSM micrograph

The day 20 biofilm formed on graphite anode was visualized in LEICA DMi 8 after staining with LIVE/ DEAD® *Bac*Light™ Bacterial Viability Kit. To analyse the proportion of live and dead cells, the CLSM imaging of the biofilm was performed with a wider excitation- emission range of 471 to 702 nm. The CLSM analysis of anodic biofilm had shown the presence of both live and dead cells. Both the channels of live and dead cells with their scales as represented in Figure 17 depict the thickness of the biofilm.

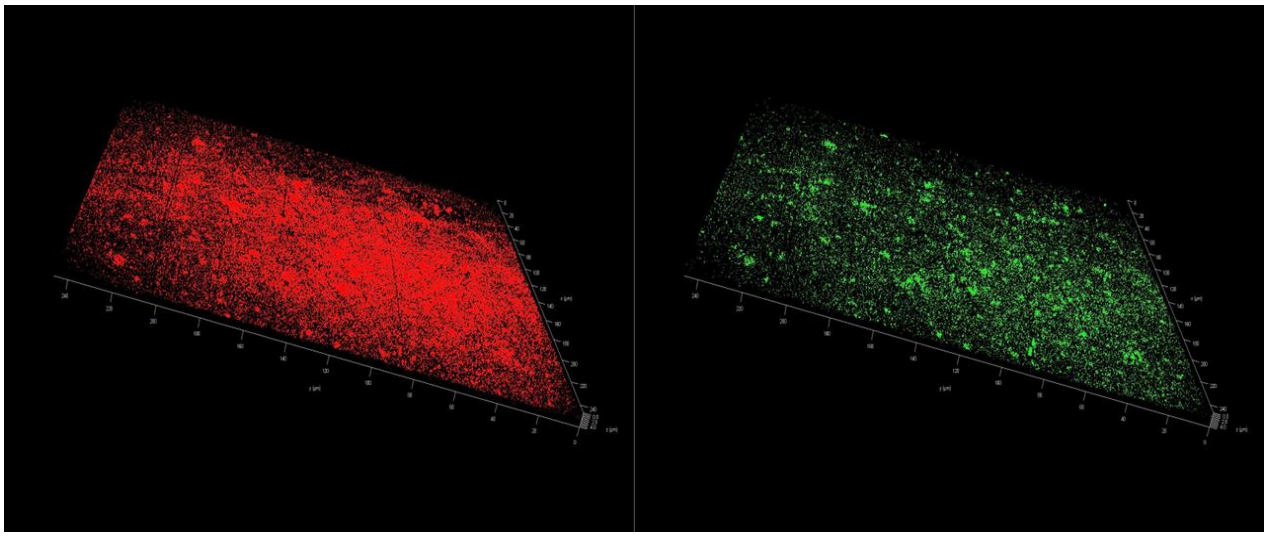
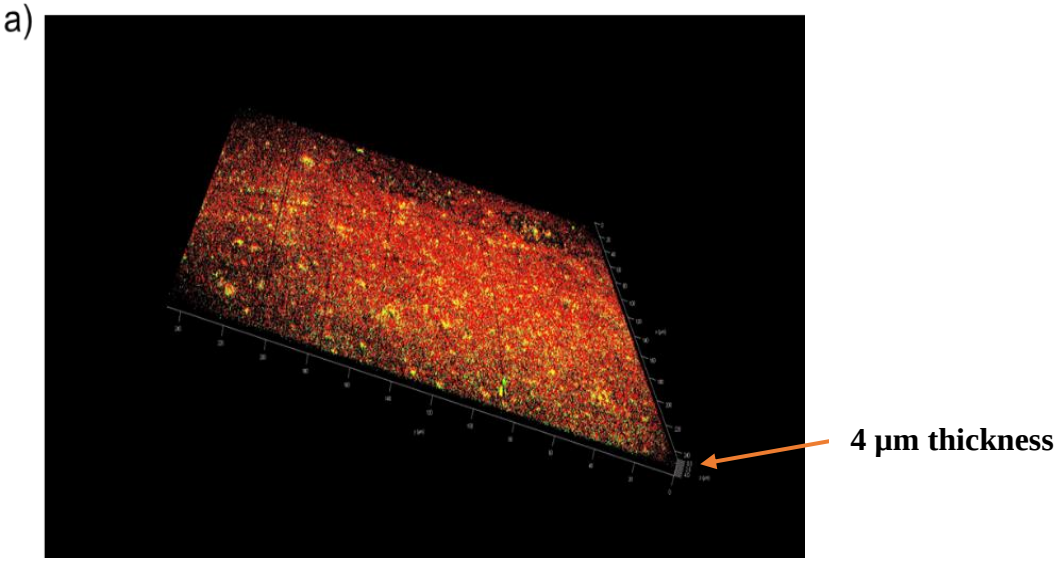


Figure 17: Channels of biofilm with their scales showing live and dead cells.

The thickness of biofilm in two -dimensional structure was approximately 4.0 µm as shown in Figure 18 (a). These stacked 2D sections of the biofilm were reconstructed into a three - dimensional image using ImageJ software as represented in Figure 18 (b).



b)

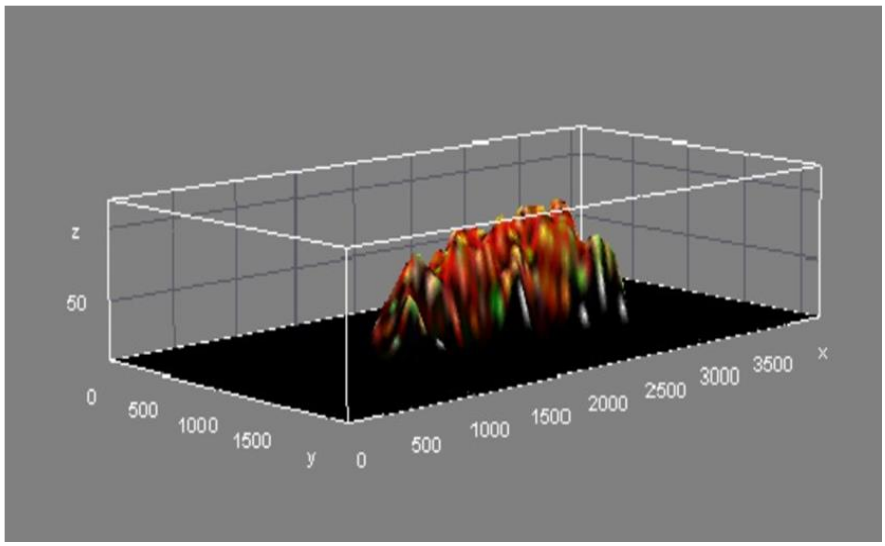


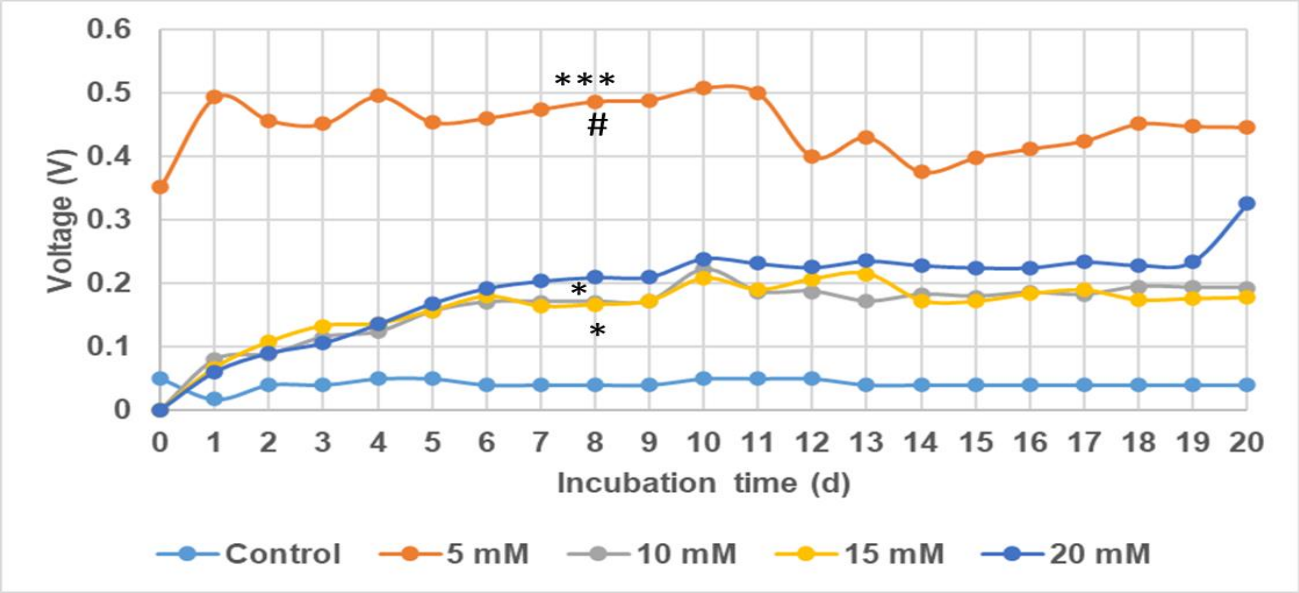
Figure 18: (a) Z stack overlayed channels showing both live and dead cells together; (b) Three - dimensional structure of biofilm

5.5 Optimization of MFC system using different sodium benzoate concentrations

The efficiency of wastewater treatment in MFC systems containing wastewater spiked with different concentration of SB like 5, 10, 15 and 20 mM was analysed based on voltage generation, COD reduction, SB degradation and growth of organisms.

5.5.1 Output voltage generation in MFC systems with different SB concentrations

On comparing the generation of output voltage by the consortium (Figure 19), it was observed that the highest voltage of about 0.5 V was produced by organisms in a short period of around 4 days in the system containing 5 mM SB concentration. Also, there was significant difference in voltage obtained from the system containing 5 mM SB when compared to 10, 15 and 20 mM SB concentrations. This shows that organisms can readily assimilate 5 mM hydrocarbon concentration but the higher concentrations may be toxic for its growth. Hence 5 mM concentration of sodium benzoate was used in all the other MFC systems including the continuous mode of MFC system.

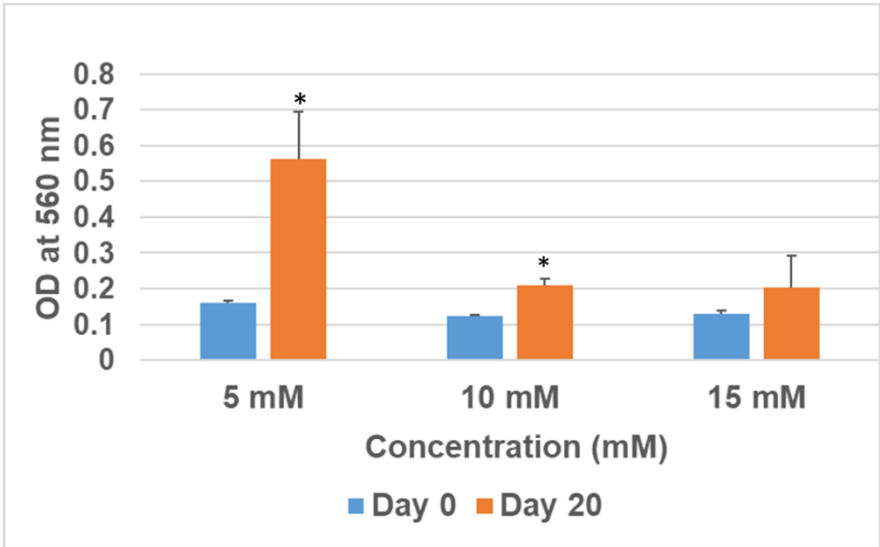


*: p value < 0.05; **: p value < 0.01; ***: p value <0.001 when compared to uninoculated control MFC system.
#: p value < 0.05 compared to 20 mM, 15 mM and 10 mM SB containing MFC system

Figure 19: Comparison of output voltage in MFC systems containing different SB concentrations

5.5.2 Growth of organisms in MFC system with different SB concentrations

As shown in figure 20, the growth of organisms was found to significantly increase in the MFC systems containing synthetic wastewater spiked with different concentrations of sodium benzoate after an incubation time of 20 days. However, the highest growth was observed in the system containing 5 mM substrate concentration.

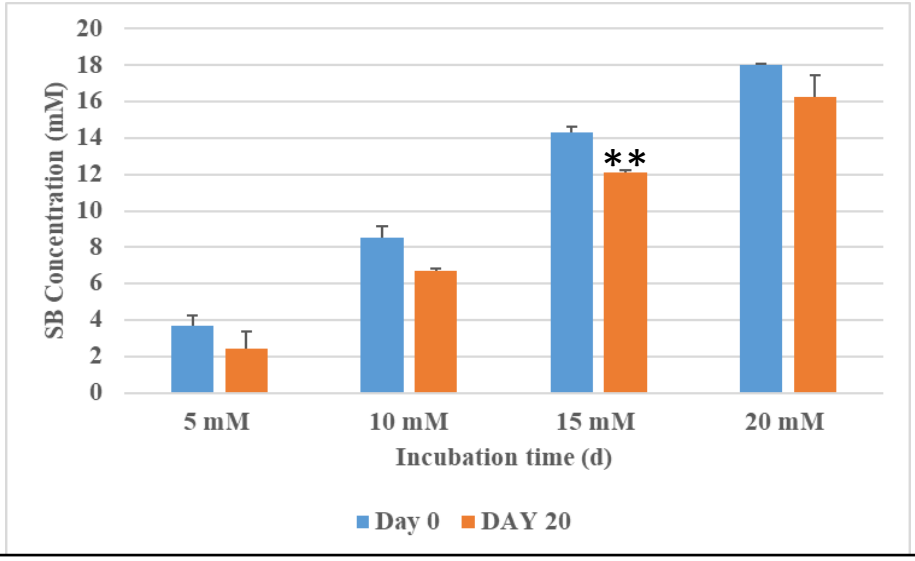


*: p value < 0.05; **: p value < 0.01; ***: p value <0.001 when compared to 0-day systems of respective SB concentrations.

Figure 20: Growth of organisms at different concentrations of SB

5.5.3 SB degradation by organisms in MFC system containing different SB concentrations

After an incubation time of 20 days, concentration of SB was determined spectrophotometrically at 230 nm. The highest reduction was observed in the MFC system containing 5 mM SB concentration which was 33.33 % (Figure 21). This was followed by 21.1%, 15.37% and 9.86% reduction in systems containing 10 mM, 15 mM and 20 mM SB concentration respectively.

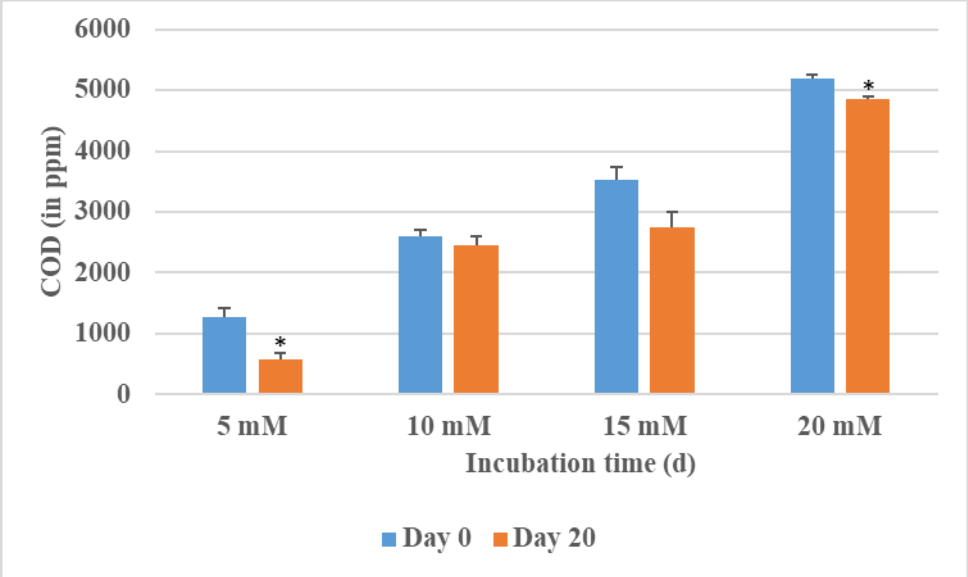


*: p value < 0.05; **: p value < 0.01; ***: p value <0.001 when compared to 0-day systems of respective SB concentrations.

Figure 21: SB degradation by organisms in systems containing different SB concentrations

5.5.4 COD reduction in MFC system containing different SB concentrations

COD concentration in the different systems was determined after 20 days of incubation. The highest COD reduction was obtained in the system containing 5 mM SB concentration which was 55.44% while degradation was comparatively less in systems containing 10 mM, 15 mM and 20 mM SB concentration as shown in Figure 22.



*: p value < 0.05; **: p value < 0.01; ***: p value <0.001 when compared to 0-day systems of respective SB concentrations.

Fig 22: COD reduction by organisms at different SB concentrations

5.5.5 Comparison of % SB reduction and SB concentration

As shown in figure 23, a high negative correlation (r = -0.97) was observed on comparing the SB concentration provided and % SB degradation obtained.

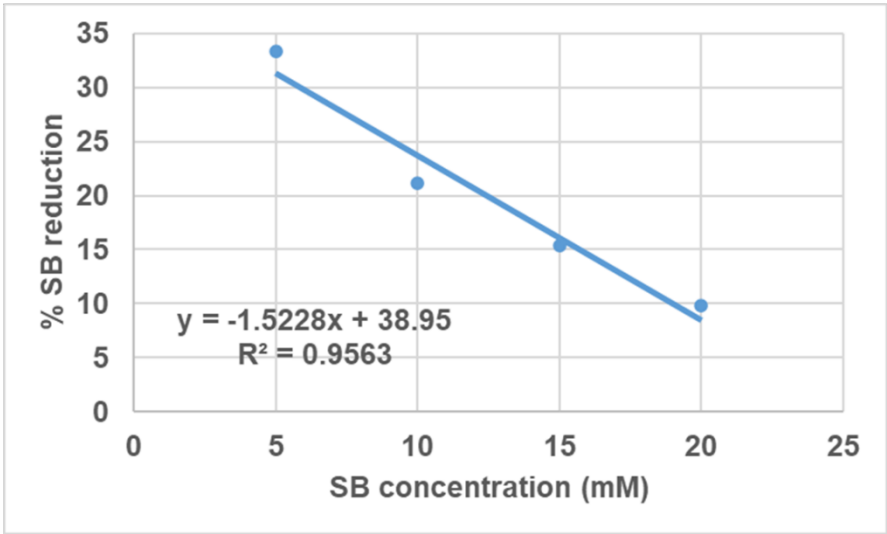


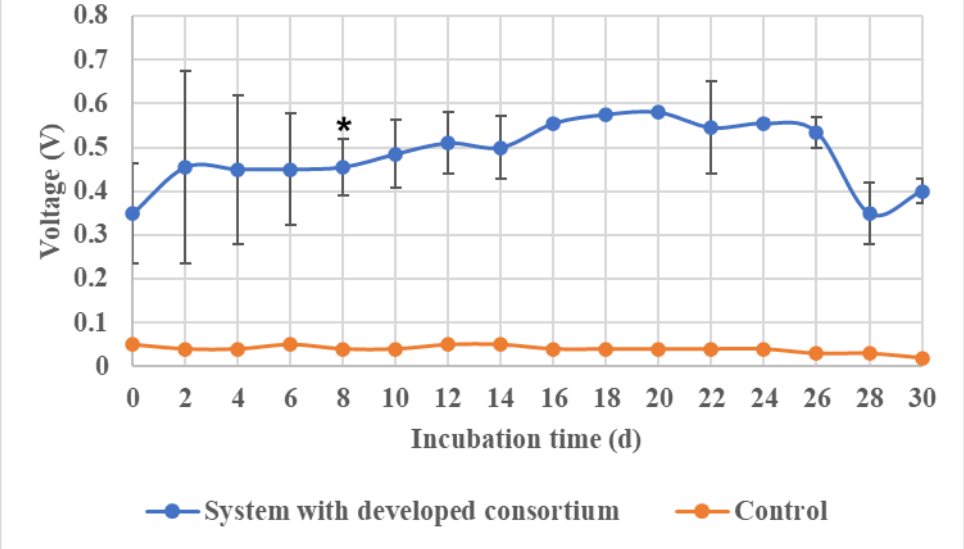
Figure 23: Correlation analysis between % SB reduction and provided SB concentration

5.6 Performance evaluation of developed bacterial consortium in MFC system

The performance of the consortium developed with the bacterial strains isolated and screened from the Kharicut canal water was evaluated in terms of voltage generation and organic load reduction in a dual chambered MFC system containing synthetic wastewater spiked with 5 mM sodium benzoate.

5.6.1 Voltage generation by the developed consortium

Voltage generated by the consortium is shown in Figure 24. It had generated a steady voltage between 0.5 to 0.6 V till 24 days of MFC run. A significant difference was observed on comparing the voltage output of the inoculated system with the uninoculated control MFC system.



*: p value < 0.05; **: p value < 0.01; ***: p value <0.001 when compared to uninoculated control MFC system.

Figure 24: Voltage generation by developed consortia in MFC system with 5 mM SB

5.6.2 Analysis of growth of organisms in the MFC system

5.6.2.1 Spectrophotometric analysis

The growth of organisms at day 0 and day 30 was determined from anolyte of the MFC system spectrophotometrically and the results are given in Figure 25. The value of optical density was higher on day 30 compared to day 0 indicating an increase in the growth of organisms after 30 days of MFC run.

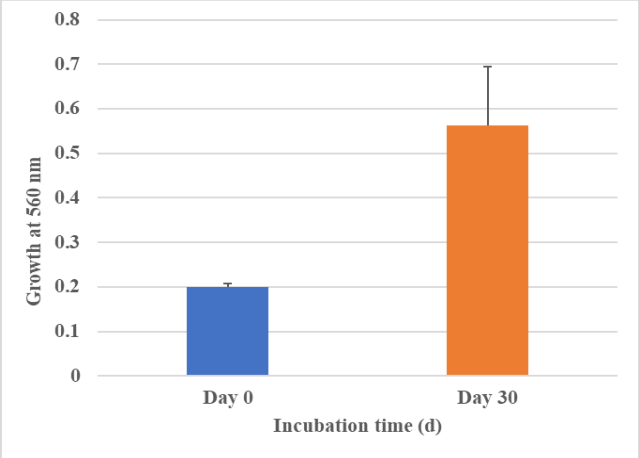
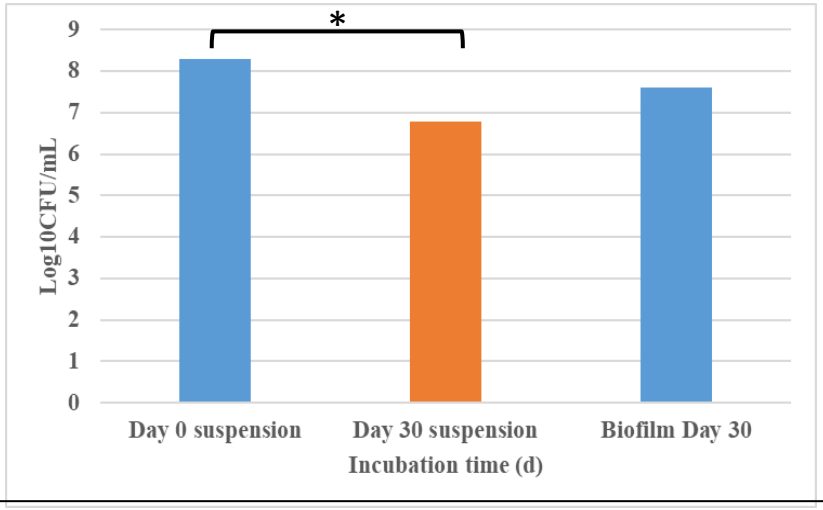


Figure 25: Growth of organisms in suspended form at day 0 and day 30 of MFC run

5.6.2.2 Evaluation of growth of organisms by total viable count

The growth of organisms was compared by analysing the total viable count of organisms in suspension and on the anodic biofilm. The analysis revealed that concentration of suspended cells was less upon 30 days of MFC run whereas concentration of cells increased in the biofilm. As shown in Figure 26, the overall growth also increased from day 0 to day 30 which was evaluated from the total viable count comparison of day 0 and day 30 samples.



*: p value < 0.05; **: p value < 0.01; ***: p value <0.001 when compared to microbial load in 0-day suspension

Figure 26: Comparison of growth of organisms in suspension and biofilm by total viable count

5.6.3 Estimation of organic load reduction by determining COD reduction and SB degradation

The efficiency of the developed consortium in reducing the organic load of the synthetic wastewater was determined by estimating reduction in COD and SB concentration in the system on 30th day of MFC run. The consortium was found to be efficient in reduction of organic load present in the wastewater. As shown in Figure 27, the concentration of SB on 0th day was 4.76 mM which was reduced to 2.76 mM on the 30th day indicating 42% SB reduction.

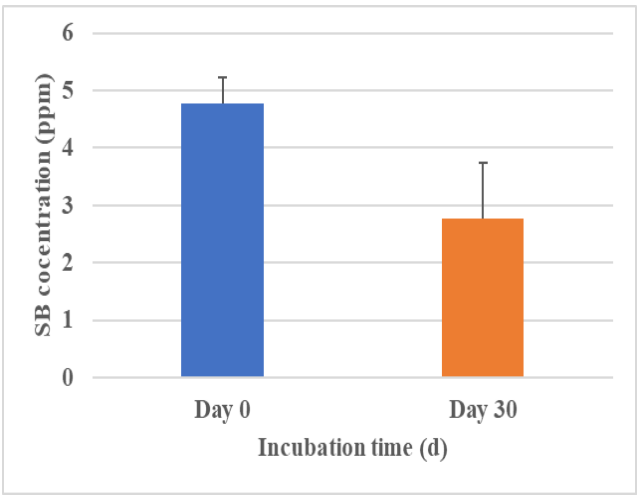
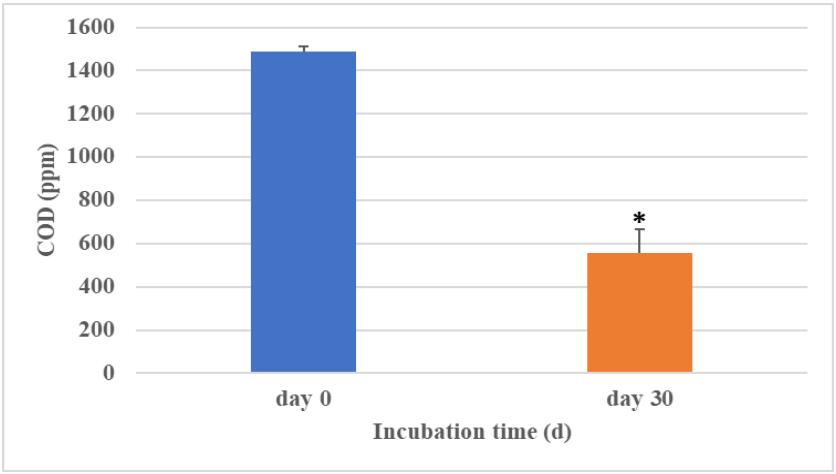


Figure 27: SB concentration in the anolyte of MFC system at day 0 and day 30 of MFC run

A similar trend was observed in COD level which significantly reduced from 1490 ppm on 0th day to 920 ppm on 30th day as shown in Figure 28 and COD reduction achieved was 38.25%.



*: p value < 0.05; **: p value < 0.01; ***: p value <0.001 when compared to 0-day MFC system

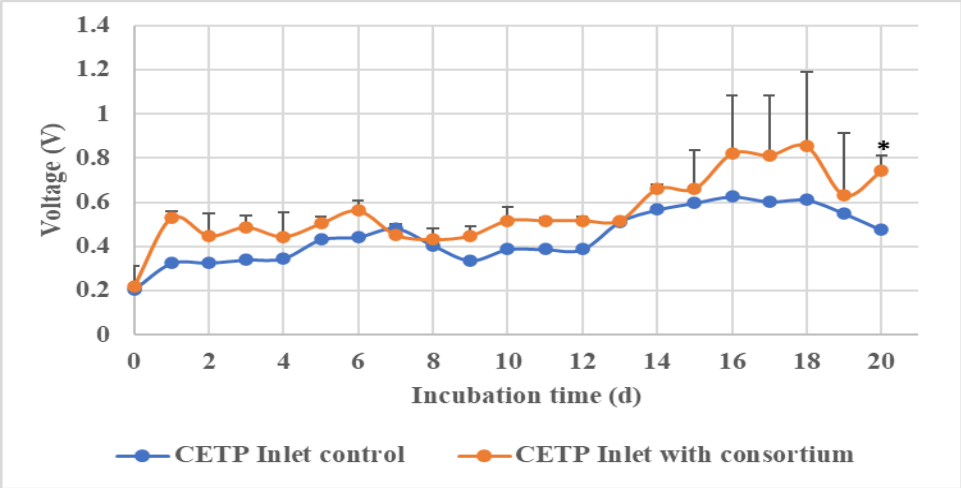
Figure 28: COD reduction observed in the synthetic wastewater in MFC system

5.7 Use of CETP wastewater in MFC

The developed consortium was analysed for its efficiency to treat actual wastewater from CETP inlet plant in terms of voltage generation and COD reduction.

5.7.1 Voltage generation from CETP Inlet wastewater

As shown in Figure 29, the highest voltage generated by the consortium in CETP Inlet wastewater was 0.8 V.

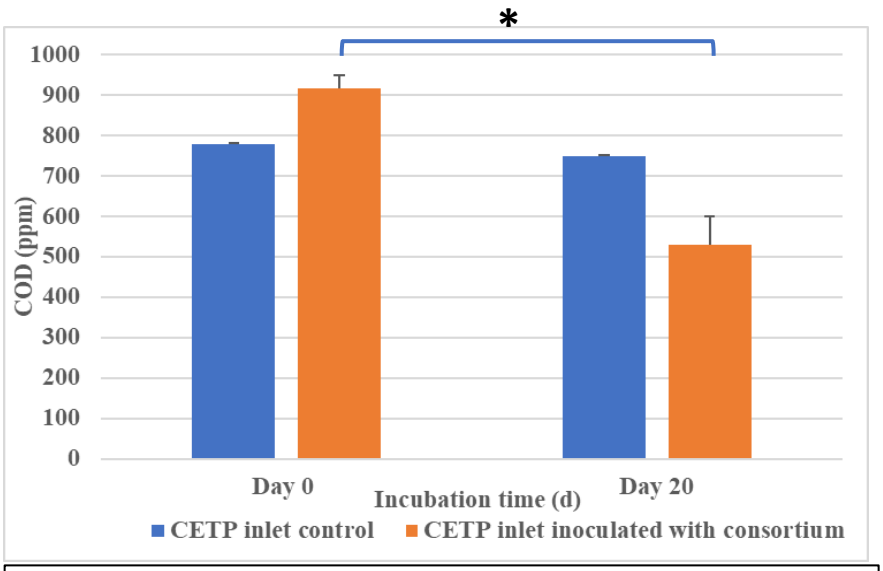


*: p value < 0.05; **: p value < 0.01; ***: p value <0.001 when compared to uninoculated control MFC system

Figure 29: Comparison of output voltage from MFC system containing CETP inlet inoculated with the developed consortium and uninoculated control

5.7.2 COD reduction in MFC system with CETP Inlet wastewater

The organic load reduction potential of the developed consortium was evaluated by determining the reduction in COD concentration after an incubation time of 20 days. A significant reduction of 42.26 % COD concentration was observed in system inoculated with the consortium as shown in Figure 30. However, the COD reduction in the system without organisms was only 3.84%.



*: p value < 0.05; **: p value < 0.01; ***: p value <0.001 when compared to 0- day MFC system

Fig 30: COD reduction by developed consortium using CETP Inlet wastewater

5.8 Development of continuous mode of microbial fuel cell system for synthetic wastewater treatment

For optimization of the continuous mode of MFC system to treat industrial wastewater, initially synthetic wastewater spiked with 5 mM aromatic hydrocarbon was used as anolyte in this study. The continuous flow of 10 mL/h was maintained in the system using peristaltic pumps. The samples were daily to investigate the extent of treatment based on COD reduction, SB degradation and growth of organisms.

5.8.1 Voltage output from continuous mode of MFC system

As can be seen from data presented in Figure 31, constant voltage output of 0.5 V was generated by chamber 4. Voltage output of IN chambers remained constant in the range of 0.4 V to 0.5 V and voltage output of OUT chambers remained constant in the range of 0.1 to 0.3 V. It is quite expected why the voltage output from OUT chambers were low, which is due to reduction in concentration of substrate reaching in these chambers.

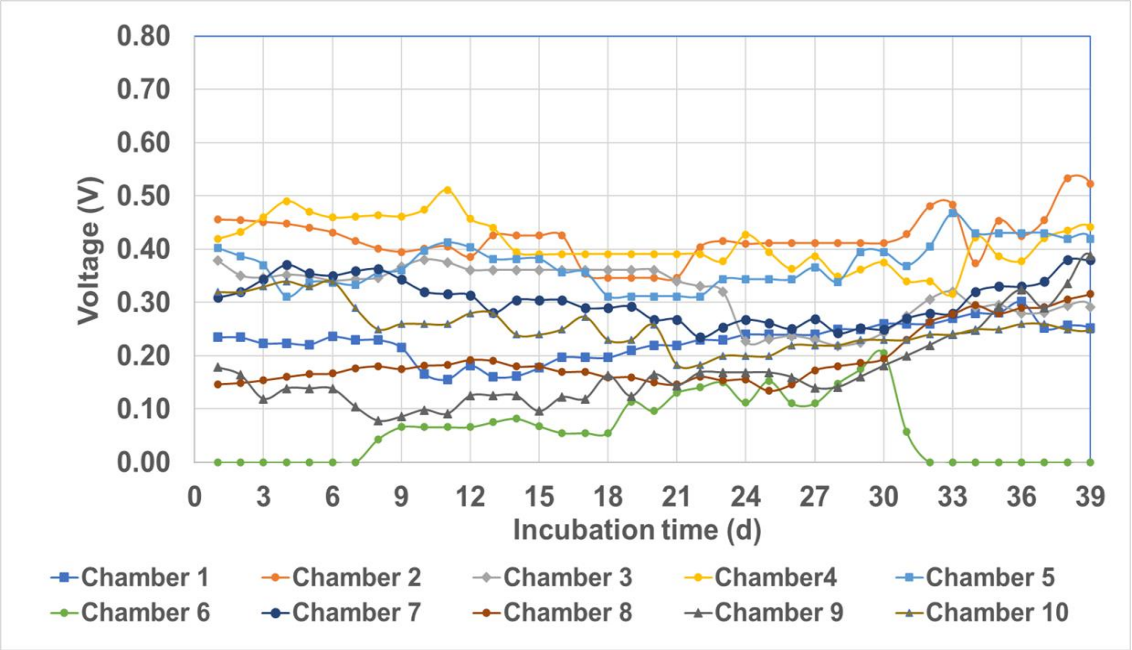


Figure 31: Voltage generation by different chambers of continuous mode of MFC system.

5.8.2 COD Reduction in wastewater treated by continuous mode of operation

COD was reduced from initial 1345 ppm to 220 ppm in 15 days of batch mode of operation. So, on day 2 of continuous mode, 20 mM SB was added as a substrate for supporting the growth of organisms.

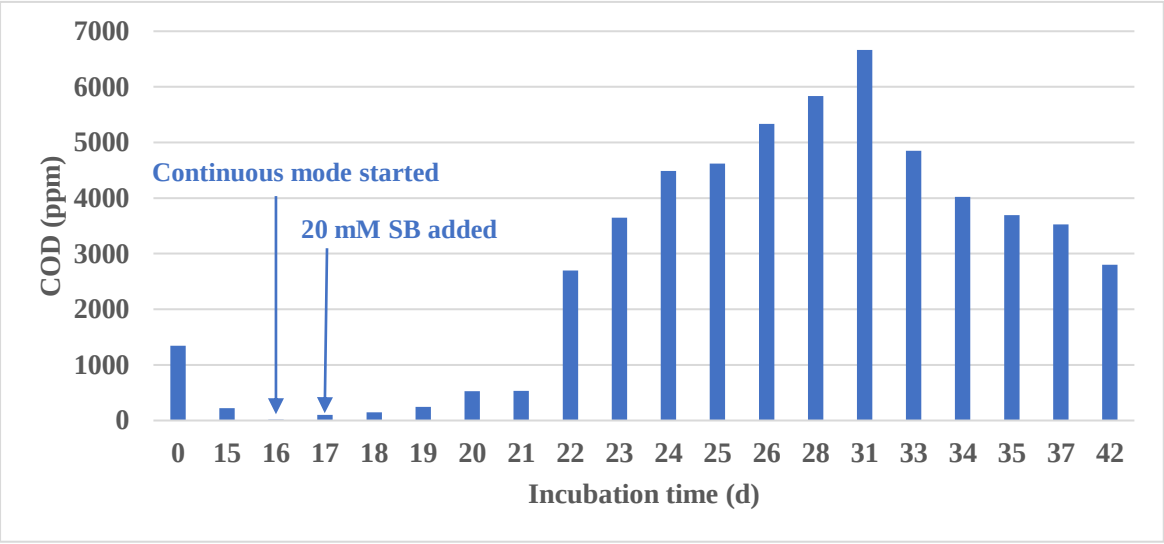


Figure 32: Analysis of COD level in samples collected on daily basis during continuous mode of MFC system run

The concentration of substrate increased gradually upto 31 days and after that it decreased upon utilization by the organisms as observed from Figure 32.

5.8.3 COD and SB degradation in wastewater of continuous mode of MFC system

The continuous mode of MFC system was operated for 15 days in batch mode before initiating the continuous mode of operation to allow the organisms to stabilize and develop a biofilm on the anodic surface. As a result, the substrate was depleted after 15 days to extremely low levels.

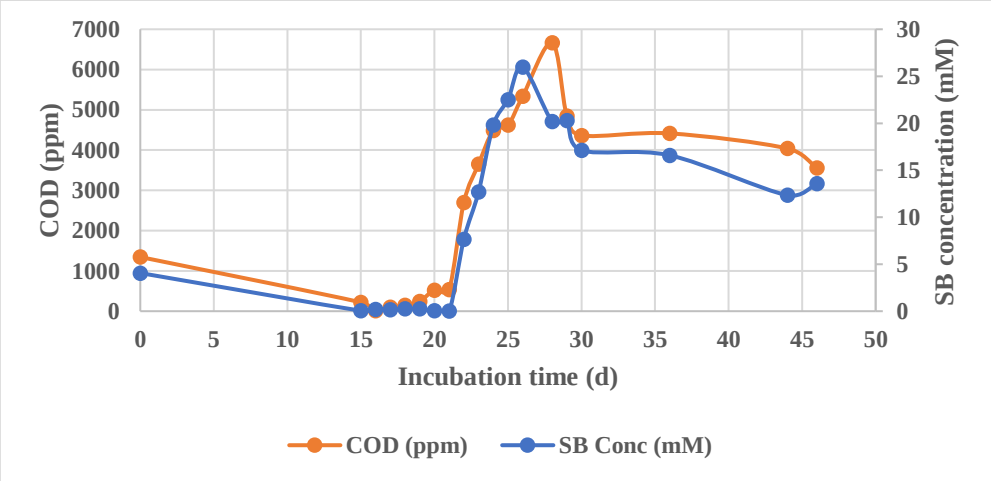


Figure 33: Analysis of COD and SB concentration in continuous mode of MFC system

To replenish the substrate and to ensure efficient treatment, 20 mM sodium benzoate (calculated concentration for the entire system was dissolved in 500 mL synthetic wastewater and fed in the system) was added on the 16th day of batch mode operation and then it was operated in continuous mode for wastewater treatment. After addition of substrate, the value of COD and SB concentration increased exponentially as seen in Figure 33 from 21st day till 30th day and then decreased slowly after 30th day as it would have been used as a source of energy by the organisms in the consortium.

5.8.4 Growth and SB concentration in continuous mode system

On comparing the pattern of growth and SB concentration, it was observed that growth of organisms was suppressed due to substrate depletion in continuous mode of MFC system (Figure 34). However, when substrate was added, growth increased further and declined upon reduction in substrate concentration.

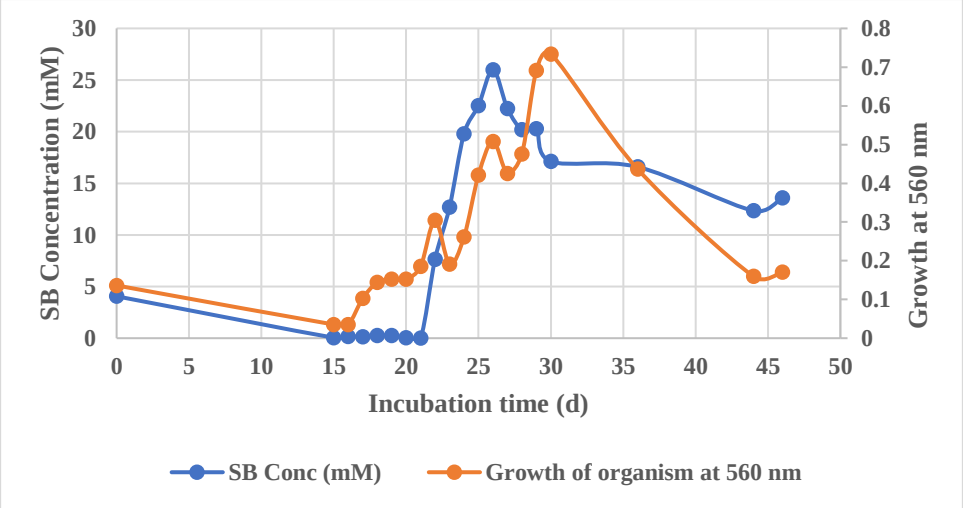


Figure 34: Comparison of growth and SB concentration in continuous mode of MFC system

Estimation of pH: The pH of all samples collected on daily basis remained constant at 7

5.8.5 Correlation analysis between different parameters of continuous mode of MFC system

Correlation analysis was performed between different parameters like growth, SB concentration and COD concentration from the samples of continuous mode of MFC system collected on daily basis.

5.8.5.1 Correlation analysis between SB concentration and COD concentration

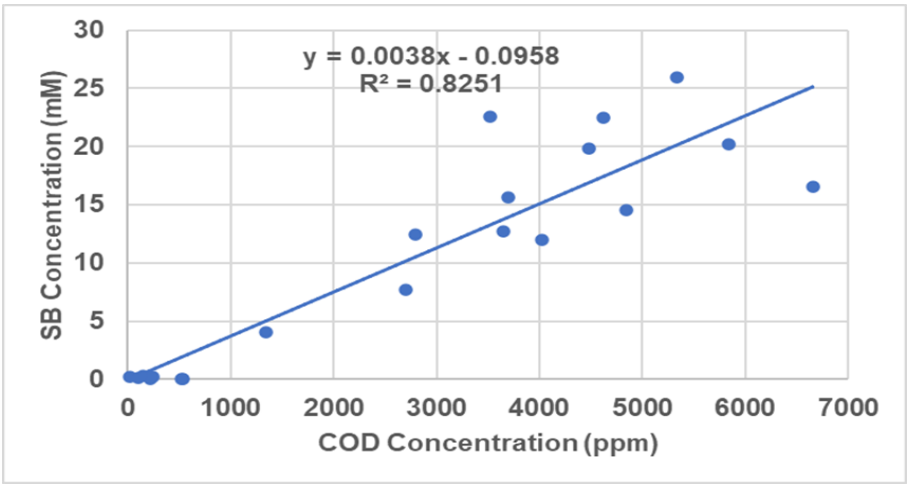


Figure 35: Correlation between SB concentration and COD concentration

20 mM SB was added on day 2 of continuous mode to support the growth of organisms. Thus, an increment in the concentration of SB and COD was observed. As shown in Figure 35, a high positive correlation (r = 0.90) was obtained between SB concentration and COD**.**

5.8.5.2 Correlation analysis between growth and SB concentration

As shown in figure 36, a positive correlation was (r = 0.77) was obtained between growth of organisms and SB concentration. Thus, an increase in growth was observed along with increase in SB concentration.

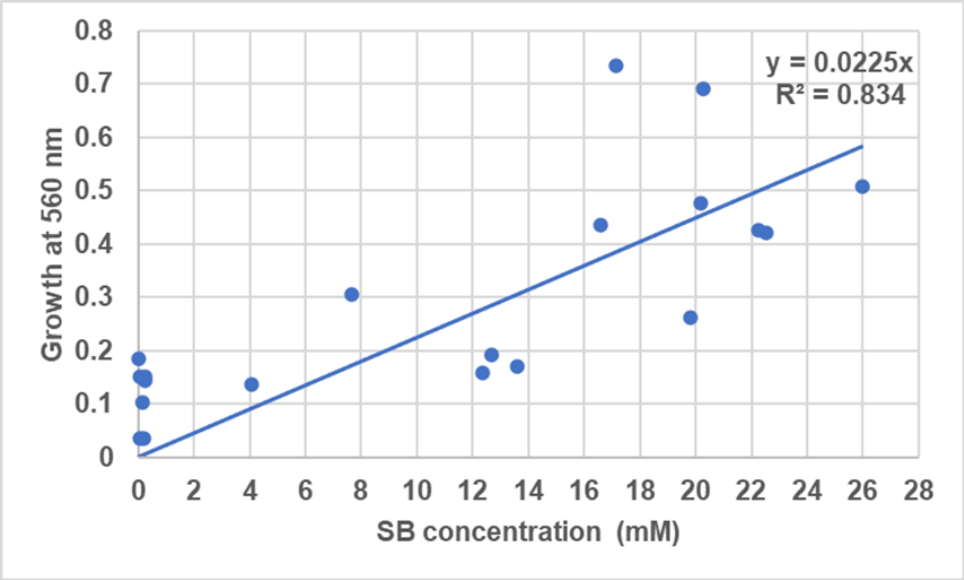


Figure 36: Correlation between growth of organisms and SB concentration

CONCLUSION

"The simpler is the insight, the more profound is the conclusion."

7. CONCLUSION

- ✓ The developed consortium had four organisms- *Stenotrophomonas maltophilia*, *Shewanella xiamenensis*, *Bacillus subtilis* and *Pseudomonas stutzeri* which were screened based on COD reduction potential, moderate EPS production and stable voltage generation.
- ✓ Analysis of carbon source utilization using GEN III Biolog® plate at 8 h and 22 h had shown that greater number of carbon sources are utilized by the organisms of the consortium at 22 h as compared to 8 h. Furthermore, at 22 h complex carbohydrates like bromo-succinic acid and galacturonic acid were utilized while at 8 h, only simple sugars like D-Fructose were assimilated by the organisms.
- ✓ Raw substrate materials containing sugars like D-Fructose, Dextrin, D- Melibiose, D-Fructose, Glucose-6-phosphate, Fructose-6-phosphate and organic acids like acetic acid, Galacturonic acid, Citric acid, L-Malic acid and Bromo succinic acid can be used for biomass development. Sugars like D- Fructose and organic acid like acetic acid are the preferred C source by all organisms at 8 h whereas at 22 h, sugars like Dextrin, D-Melibiose, D-Fructose, Glucose-6-phosphate, Fructose-6-phosphate and organic acids like Galacturonic acid, Citric acid, L-Malic acid and Bromo succinic acid are the preferred C sources by all organisms.
- ✓ Growth curve analysis in different conditions had demonstrated that aerobic and shaking condition was the best environment for growth of organisms.
- ✓ All the four organisms had shown a similarity in growth pattern in both aerobic static condition and anaerobic static condition. This growth behaviour would enable their efficient survival in anaerobic conditions of anodic chamber in MFC system without affecting their growth rate.
- ✓ In the optimization of different substrate concentrations in MFC system, the system containing 5 mM sodium benzoate had generated significantly high voltage output when compared to the uninoculated control as well as with respect to systems containing 10mM, 15 mM and 20mM SB concentrations. So, it was considered to be optimum concentration of substrate in MFC system.
- ✓ As the provided SB concentration increased, the % SB reduction decreased.
- ✓ Upon prolonged incubation of MFC, the suspended bacteria are transformed into biofilm attached cells as evidenced by reduction in suspended cell population.

- ✓ The inoculated consortium was able to reduce the organic load of the wastewater which was observed by the reduction of COD and SB concentration on 30^{th} day as compared to 0^{th} day. The achieved COD reduction was 38.25% while reduction in SB concentration was 42%.
- ✓ A high voltage output of 0.8 V was generated by the organisms of the consortium by using CETP inlet wastewater.
- ✓ "IN" chambers were observed to generate a higher voltage output as compared to "OUT" chambers. This may be due to the lower concentration of substrate reaching to the OUT chambers as it moves from the IN chambers to the OUT chambers.
- ✓ A positive correlation was observed in growth and SB concentration where growth increased upon increase of SB concentration and decreased upon reduction in SB concentration.
- ✓ A positive correlation was also obtained between SB concentration and COD concentration.

FUTURE ASPECTS

"The future holds endless potential."

8. FUTURE ASPECTS

- Optimization of growth parameters of bacteria present in MFC may enable them to improve treatment efficiency and electricity generation capability of MFC system.

- Successful implementation of continuous mode of MFC system at lab scale could facilitate its development to pilot scale for treatment of wastewater and electricity generation

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9. REFERENCES

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ANNEXURE

1. Composition of Phosphate Buffer Saline used as catholyte.

Table 5: Composition of Phosphate Buffer

Ingredients	Concentration (g/L)
KH_2PO_4	1.2
K_2HPO_4	1.8
NaCl	10
pH	7.0

2. Composition of Basal media as a part of synthetic wastewater

Table 6: Components of Basal media in synthetic wastewater

Ingredients	Concentration (mg/L)
KH_2PO_4	50
K_2HPO_4	25
H_3BO_3	0.1
$CoCl_2.6H_2O$	0.52
$(NH_4)_3. Mo_7O_{24}. 9H_2O$	0.52
NH_2Cl	100

3. Composition of Salt solution as a part of synthetic wastewater

Table 7: Components of Salt Solution in Synthetic wastewater

Ingredients	Concentration (mg/L)
$NiSO_4. 6H_2O$	0.5
$MnSO_4. 7H_2O$	0.5
$ZnSO_4. 7H_2O$	0.1
$MgSO_4. 7H_2O$	20
$CuSO_4. 5H_2O$	0.004
$FeSO_4. 7H_2O$	10

$CaCl_2.7H_2O$	70
$NaHCO_3$	480

4. Standard curve for SB concentration

As shown in Figure 37, the standard curve of O.D. vs Concentration (mM) was plotted for SB concentration to determine the concentration of unknown samples.

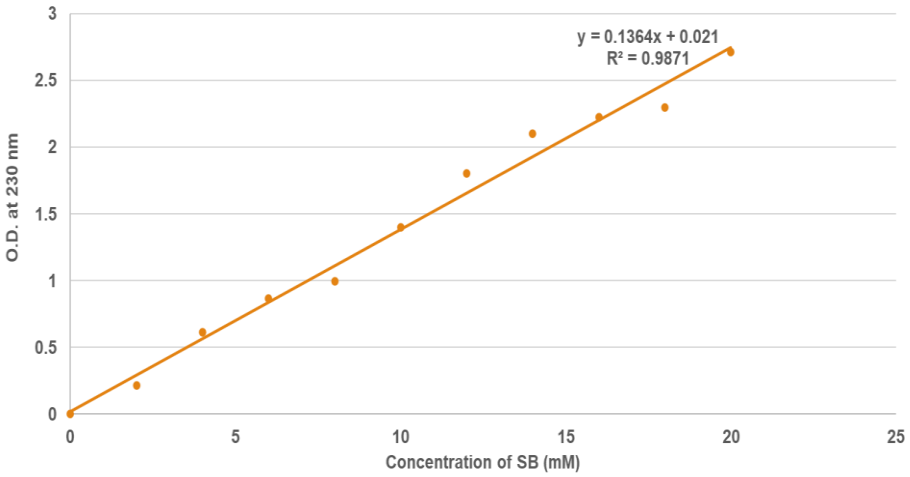

Figure 37: Standard curve for determination of SB concentration

MFC Thesis

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